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(54)【発明の名称】機能的核酸の同定方法

(57)【要約】
本発明は所望の表現型に機能的に関連した核酸分子を同定するための方法に関する。

【特許請求の範囲】
【請求項１】
以下の工程：
（ａ）親細胞のポピュレーションを準備し、その際、前記細胞ポピュレーションが実質的に所望の表現型を欠損し、
（ｂ）場合により前記の細胞ポピュレーションに細胞ゲノムの再配置及び／又は突然変異をもたらす処置を施し、
（ｃ）前記の（ｂ）からの細胞ポピュレーションに所望の表現型のための選択処置を施し、
（ｄ）前記の所望の表現型を表す細胞を同定し、かつ場合により特徴付け、
（ｅ）前記の所望の表現型を表す細胞からタンパク質及び／又はｍＲＮＡを得て、
（ｆ）前記の所望の表現型を表す細胞において遺伝子発現を調査し、
（ｇ）前記の所望の表現型を表す細胞における遺伝子発現と所望の表現型を実質的に欠損している細胞における遺伝子発現とを比較する
を含む、所望の表現型と機能的に関連した核酸分子を同定するための方法。
【請求項２】
所望の表現型を癌細胞特性から選択する、請求項１記載の方法。
【請求項３】
癌細胞特性が浸潤性、転移、接触阻害の欠損、細胞外基質所要量の欠損、成長因子の独立性、血管形成誘導、免疫防御の回避、抗アポトーシス及び／又は腫瘍マーカーレベルの増大から選択される、請求項２記載の方法。
【請求項４】
所望の表現型が抗アポトーシスである、請求項２記載の方法。
【請求項５】
所望の表現型が分泌されるタンパク質の産生、病原に対する感受性又は抵抗性、老化、細胞機能の調節及びシグナル伝達経路の調節から選択される、請求項１記載の方法。
【請求項６】
常にゲノム再配置及び突然変異誘発のプロセスにある親細胞を選択する、請求項１から５までのいずれか１項記載の方法。
【請求項７】
親細胞が不死化細胞又は形質転換された細胞である、請求項６記載の方法。
【請求項８】
実質的に安定なゲノムを有する親細胞を選択する、請求項１から５までのいずれか１項記載の方法。
【請求項９】
工程（ｂ）が突然変異誘発処置を含む、請求項８記載の方法。
【請求項１０】
前記の突然変異誘発処置が放射線照射、化学的突然変異誘発及びその組み合わせから選択される、請求項９記載の方法。
【請求項１１】
工程（ｄ）がセルソーティング処置を含む、請求項１から１０までのいずれか１項記載の方法。
【請求項１２】
前記のセルソーティング処置が蛍光活性化セルソーティング処置（ＦＡＣＳ）である、請求項１１記載の方法。
【請求項１３】
工程（ｅ）でｍＲＮＡを得ること及び該ｍＲＮＡ又はそれから生成される核酸を核酸アレイとハイブリダイズさせることを含む、請求項１から１２までのいずれか１項記載の方法。
【請求項１４】

ｍＲＮＡから生成される核酸がｃＤＮＡ及びｃＲＮＡからなる群から選択される、請求項１３記載の方法。
【請求項１５】
前記の核酸アレイが、複数の異なる核酸分子を固定されて有する固体担体を含む、請求項１３又は１４記載の方法。
【請求項１６】
前記の核酸アレイがゲノムＤＮＡアレイ、ｃＤＮＡアレイ及びオリゴヌクレオチドアレイのアレイから選択される、請求項１３から１５までのいずれか１項記載の方法。
【請求項１７】
前記の核酸アレイがキナーゼ、ホスファターゼ、酵素及び受容体から選択される機能的な細胞性ポリペプチド又はその部分をコードする核酸を含む、請求項１３から１６までのいずれか１項記載の方法。
【請求項１８】
工程（ｅ）においてタンパク質を得ること及び所望の表現型を表す細胞におけるその細胞含量を分析することを含む、請求項１から１２までのいずれか１項記載の方法。
【請求項１９】
前記の分析が２Ｄゲル電気泳動、質量分析及び／又はタンパク質アレイへの結合を含む、請求項１８記載の方法。
【請求項２０】
分析の前にタンパク質混合物の複合性を低下させるために前処理工程を実施する、請求項１８又は１９記載の方法。
【請求項２１】
更に、所望の表現型と関連する複数の遺伝子（遺伝子クラスター）の同定を含む、請求項１から２０までのいずれか１項記載の方法。
【請求項２２】
更に、規定の遺伝子又は遺伝子クラスターと所望の表現型との関連を調査する確認工程を含む、請求項１から２１までのいずれか１項記載の方法。
【請求項２３】
確認工程が優性阻害型突然変異体の作成を含む、請求項２２記載の方法。
【請求項２４】
更に、所望の表現型と関連する規定の遺伝子又は遺伝子クラスターについて試験物質の活性を測定するスクリーニング処置を含む、請求項１から２３までのいずれか１項記載の方法。
【請求項２５】
所望の表現型と関連する遺伝子又は遺伝子クラスターの発現プロフィールの作成のための、請求項１から２４までのいずれか１項記載の方法の使用。
【請求項２６】
発現プロフィールを生物学的試料における発現プロフィールと比較する、請求項２５記載の使用。
【請求項２７】
試料をヒトの患者から得る、請求項２６記載の使用。
【請求項２８】
ターゲットとしての、第１表、第２表及び第５表に示される核酸又はその断片又は前記の核酸によってコードされるペプチド又はポリペプチド又は断片の使用。
【請求項２９】
診断的用途のための請求項２８記載の使用。
【請求項３０】
治療的用途のための請求項２８記載の使用。
【請求項３１】
新規の医薬物質を同定するためのスクリーニング処置のための請求項２８記載の使用。

【発明の詳細な説明】
【０００１】
本発明は所望の表現型と機能的に関連する核酸分子を同定するための方法に関する。
【０００２】
アポトーシスの実行装置について多くの情報が集められている（Ｈｅｎｇａｒｔｎｅｒ，Ｎａｔｕｒｅ ４０７ （２０００），７７０－７７６）。しかしながらアポトーシスの開始をコントロールするシグナルについてのデータは最近になって蓄積され始めたにすぎない（Ｒｉｃｈ ｅｔ ａｌ．，Ｎａｔｕｒｅ ４０７ （２０００），７７７－７８３）。アポトーシス関連遺伝子又は他の特異的な表現型と関連する遺伝子を同定するための従来の方法は時間がかかるものであった。例えばＨｕｄｚｉａｋ他（Ｃｅｌｌ Ｇｒｏｗｔｈ ａｎｄ Ｄｉｆｆｅｒｅｎｔｉａｔｉｏｎ １２９ （１９９０），１２９－１３４）は腫瘍壊死因子－αを使用してＮＩＨ ３Ｔ３繊維芽細胞における形質転換及びｍｅｔ癌原遺伝子増幅のための選択方法を記載している。この方法を、他のチロシンキナーゼを含むびまん性腫瘍成長に関連する他の遺伝子産物を同定するために使用してよいことが示唆されている。しかしながら、かかる遺伝子の同定のための迅速又は信頼性のある方法は提供されていない。
【０００３】
本発明によれば機能的核酸分子を同定するための新規の方法が提供される。この方法はゲノム進化の概念をベースとしており、従って突然変異誘発及び／又はゲノム再配置工程に引き続いて所望の表現型を表す細胞クローンを選択することを必要とする。バイオインフォマティクス指向の遺伝子ソーティングに関連する引き続いてのトランスクリプトーム分析によって、選択された細胞特性のためだけでなく、付与される細胞表現型を左右する全シグナル経路のために重要な遺伝子の包括的な同定が可能である。この方法は、選択方法が利用可能な広範な細胞特性に対して使用できる。
【０００４】
このように本発明の対象は以下の工程：
（ａ）親細胞のポピュレーションを準備し、その際、該細胞ポピュレーションが実質的に所望の表現型を欠損し、
（ｂ）場合により前記の細胞ポピュレーションに細胞ゲノムの再配置及び／又は突然変異をもたらす処置を施し、
（ｃ）前記の（ｂ）からの細胞ポピュレーションに所望の表現型のための選択処置を施し、
（ｄ）前記の所望の表現型を表す細胞を同定し、かつ場合により特徴付け、
（ｅ）前記の所望の表現型を表す細胞からタンパク質及び／又はｍＲＮＡを得て、
（ｆ）前記の所望の表現型を表す細胞において遺伝子発現を調査し、
（ｇ）前記の所望の表現型を表す細胞における遺伝子発現と所望の表現型を実質的に欠損している細胞における遺伝子発現とを比較する
を含む、所望の表現型と機能的に関連する核酸分子を同定するための方法である。
【０００５】
本発明の方法において実質的に任意の型の親細胞（例えば細胞系統又は初代細胞）を使用できる。最も重要なことには、これらの細胞が所望の選択特性を欠損するか、又は僅かに弱くその特性を表すべきである。出発細胞の有利な例は真核細胞、例えば哺乳動物細胞、特にヒトの細胞である。
【０００６】
細胞、有利には所望の表現型を表す細胞クローンを作成するために、親細胞に細胞ゲノムの再配置及び／又は突然変異をもたらす処置を施してよい。この工程は親細胞の誘導を含む、ゲノム再配置及び／又は突然変異誘発する進化処置である。形質転換された細胞、例えば腫瘍細胞、例えばＨｅｌａ細胞又は不安定性に対する低い閾値を有する正常細胞、例えば不死化細胞、例えばＮＩＨ ３Ｔ３細胞の場合には特に誘導は必要ない。それというのもこれらの細胞は常にゲノム再配置及び突然変異誘発のプロセスにあるからである。親

細胞培養を、クローン又は有利には多ウェルプレート、例えば９６ウェルマイクロタイタープレート中に細分された培養の形のいずれかで選択条件にさらすことで十分であり、又は選択に致死的条件を要する場合には細胞単層の曝露で十分である。しかしながら実質的に安定なゲノムを有する親細胞を使用してもよいことに留意すべきである。しかしながらこれらの細胞は所望のゲノム再配置及び／又は突然変異誘発を得るために特定の誘導を必要とする。

【０００７】

有利な実施態様において、該方法の工程（ｂ）は突然変異誘発処置を含む。この突然変異誘発処置は放射線照射、例えばＵＶ又はγ－線による照射、化学的突然変異誘発、例えばＮ－メチルマレイミド又はエチルマレイミドでの処理による化学的突然変異誘発又はその組み合わせから選択できる。

【０００８】

細胞ゲノムの再配置及び突然変異を達成した後に細胞ポピュレーションに所望の表現型についての選択処置を施す。選択の後に、細胞、例えば所望の表現型を表す個々の細胞クローンを同定し、かつ場合により特徴付けする。前記の同定は形態学的測定法及び／又はセルソーティング法、例えば蛍光活性化セルソーティング（ＦＡＣＳ）による方法を含んでよい。これらの細胞を増大させ、引き続き所望の表現型／特性を検証及び／又は定量してよい。

【０００９】

引き続き所望の表現型を表す細胞からタンパク質及び／又はｍＲＮＡを得る。この材料を、所望の表現型を表す細胞における遺伝子発現を調査するため及び前記の細胞における遺伝子発現と所望の表現型を実質的に欠損している細胞における遺伝子発現とを比較するために使用してよい。

【００１０】

有利な実施態様において、所望の表現型を表す細胞からｍＲＮＡを得る。該ｍＲＮＡを選択された遺伝的に改変された細胞クローンから抽出し、かつ直接又は別の核酸、例えばｃＤＮＡ又はｃＲＮＡへの変換の後のいずれかに核酸アレイとのハイブリダイゼーションのためのプローブとして使用してよい。該アレイとのハイブリダイゼーションのために使用される核酸、例えばｍＲＮＡ、ｃＤＮＡ又はｃＲＮＡは通常、アレイにおける部位特異的ハイブリダイゼーションを測定するために標識されている。該アレイは固体担体、例えばフィルタ、チップ、スライドなどであり、該担体上の特定の位置に複数の種々の核酸分子をそこに固定されて有する。核酸アレイはゲノムＤＮＡアレイ、ｃＤＮＡアレイ及びオリゴヌクレオチドアレイから選択されてよい。有利には機能的な細胞性ポリペプチド又はその一部、より有利にはキナーゼ、ホスファターゼ、酵素及び受容体から選択されるポリペプチドをコードする核酸を有するアレイを使用してよい。選択される細胞クローンにおける遺伝子発現の尺度としてのアレイ上でのハイブリダイゼーションは公知の方法により、例えばホスホイメージャーを使用する画像分析によって測定できる。幾つかのケースにおいて細胞の所望の新規の特性は、例えば細分化された培養の調整培地の大規模高処理量アッセイ分析によって測定できる。

【００１１】

ｍＲＮＡ分析による発現プロファイリングに加えて又はその代替として、親細胞系統及び同定されたクローン又はそれらの上清と比較された、同定されたクローンのタンパク質含量における差異を測定するプロテオミクスアプローチを適当な方法、例えば２Ｄゲル電気泳動によって実施してよい。親細胞系統及び同定されたクローンにおける濃度が異なるタンパク質は２Ｄゲル中で異なって染色されたスポットを示す。更にリン酸化のようなタンパク質変性はこの方法によって検出できる。一度、タンパク質混合物の複雑性を低減させるために該分析工程の前に細胞性タンパク質の分離を実施してもよい。例えばカラムクロマトグラフィー工程を実施してよく、該工程はキナーゼ（ＡＴＰカラムを使用する親和性クロマトグラフィーによって）又はグリコシル化されたタンパク質（レクチンカラムを使用して）を精製し、次いでこれを更に２Ｄゲル電気泳動によって分離してよい。タンパク

質濃度の差異を分析するためのいかなる別の方法（タンパク質チップ、質量分析）を使用してもよい。

【００１２】

所望の表現型を表す細胞における遺伝子発現結果を所望の表現型を実質的に欠損する細胞における、有利には親細胞における遺伝子発現と比較する。更に該遺伝子発現結果をクラスター検出プログラムによって分析してよい。この分析は所望の細胞表現型を与える遺伝子発現における複数の可能な変化を明らかにする。

【００１３】

本発明の方法の用途は非常に広範であり、かつ実質的に選択できかつ／又はアッセイで測定できる全ての細胞特性を含む。例えば所望の表現型は癌細胞特性、例えば浸潤性、転移、接触阻害の欠損、細胞外基質所要量の欠損、成長因子の独立性、血管形成誘導、免疫防御の回避、抗アポトーシス及び／又は腫瘍マーカーレベルの増大から選択されてよい。

【００１４】

特に有利な実施態様において、所望の表現型は抗アポトーシスである。別の用途は、公知の腫瘍マーカーについて癌細胞をソーティングすることによる癌関連遺伝子の解明である。しばしば腫瘍マーカーは結果であり、細胞の腫瘍原性の原因ではなく、従って医薬物質ターゲットとして適さない。しかしながら該マーカーと癌表現型との相関が確立されているので、増大するマーカー発現について細胞をソーティングすることはまた該マーカーに連係し、癌表現型を引き起こす遺伝子をソーティングすることにもなる。これらの遺伝子は親細胞系統及びソーティングされた細胞における発現プロフィールを比較することによって同定でき、かつ該遺伝子は潜在的な医薬物質ターゲットである。

【００１５】

選択的に、所望の表現型は他の特性、例えば分泌性タンパク質、例えばインスリン、成長ホルモン、インターフェロンなどの産生、病原、例えばウイルス、例えばＨＣＶ、ＨＢＶ又は他の病原に対する感受性又は抵抗性、老化及び細胞機能の調節、すなわち特定の細胞機能を調節する遺伝子の同定、例えばアップレギュレートされたインスリン受容体活性を有する細胞クローンについてのスクリーニングを含むインスリン受容体活性の負のレギュレーターの同定から選択されてよい。

【００１６】

更に有利な実施態様はシグナル伝達経路の要素の同定、一般にそれぞれのシグナルをより良好に伝達できる細胞をソーティングすることである。例えば特にＥＧＦ受容体ファミリー、例えばＥＧＦＲ、ＨＥＲ２及びＨＥＲ３の受容体の受容体チロシンキナーゼ（ＲＴＫ）のシグナル伝達経路の要素の同定は適当な受容体タンパク質、例えば緑色蛍光タンパク質（ＧＦＰ）を、それぞれの受容体のリガンドによる刺激に応答するプロモーター（例えばＥＧＦ刺激のためのｃ－ｆｏｓプロモーターなど）のコントロール下に発現する細胞系統を作成することによって実施できる。次いでリガンドによる受容体の刺激はＧＦＰの転写をもたらし、かつ例えばＦＡＣＳ機器によって検出可能な増大した緑色蛍光が引き起こされる。最も高い蛍光誘導を示す細胞のソーティングは親細胞ポピュレーションよりも強力にリガンド指向シグナルに応答する細胞を富化する。両方の細胞ポピュレーションの発現パターンの分析は、変動する発現がシグナルと異なる反応を担い、従ってシグナル伝達経路に影響を及ぼす遺伝子を同定する。この戦略は蛍光出力を生成できるいかなるシグナルにも適用できる。

【００１７】

以下に、本発明をｃＤＮＡアレイを使用する抗アポトーシス性核酸の同定に関してより詳細に記載する。しかしながらこの実施態様は本発明の方法についての概略にすぎず、限定として解釈すべきではないことを留意すべきである。

【００１８】

アポトーシスの調節に関連する核酸を同定するために、本発明の方法をアポトーシス感受性細胞及びアポトーシス抵抗性細胞において分化的に発現される遺伝子の同定のために使用した。

【００１９】
アポトーシスをＦａｓ活性化によってヒトの子宮頚癌細胞系統ＨｅｌａＳ３において誘導した。Ｆａｓの活性化はカスパーゼ－８の自己触媒的な活性化をもたらし、従ってアポトーシスを引き起こす。Ｆａｓ活性化のために親細胞を抗Ｆａｓ抗体と一緒にインキュベートした。
【００２０】
選択処置の後に、僅か少量の生細胞が存在した。これらの細胞は親細胞系統よりも高いアポトーシスへの抵抗性を有した。生存細胞をクローンとして増幅させた。ｍＲＮＡをこれらのクローン及び親細胞系統から単離し、引き続きｃＤＮＡに逆転写した。次いでｃＤＮＡアレイをクローン及び親細胞系統からのｃＤＮＡとハイブリダイズさせ、こうしてアレイ上で遺伝子発現を測定した。アレイ上の配列は有利にはキナーゼ及びホスファターゼをコードする約１０００の遺伝子から得た。親細胞系統の発現及びクローンの発現を比較することによって、増大された発現を示す（２倍を上回る増大）約２００の遺伝子がクローンの少なくとも１０％で同定された。これらはクローンのアポトーシス抵抗性と関連する核酸である（第１表及び第２表）。第１表はアポトーシス抵抗性クローンで誘導される遺伝子のリストであり、抗アポトーシス機能に連係していない。第２表は従来公知の抗アポトーシス機能を有するアポトーシス抵抗性クローンにおいて誘導される遺伝子のリストである。
【００２１】
親細胞系統、例えばＨｅｌａＳ３及び所望の表現型を有するクローン、例えばアポトーシス抵抗性クローンにおいて分化的に発現される遺伝子の同定のための改善された方法、例２に記載されるような評価方法を適用してよい。親細胞系統で分析される各核酸について複数の測定値を測定し、そこから平均値及び標準偏差を計算できる。例えばＲＮＡを少なくとも２回、少なくとも２つの独立した調製において親細胞から単離してよい。各調製からの材料を少なくとも２つの核酸アレイとのハイブリダイゼーションのために使用する。アレイ上に生じるスポットに関する前記の値の平均を計算し、かつ標準偏差を決定する。所望のクローンからの材料を１つの核酸アレイとハイブリダイズさせる。その値が予定されたカットオフを上回る場合に、遺伝子が所望のクローンにおいて分化的に発現されたと考慮する。アップレギュレートされた遺伝子についてのカットオフは有利には親細胞のそれぞれの値の平均と２倍の標準偏差との和である。ダウンレギュレートされた遺伝子についてのカットオフは有利には親細胞のそれぞれの値の平均からの２倍の標準偏差の差である。この方法を使用して、該実験方法において固有のエラーを校正できる。核酸アレイの調製の間に生じるこれらの誤差が標準偏差を決定するので、標準偏差からはずれる所望のクローンのいかなる値も分化的に発現された遺伝子を表す。従ってまた任意のカットオフを使用することによって検出できない遺伝子発現における小さな差異も検出できる。この改善された評価方法によって得られた値を第５表に示す。
【００２２】
このように本願の対象は第１表、第２表及び第５表、有利には第１表及び第５表に示されるような核酸及びこれらの核酸によってコードされるポリペプチドの、診断的用途及び治療的用途、アポトーシス過程の不全に関連する疾患、例えば腫瘍のための“ターゲット”としての使用である。更に該核酸及び遺伝子産物はアポトーシス性／抗アポトーシス処置の新規のモジュレーター、特に医薬物質の同定のためのスクリーニング法におけるターゲットとして適当である。該医薬物質は生体分子、例えば該遺伝子産物に対する抗体、酵素インヒビター又は低分子の非生物学的な医薬物質であってよい。医薬物質スクリーニングの方法は細胞ベースの系を含み、その際、目的の標的核酸を過剰発現する細胞を使用するか、又は該方法は分子ベースの系を含み、その際、目的のポリペプチドを部分的に精製された又は実質的に精製及び単離された形で使用する。特定のスクリーニング方法は当業者に公知であり、本願に詳細に記載する必要はない。しかしながら高い処理量のスクリーニングアッセイは使用してよいことを留意すべきである。
【００２３】

更に細胞系統にわたる発現パターンは類似している遺伝子の幾つかの群又はクラスターが同定された。アポトーシス抵抗性クローンのクラスターを第３表に示す。扁平上皮癌細胞系統におけるクラスターを第４表に示す。かかるクラスターの同定は診断的用途及び／又は治療的用途並びにスクリーニング法における活性剤の特定の組み合わせの使用を可能にする。このように本発明の有利な実施態様によれば、幾つかのターゲットの存在及び／又は活性を調節可能な医薬物質の組み合わせを効能を増大させるために使用できる。

【００２４】

更に本発明の方法は所望の表現型と関連する遺伝子及び特に遺伝子クラスターの発現プロフィールの作成を可能にする。これらの発現プロフィールを特定の生物学的試料（該試料は患者、例えばヒトの患者、特に腫瘍患者から得られる体液又は組織試料であってよい）における発現プロフィールと比較してよい。本発明による方法によって得られる発現プロフィールと生物学的試料における発現プロフィールとの比較は特に個々の患者に適合された改善された診断、モニタリング及び／又は治療の戦略の進展を可能にする。

【００２５】

これらの実験において、クローンにおける増大された発現を有するタンパク質の触媒活性の阻害がアポトーシスの促進された増大をもたらすことが裏付けられた。また親細胞系統においてもその阻害は増大されたアポトーシスをもたらした。このことはクローンのアポトーシス抵抗性について同定された核酸及びタンパク質の重要性を概説し、かつ阻害特異性を裏付けている。

【００２６】

更に本発明を以下の実施例及び図面においてより詳細に記載する。

【００２７】

図１はアップレギュレートされたキナーゼの阻害を示している。

【００２８】

細胞をＦＣＳを含まず、かつ１００ｎｇ／ｍｌの抗Ｆａｓ抗体ＣＨ－１１で処理された、インヒビターを含む及び含まないハムＦ１２培地中で増殖させた。アポトーシスを実施例に記載されるようなＦＡＣＳ分析によって測定した。ＳＵ５４０２：１０μＭ、ＡＧ１２９５：１μＭ、ＳＢ２０３５８０：１０μＭ、ＰＤ９８０５９：２５μＭ。

【００２９】

図２は優性阻害型突然変異体及びアンチセンス構築物によるｐｙｋ－２の阻害を示している。

【００３０】

図３はクローンのアポトーシス感受性を示している。７０％の集密的な細胞を２４時間、ＦＣＳを含まない培地中で飢餓状態にし、引き続き１００ｎｇ／ｍｌのＣＨ－１１を添加した。１６時間のインキュベーション後に細胞核を低張バッファー中で染色し、かつＦＡＣＳによって分析した。ｓｕｂ－Ｇ１ピークのパーセンテージを導き出した。ＦＣＳ無しでのアポトーシス率をＦＣＳありでの率から減算した。

【００３１】

図４は他のアポトーシスインデューサーでのアポトーシス感受性を示している。７０％の集密的な細胞を２４時間ＦＣＳを含まない培地中で飢餓状態にし、引き続き１０μｇ／ｍｌのシスプラチナム又はＴＮＦ－αと０．１μｇ／ｍｌのシクロヘキシミドを細胞に添加した。１６時間後に細胞核をヨウ化プロピジウムで染色し、かつＦＡＣＳによって分析した。５０ｎＭのタキソールを３時間で細胞に添加し、引き続き培地を１０％のＦＣＳを有する新鮮な培地によって交換した。２日後にｓｕｂ－Ｇ２細胞のパーセンテージを導き出した。ＦＣＳ無しでのアポトーシス率をＦＣＳありでの率から減算した。この値をそれぞれのＨｅｌａＳ３細胞のパーセンテージとして表現した。

【００３２】

ウイルス上清をフェニックスＡ（Ｐｈｏｅｎｉｘ Ａ）パッケージング細胞系統及びベクターｐＬＸＳＮ中にクローニングされたそれぞれのクローニングされた構築物（ｐｙｋ－２野生型又はｐｙｋ－２ＫＭ突然変異体を発現する）を使用して作成した。ＨｅｌａＳ３

及びクローン１４を一晩感染させた。培地を翌日に交換し、２日後に細胞を２４時間ＦＣＳを含まない培地中で飢餓状態にし、それから一晩１００ｎｇ／ｍｌのＣＨ－１１を添加した。アポトーシスを図１に記載されるように測定した。

【００３３】

例１

１．材料及び方法

１．１　アポトーシス抵抗性クローンの選択

子宮頚癌細胞系統ＨｅｌａＳ３（ＡＴＣＣ　ＣＣＬ－２．２）を１０ｃｍの細胞培養皿（10^5細胞）で１０％のＦＣＳを含有するハムＦ１２成長培地にプレーティングした。翌日に該培地を１００ｎｇ／ｍｌのアポトーシス活性化抗Ｆａｓ抗体ＣＨ－１１（Ｃｏｕｌｔｅｒ　Ｉｍｍｕｎｏｔｅｃｈ）を補ったＦＣＳ不含の培地と交換した。３日後に殆どの細胞が死んだら、培地をもう一度抗体を含まない１０％のＦＣＳを含有する培地と交換した。生存している細胞を３週間クローンとして培養した。該クローンをピックアップし、かつ増幅させた。

【００３４】

１．２　アポトーシスアッセイ

親細胞系統ＨｅｌａＳ３から又はクローンからそれぞれ得られるウェルあたり５００００細胞を１２ウェル細胞培養皿中で２日間、１０％のＦＣＳを含有するハムＦ１２培地中で増殖させた。３日目に、該細胞を１ｍｌのハムＦ１２培地で２回洗浄し、かつ次いで培地を１ｍｌのハムＦ１２培地と交換した。翌日に該培地にそれぞれのインヒビター及び１００～２００ｎｇ／ｍｌのＣＨ－１１を供給した。その翌日に培地をデカンテーションし、かつエッペンドルフチューブに移した。該細胞を２００μｌのＰＢＳで１回洗浄し、該ＰＢＳをそれぞれのエッペンドルフチューブに移した。次いで残りの細胞をまたそれぞれのエッペンドルフチューブにＰＢＳ中のＥＤＴＡ／トリプシンでの処理後に移した。該細胞を遠心分離によってペレット化させ、５００μｌの低張バッファー（０．１％のクエン酸ナトリウム、０．１％のトライトンＸ１００、２０μｇ／ｍｌのヨウ化プロピジウム）中で懸濁し、かつ４℃で２～２４時間インキュベートした。得られた細胞核をＦＡＣＳによって分析した。

【００３５】

１．３　ＦＡＣＳ（蛍光活性化セルソーティング）－アポトーシス性核の測定のための分析

単一核のヨウ化プロピジウム蛍光をＦＡＣＳＣａｌｉｂｕｒ（Ｂｅｃｔｏｎ　Ｄｉｃｋｉｎｓｏｎ）サイトメーターを使用して測定した。前方散乱光（ＦＳＣ）及び側方散乱光（ＳＳＣ）を同時に記録した。ＦＳＣピークを１０２４チャンネルの線形スケールでのチャンネル５００で、かつ赤色蛍光ピークを対数スケールのチャンネル２００で調節した。ＦＳＣカットオフ値を、供給物なしのネガティブコントロールの最も大きな核の９５％にゲーティングすることによって測定した。これらの核をＧ１／Ｇ０ピークとチャンネル１０の間の副二倍体（ｓｕｂｄｉｐｌｏｉｄ）シグナルが存在する場合にアポトーシス性として分類した。

【００３６】

１．４　ｃＤＮＡの調製

全ＲＮＡを、グアニジニウムイソチオシアネートによって細胞を溶解し、かつ引き続き酸フェノールで抽出することによって単離した（分子生物学における最近のプロトコール）。ｍＲＮＡを標準的方法によってオリゴ－ｄＴセルロースに結合させることによって単離した（分子生物学における最近のプロトコール）。

【００３７】

Ｃａｐファインダープライマー（ＣＡＰ　ｆｉｎｄｅｒ　ｐｒｉｍｅｒ）Ｋ１及びＫ２（クロンテック　Ｉｎｃ．，　ＵＳＡ）及びＡＭＶ逆転写酵素（Ｒｏｃｈｅ　Ｄｉａｇｎｏｓｔｉｃｓ）を使用して逆転写によってｍＲＮＡからｃＤＮＡを合成し、かつＰＣＲ精製キット（Ｑｉａｇｅｎ）を使用して精製した。３μｇのｍＲＮＡから、一本鎖ＤＮＡ及び一本

鎖ＲＮＡからなる５０μｌのｃＤＮＡを得た。
【００３８】
１．５　ｃＤＮＡアレイの調製
ｐ－Ｂｌｕｅｓｃｒｉｐｔ中にクローニングされたｃＤＮＡをナイロンメンブレン上にＢｉｏＧｒｉｄスポッター（ＢｉｏＲｏｂｏｔｉｃｓ，ＵＫ）でスポッティングした。２５０ｎｇのＤＮＡを１スポットあたりに使用した。約二分の一の遺伝子について、２つ以上のプローブを使用し、かつ各プローブを２回スポッティングした。以下の記号を使用した：
ＹＫ＝チロシンキナーゼ
ＳＴＫ＝セリン／トレオニンキナーゼ
ＰＰ＝ホスファターゼ
Ｌｉｇ＝リガンド
ＵＫ＝未知のキナーゼ
ＵＰ＝未知のホスファターゼ
ＯＴ＝その他
例：
ＹＫ＿１ｂ＿Ａｂｌ＿２＝チロシンキナーゼ１，プローブｂ、スポット２
１．６　ｃＤＮＡの放射活性標識
５μｌのｃＤＮＡをメガプライムラベリングキット（Ｍｅｇａｐｒｉｍｅ Ｌａｂｅｌｌｉｎｇ Ｋｉｔ）（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ）を使用して５０μのＣｉα^{3} ^{3}Ｐ－ＡＴＰで標識し、ＰＣＲ精製キット（Ｑｉａｇｅｎ）を使用して精製した。こうして得られたｃＤＮＡをＣＯＴ－ＤＮＡ（Ｒｏｃｈｅ Ｄｉａｇｎｏｓｔｉｃｓ）とハイブリダイズさせて、ｃＤＮＡアレイに非特異的に結合しうる繰り返し配列をブロッキングした。
【００３９】
１．７　ｃＤＮＡアレイのハイブリダイゼーション
ｃＤＮＡアレイをプレハイブリダイゼーション溶液（５０×デンハルト、１０×ＳＳＣ、０．２５ＭのNa_3PO_4、ｐＨ６．８、５０ｍＭの$Na_4P_2O_7$、０．１ｍｇ／ｍｌのｔＲＮＡ（パン酵母、Ｒｏｃｈｅ Ｄｉａｇｎｏｓｔｉｃｓ）中で６８℃において４時間又は一晩プレハイブリダイゼーションさせた。
【００４０】
引き続きｃＤＮＡアレイをハイブリダイゼーションバッファー（５×ＳＳＣ、０．１％のＳＤＳ、０．１ｍｇ／ｍｌのｔＲＮＡ）中で標識されたｃＤＮＡと１６時間ハイブリダイズさせた。該ｃＤＮＡアレイを以下のように洗浄した：
２×２０分Ｗ１（２×ＳＳＣ、０．１％のＳＤＳ）４２℃
１×２０分Ｗ２（０．２×ＳＳＣ、０．１％のＳＤＳ）４２℃
１×６０分Ｗ２　６５℃
ｃＤＮＡアレイをホスホイメージャープレート（Ｆｕｊｉｆｉｌｍ）上で４８時間さらし、かつ引き続きホスホイメージャー（Ｂａｓ－２５００，Ｆｕｊｉｆｉｌｍ）上で分析した。
【００４１】
１．８　ｃＤＮＡアレイの分析
フィルタ上のスポット容量をアレイヴィジョンソフトウェア（Ａｒｒａｙ Ｖｉｓｉｏｎ ｓｏｆｔｗａｒｅ）（Ｖ５．１，Ｉｍａｇｉｎｇ Ｒｅｓｅａｒｃｈ Ｉｎｃ．）を使用して測定した。全ての更なる計算はエクセル（Ｍｉｃｒｏｓｏｆｔ Ｃｏｒｐ．）で実施した。
【００４２】
ｃＤＮＡアレイのより良好な内部比較のために、規格化法を以下のように実施した：アレイ上の各スポットからバックグラウンド（アレイのｐ－Ｂｌｕｅｓｃｒｉｐｔ値の平均）を減算し、かつアレイ中の全てのスポット容量の和によって除算した。こうして得られた

値を１００００で乗じた。
【００４３】
親細胞系統ＨｅｌａＳ３及びアポトーシス抵抗性クローンにおいて分化的に発現される遺伝子の同定のために、クローンの値及び親細胞系統の種々のアレイ（参照アレイ）の平均値からの商を計算した。０．１未満の全ての規格化された値を該計算のために０．１に設定した。０とは異なる全ての値の９０％は前記の値より高かった。それぞれの遺伝子を、パーセンテージが少なくとも１００％と異なる場合に分化的に発現されたと定義した。かかる遺伝子だけを分析し、その際、該アレイ上のそれぞれのスポットについての参照アレイ上の値の偏差は十分に小さかった。以下のフィルタをこれらの遺伝子を除外するために使用した。
【００４４】
参照アレイ及びそれぞれのクローンの１つのスポットについての値が２．５より小さい場合に、参照アレイの互いの偏差は０．２～５の範囲でなければならない。
【００４５】
参照アレイの値が２．５より小さく、クローンの値が２．５より大きいか、又はその逆の場合に、参照アレイの互いの偏差は０．３～３の範囲である必要がある。
【００４６】
参照アレイの値及びクローンの値の両方が２．５より大きい場合に、参照アレイの互いの偏差は０．５～２の範囲である必要がある。
【００４７】
１．９　遺伝子クラスタリング
遺伝子クラスタリングのためにプログラムＣｌｕｓｔｅｒ（Ｍｉｃｈａｅｌ Ｅｉｓｅｎ, Ｓｔａｎｆｏｒｄ Ｕｎｉｖｅｒｓｉｔｙ）を使用した。クローンの値及び親細胞系統のそれぞれのアレイの平均値からの商を使用した。参照アレイ上の値で高い偏差を示すスポットを排除した。この目的のために、誘導された遺伝子の同定で既に適用されたフィルタを使用した。１９２２個のスポットから１４５１が残った。これらの値を対数的にクラスターに移行し、かつ更にクローンの少なくとも８０％の値が０と異なるスポット上でフィルタした。こうして得られる５２０個のスポットを階層的クラスタアルゴリズムを介して分析した。
【００４８】
発現パターン及びクラスターの全体の類似性は１と－１との間の値を有する相関係数に反映する。１の相関係数は、発現パターンが同一であることを意味し、０はこれらが完全に独立していることを意味し、かつ－１は互いの反対であることを意味する。
【００４９】
２．結果
２．１　アポトーシス抵抗性クローンはＣＨ－１１抗体を使用してＨｅｌａＳ３の選択によって得られる
４０個のクローンがＣＨ－１１抗体での選択の後に得られた。これらのクローンの２０個をＣＨ－１１に対するその感受性に関して試験した。クローンが抵抗性である程度は個々のクローンの間で異なるが、そのどれもが完全にアポトーシス抵抗性でなく、このことはアポトーシス機関が機能的であることを示唆している。これらのクローンはＴＮＦ－α及びシスプラチンによって誘導されるアポトーシスに屈折性を有している。
【００５０】
２．２　多くの遺伝子がアポトーシス抵抗性クローンにおいて増大された発現を示す
第１表及び第２表はアポトーシス抵抗性クローンにおいて増大された発現を示す遺伝子のリストを示している。更にそれぞれのクローンのジーンバンクのアクセッション番号、発現が増大された発現についてのカットオフを上回るクローンの数及びカットオフを上回る平均パーセンテージが示されている。
【００５１】
殆どの分析された遺伝子はタンパク質ホスファターゼ及びキナーゼ、すなわち細胞調節の

ために重要な酵素をコードしている。
【００５２】
こうして決定された誘導されたクローンの幾つかは依然としてアポトーシス及び／又は腫瘍形成に関連していない（第１表）。他の遺伝子、例えばＣＡＭＫＫ（カルモジュリン依存性キナーゼキナーゼ）、ＥＧＦＲ（上皮成長因子受容体）、Ｂｃｒ（ブレークポイントクラスター領域）、ＦＧＦＲ－１（繊維芽細胞成長因子受容体１）、Ｎｉｋ（ＮＦκＢ－相互作用キナーゼ）及びＤＡＰＫ（死に関連するプロテインキナーゼ）は既にアポトーシス関連遺伝子として公知である。
【００５３】
２．３　遺伝子クラスタリングは共通して調節される遺伝子の群を示す
発現データのクラスタリングによって、共通してアップレギュレート又はダウンレギュレートされる遺伝子の群が見いだされた。共通の調節は遺伝子の共通の機能を示唆している。こうして単一のアポトーシス調節遺伝子だけでなく、複数の遺伝子からなるシグナル伝達カスケードも見いだされる。アポトーシス抵抗性クローンにおいて同定されるクラスターを第３表に示す。遺伝子のクラスタリングはアップレギュレートされた遺伝子をグループ化することを可能にし、かつ単一の遺伝子だけの代わりに種々の抗アポトーシスシグナル経路を導き出すことを可能にする。アポトーシス抵抗性クローンに見いだされたクラスターはまた扁平上皮細胞癌細胞系統の発現データに部分的に見いだされうる（第４表）。スクリーニングによって生理学的に関連するアポトーシスクラスターは腫瘍発達のために重要であり、従って医薬物質ターゲットとして提供できることを見いだすことができることを示唆している。
【００５４】
クラスター１は多くのクローン、例えばＣＡＭＫＫ、ＵＫ１１（未知のキナーゼ１１）、ＰＴＰα（タンパク質チロシンホスファターゼα）及びＰＲＫ（増殖関連キナーゼ）において誘導される幾つかの遺伝子を有する。
【００５５】
クラスター２は高度に相関した発現を示す３つの遺伝子、すなわちセリン／トレオニンホスファターゼＶＨ２、ＴＩＭＰ（メタロプロテイナーゼ１）及びＭＭＰ－１５（基質メタロプロテイナーゼ１５）を有する。興味深いことに、酵素（ＭＭＰ－１５）及び潜在的なインヒビター（ＴＩＭＰ－１）は共通して調節される。
【００５６】
クラスター３はとりわけ膜結合チロシンホスファターゼＬａｒ及びプロアポトーシス性セリン／トレオニンキナーゼＤＡＰキナーゼを含む。
【００５７】
クラスター４において、ＢＣＲ、ｐ３８の潜在的なインヒビター及びＪＮＫシグナル経路及びｐ３８のアクチベーター、すなわちＭＡＰＫＫ－３（マイトジェン活性化キナーゼキナーゼ３）は共通して調節される。
【００５８】
２．４　誘導された遺伝子の阻害はアポトーシスを増大させる
誘導された遺伝子が事実アポトーシスのモジュレーターであることを示すために、選択された酵素を特異的インヒビターによって阻害し、かつアポトーシスを誘導した。以下の酵素のためのインヒビターを使用した：
－　ＳＵ５４０２はＦＧＦ受容体を阻害するが、定義されたＦＧＦ受容体のために特異的でない
－　ＡＧ１２９５はＰＤＧＦ受容体を阻害する
－　ＳＢ２０３５８０はｐ３８ＭＡＰキナーゼを阻害する
ＰＤ９８０５９はＭＡＰキナーゼキナーゼ１を阻害し、またこれはＭＡＰキナーゼＥＲＫ１及びＥＲＫ２を活性化する。このインヒビターはＳＢ２０３５８０のためのコントロールとして使用される。それというのもＳＢ２０３５８０もＥＲＫ１及びＥＲＫ２を部分的に阻害するからである。更にＥＲＫ２は該クローンにおいて増大された発現を示す。Ｈｅ

ｌａＳ３、クローン１４及びクローン２０（部分的）のための結果を図１に示す。

【００５９】

ＨｅｌａＳ３細胞におけるＦＧＦ受容体の阻害は約５０％のアポトーシスにおける増大をもたらすことが判明した。クローン１４及び５において、ＳＵ５４０２はそれぞれ約３００％又は５０％の増大をもたらす。このように２つのＦＧＦ受容体（クローン１４、ＦＧＦＲ－１及びＦＧＦＲ－３）の増大された発現を有するクローンにおいて、ＦＧＦ受容体の阻害はアポトーシスの高められた増大をもたらす。ＦＧＦ受容体のいかなる増大された発現も示さないクローン５において、アポトーシスにおける増大は親細胞系統ＨｅｌａＳ３に匹敵する。

【００６０】

ＰＤＧＦ受容体の阻害はＨｅｌａＳ３において約３０％の増大をもたらす。ＰＤＧＦ受容体の増大された発現を示すクローン１４において、該阻害は多くのアポトーシス細胞の二倍化をもたらす。それに対して、いかなる検出可能なＰＤＧＦ受容体も含有しないクローン５はＡＧ１２９５での処理後にアポトーシスの３０％だけの増大を示す。

【００６１】

ｐ３８ＭＡＰキナーゼを阻害する。それというのもＢＣＲ、ｐ３８ＭＡＰキナーゼシグナル経路のインヒビター及びｐ３８アクチベーターであるＭＡＰＫＫ－３（ＭＥＫ－３）は該クローンにおいて増大された発現を示すからである。更に両方の遺伝子は１つのクラスターにグループ化される。

【００６２】

ＨｅｌａＳ３におけるｐ３８阻害はアポトーシスの２５％の増大をもたらす。増大されたＭＥＫ－３を示すクローン１４において、ｐ３８の阻害はアポトーシスの６０％の増大をもたらす。それに対してＭＥＫ－１の阻害はアポトーシス率の二倍化をもたらす。ＨｅｌａＳ３と比較された、ｐ３８の阻害後のアポトーシスの増大及びＭＥＫ－１の阻害後の一定のアポトーシスはＥＲＫ１／２の阻害及びｐ３８の付加的な阻害によって説明できる。

【００６３】

ＨｅｌａＳ３と類似のレベルでＭＥＫ－３を発現するクローン２０において、ＳＢ２０３５８０での処理だけがアポトーシスの僅かな増大をもたらすにすぎない。それに対してＰＤ９８０５９での処理はアポトーシス率を三倍にする。このようにＳＢ２０３５８０はこの系において特異的に作用し、ｐ３８の阻害後のアポトーシスの増大における差異はｐ３８アクチベーターＭＥＫ－３の発現と相関している。

【００６４】

これらの阻害実験は結果的に、本発明のアポトーシスに関連する遺伝子を同定する方法が効果的であることを裏付けている。

【００６５】

２．５　優性阻害型突然変異体又はアンチセンス鎖の導入による阻害

アポトーシス抵抗性クローンにおいてアップレギュレートされるそれぞれの酵素を、優性阻害型突然変異体又はアンチセンス鎖を導入することによって阻害することもできる。図２は、例えば野生型のｐｙｋ－２が、ＨｅｌａＳ３への増大の際に増大された抵抗性を与えることを示している。ｐｙｋ－２のより高度な発現を有するクローン１４において、野生型酵素の導入は効果的でないが、酵素の活性中心においてメチオニンに突然変異されたリジンを有する突然変異体（ｐｙｋ－２ＫＭ）は該クローンの表現型を回復する。アンチセンス構築物は相応の効果を有するが、より弱い効果を有する。

【００６６】

例２

実験法を例１に記載されるように実施した。

【００６７】

親細胞系統ＨｅｌａＳ３及びアポトーシス抵抗性クローンで分化的に発現される遺伝子の同定のために、以下の評価法を適用した。親細胞系統ＨｅｌａＳ３のｃＤＮＡアレイ上の各スポットについて、４つの値を以下のように決定した。ＲＮＡをＨｅｌａＳ３から２つ

の独立した調製において２回単離した。各ＲＮＡ調製物を使用してｃＤＮＡを合成し、かつ各ｃＤＮＡを２つのｃＤＮＡアレイとハイブリダイズさせた。ｃＤＮＡアレイ上に生じるスポットについてのこれらの４つの値の平均を計算し、かつ標準偏差を測定した。各アポトーシス抵抗性クローンのｃＤＮＡを１つのｃＤＮＡアレイとハイブリダイズさせた。遺伝子は、その値が以下のカットオフを上回る場合にアポトーシス抵抗性クローン中で分化的に発現されたと考慮した。アップレギュレートされた遺伝子のカットオフはそれぞれのＨｅｌａＳ３値の平均と２倍の標準偏差との和であった。従って、ダウンレギュレートされた遺伝子のカットオフはそれぞれのＨｅｌａＳ３値の平均から２倍の標準偏差を引いたものであった。アップ又はダウンレギュレートの規模をカットオフを上回る／下回るパーセンテージとして表した。例えばアップレギュレートされる遺伝子についてのカットオフを１００％上回る値はカットオフと比較して２倍の誘導を意味し、かつダウンレギュレートされた遺伝子についてのカットオフを１００％下回る値は抵抗性クローンでのその値の二分を意味する。

【００６８】

遺伝子クラスタリングのためにプログラムＣｌｕｓｔｅｒ（Ｍｉｃｈａｅｌ Ｅｉｓｅｎ, Ｓｔａｎｆｏｒｄ Ｕｎｉｖｅｒｓｉｔｙ）を使用してよい。４つの参照アレイ及び２０個のアポトーシス抵抗性クローンのアレイの正規化された値を使用した。２４個の調査されるアレイの少なくとも２０で１より大きい値を有する遺伝子をフィルタし、かつ以下の計算のために使用した。遺伝子クラスタリングのためにプログラムＣｌｕｓｔｅｒ（Ｍｉｃｈａｅｌ Ｅｉｓｅｎ, Ｓｔａｎｆｏｒｄ Ｕｎｉｖｅｒｓｉｔｙ）を使用してよい。４つの参照アレイ及び２０個のアポトーシス抵抗性クローンのアレイの正規化された値を使用した。２４個の調査されるアレイの少なくとも２０個において１より大きい値を有する遺伝子はフィルタし、かつ以下の計算のために使用した。値がクラスタリングが信頼できないバックグラウンドに非常に近い遺伝子のクラスタリングを回避するために、１のカットオフを利用した。こうして２４００個のスポットから、階層型クラスターアルゴリズムを介して分析された５２０が残った。

【００６９】

結果を第５表に示す。

【００７０】

【外１】

参考文献：

1. Will, H. and B. Hinzmann, *cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment.* Eur J Biochem, 1995. **231**(3): p. 602-8.
2. Butler, G.S., et al., *Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases.* Eur J Biochem, 1997. **244**(2): p. 653-7.
3. Hu, M.C., et al., *Protein phosphatase X interacts with c-Rel and stimulates c-Rel/nuclear factor kappaB activity.* J Biol Chem, 1998. **273**(50): p. 33561-5.
4. Kersh, G.J. and P.M. Allen, *Essential flexibility in the T-cell recognition of antigen.* Nature, 1996. **380**(6574): p. 495-8.
5. Kohno, K., et al., *Amino acid sequence of mammalian elongation factor 2 deduced from the cDNA sequence: homology with GTP-binding proteins.* Proc Natl Acad Sci U S A, 1986. **83**(14): p. 4978-82.
6. Geiger, B., *Intermediate filaments. Looking for a function.* Nature, 1987. **329**(6138): p. 392-3.
7. Didsbury, J.R., Y.S. Ho, and R. Snyderman, *Human Gi protein alpha-subunit: deduction of amino acid structure from a cloned cDNA.* FEBS Lett, 1987. **211**(2): p. 160-4.
8. Bokoch, G.M. and A.G. Gilman, *Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin.* Cell, 1984. **39**(2 Pt 1): p. 301-8.
9. Sullivan, K.A., et al., *Inhibitory and stimulatory G proteins of adenylate cyclase: cDNA and amino acid sequences of the alpha chains.* Proc Natl Acad Sci U S A, 1986. **83**(18): p. 6687-91.

【００７１】

【外２】

10. Williams, N.G., H. Zhong, and K.P. Minneman, *Differential coupling of alpha1-, alpha2-, and beta-adrenergic receptors to mitogen-activated protein kinase pathways and differentiation in transfected PC12 cells.* J Biol Chem, 1998. 273(38): p. 24624-32.
11. Mukai, H. and Y. Ono, *A novel protein kinase with leucine zipper-like sequences: its catalytic domain is highly homologous to that of protein kinase C.* Biochem Biophys Res Commun, 1994. 199(2): p. 897-904.
12. Wick, M.J., et al., *Mechanism of phosphorylation of protein kinase B/Akt by a constitutively active 3-phosphoinositide-dependent protein kinase-1.* J Biol Chem, 2000. 275(51): p. 40400-6.
13. Takahashi, M., et al., *Proteolytic activation of PKN by caspase-3 or related protease during apoptosis.* Proc Natl Acad Sci U S A, 1998. 95(20): p. 11566-71.
14. Watanabe, G., et al., *Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho.* Science, 1996. 271(5249): p. 645-8.
15. Mukai, H., et al., *Activation of PKN, a novel 120-kDa protein kinase with leucine zipper-like sequences, by unsaturated fatty acids and by limited proteolysis.* Biochem Biophys Res Commun, 1994. 204(1): p. 348-56.
16. Maru, Y. and O.N. Witte, *The BCR gene encodes a novel serine/threonine kinase activity within a single exon.* Cell, 1991. 67(3): p. 459-68.
17. Diekmann, D., et al., *Bcr encodes a GTPase-activating protein for p21rac.* Nature, 1991. 351(6325): p. 400-2.
18. Galaktionov, K. and D. Beach, *Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins.* Cell, 1991. 67(6): p. 1181-94.
19. Honda, R., et al., *Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase.* FEBS Lett, 1993. 318(3): p. 331-4.
20. Sebastian, B., A. Kakizuka, and T. Hunter, *Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15.* Proc Natl Acad Sci U S A, 1993. 90(8): p. 3521-4.
21. Miyata, H., et al., *Overexpression of CDC25B overrides radiation-induced G2-M arrest and results in increased apoptosis in esophageal cancer cells.* Cancer Res, 2001. 61(7): p. 3188-93.
22. Zuo, Z., et al., *Ser/Thr protein phosphatase type 5 (PP5) is a negative regulator of glucocorticoid receptor-mediated growth arrest.* Biochemistry, 1999. 38(28): p. 8849-57.
23. Silverstein, A.M., et al., *Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin.* J Biol Chem, 1997. 272(26): p. 16224-30.
24. Chen, M.X., et al., *A novel human protein serine/threonine phosphatase, which possesses four tetratricopeptide repeat motifs and localizes to the nucleus.* Embo J, 1994. 13(18): p. 4278-90.

【００７２】
【外３】

25. Lumeng, C., et al., *Interactions between beta 2-syntrophin and a family of microtubule-associated serine/threonine kinases.* Nat Neurosci, 1999. **2**(7): p. 611-7.
26. Walden, P.D. and N.J. Cowan, *A novel 205-kilodalton testis-specific serine/threonine protein kinase associated with microtubules of the spermatid manchette.* Mol Cell Biol, 1993. **13**(12): p. 7625-35.
27. Yoshiyama, K., et al., *CD156 (human ADAM8): expression, primary amino acid sequence, and gene location.* Genomics, 1997. **41**(1): p. 56-62.
28. Schlomann, U., et al., *Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration.* J Neurosci, 2000. **20**(21): p. 7964-71.
29. Wang, Z., R. Juttermann, and P.D. Soloway, *TIMP-2 is required for efficient activation of proMMP-2 in vivo.* J Biol Chem, 2000. **275**(34): p. 26411-5.
30. Zucker, S., et al., *Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP).* J Biol Chem, 1998. **273**(2): p. 1216-22.
31. Hayakawa, T., et al., *Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2).* J Cell Sci, 1994. **107**(Pt 9): p. 2373-9.
32. Wang, X.S., et al., *MAPKKK6, a novel mitogen-activated protein kinase kinase kinase, that associates with MAPKKK5.* Biochem Biophys Res Commun, 1998. **253**(1): p. 33-7.
33. Kratzschmar, J., L. Lum, and C.P. Blobel, *Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence.* J Biol Chem, 1996. **271**(9): p. 4593-6.
34. Nath, D., et al., *Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells.* J Cell Sci, 1999. **112**(Pt 4): p. 579-87.
35. Knecht, R., et al., *Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck.* Cancer Res, 1999. **59**(12): p. 2794-7.
36. Smits, V.A., et al., *Polo-like kinase-1 is a target of the DNA damage checkpoint.* Nat Cell Biol, 2000. **2**(9): p. 672-6.
37. Toyoshima-Morimoto, F., et al., *Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase.* Nature, 2001. **410**(6825): p. 215-20.
38. Schmucker, D. and S.L. Zipursky, *Signaling downstream of eph receptors and ephrin ligands.* Cell, 2001. **105**(6): p. 701-4.
39. Miao, H., et al., *Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway.* Nat Cell Biol, 2001. **3**(5): p. 527-30.
40. Miao, H., et al., *Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation.* Nat Cell Biol, 2000. **2**(2): p. 62-9.
41. Welsh, M., et al., *Shb is a ubiquitously expressed Src homology 2 protein.* Oncogene, 1994. **9**(1): p. 19-27.

【００７３】

【外４】

42. Oishi, K., et al., *Identification and characterization of PKNbeta, a novel isoform of protein kinase PKN: expression and arachidonic acid dependency are different from those of PKNalpha.* Biochem Biophys Res Commun, 1999. **261**(3): p. 808-14.
43. Gu, M., I. Warshawsky, and P.W. Majerus, *Cloning and expression of a cytosolic megakaryocyte protein-tyrosine-phosphatase with sequence homology to retinaldehyde-binding protein and yeast SEC14p.* Proc Natl Acad Sci U S A, 1992. **89**(7): p. 2980-4.
44. Stebbins, C.E., W.G. Kaelin, Jr., and N.P. Pavletich, *Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function.* Science, 1999. **284**(5413): p. 455-61.
45. Maxwell, P.H., et al., *The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis.* Nature, 1999. **399**(6733): p. 271-5.
46. Kibel, A., et al., *Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C.* Science, 1995. **269**(5229): p. 1444-6.
47. Sakano, S., et al., *Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase).* Oncogene, 1994. **9**(4): p. 1155-61.
48. Rane, M.J., et al., *p38 Kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils.* J Biol Chem, 2001. **276**(5): p. 3517-23.
49. Rouse, J., et al., *A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins.* Cell, 1994. **78**(6): p. 1027-37.
50. Gurney, M.E., et al., *Neuroleukin: a lymphokine product of lectin-stimulated T cells.* Science, 1986. **234**(4776): p. 574-81.
51. Faik, P., et al., *Mouse glucose-6-phosphate isomerase and neuroleukin have identical 3' sequences.* Nature, 1988. **332**(6163): p. 455-7.
52. Chaput, M., et al., *The neurotrophic factor neuroleukin is 90% homologous with phosphohexose isomerase.* Nature, 1988. **332**(6163): p. 454-5.
53. Gurney, M.E., et al., *Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and sensory neurons.* Science, 1986. **234**(4776): p. 566-74.
54. Wang, H., et al., *Characterization of PCP-2, a novel receptor protein tyrosine phosphatase of the MAM domain family.* Oncogene, 1996. **12**(12): p. 2555-62.
55. Frigerio, J.M., J.C. Dagorn, and J.L. Iovanna, *Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs.* Biochim Biophys Acta, 1995. **1262**(1): p. 64-8.
56. Yan, H., et al., *A novel receptor tyrosine phosphatase-sigma that is highly expressed in the nervous system.* J Biol Chem, 1993. **268**(33): p. 24880-6.
57. Elchebly, M., et al., *Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma.* Nat Genet, 1999. **21**(3): p. 330-3.
58. Nagase, T., et al., *Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121-KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1.* DNA Res, 1995. **2**(4): p. 167-74, 199-210.

【００７４】
【外５】

59. Benovic, J.L., et al., *Beta-adrenergic receptor kinase: primary structure delineates a multigene family.* Science, 1989. **246**(4927): p. 235-40.
60. Benovic, J.L., et al., *Purification and characterization of the beta-adrenergic receptor kinase.* J Biol Chem, 1987. **262**(19): p. 9026-32.
61. Colamonici, O.R., et al., *p135tyk2, an interferon-alpha-activated tyrosine kinase, is physically associated with an interferon-alpha receptor.* J Biol Chem, 1994. **269**(5): p. 3518-22.
62. Stahl, N., et al., *Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components.* Science, 1994. **263**(5143): p. 92-5.
63. Bernards, A., *Predicted tyk2 protein contains two tandem protein kinase domains.* Oncogene, 1991. **6**(7): p. 1185-7.
64. Wang, B., et al., *Molecular cloning and characterization of a novel human receptor protein tyrosine phosphatase gene, hPTP-J: down-regulation of gene expression by PMA and calcium ionophore in Jurkat T lymphoma cells.* Biochem Biophys Res Commun, 1997. **231**(1): p. 77-81.
65. Takeda, K., et al., *Essential role of Stat6 in IL-4 signalling.* Nature, 1996. **380**(6575): p. 627-30.
66. Masuda, A., et al., *Interleukin-15 Prevents Mouse Mast Cell Apoptosis through STAT6-mediated Bcl-xL Expression.* J Biol Chem, 2001. **276**(28): p. 26107-13.
67. Kato, Y., et al., *BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C.* Embo J, 1997. **16**(23): p. 7054-66.
68. Kato, Y., et al., *Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor.* Nature, 1998. **395**(6703): p. 713-6.
69. Zhou, G., Z.Q. Bao, and J.E. Dixon, *Components of a new human protein kinase signal transduction pathway.* J Biol Chem, 1995. **270**(21): p. 12665-9.
70. Siliciano, J.D., T.A. Morrow, and S.V. Desiderio, *itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2.* Proc Natl Acad Sci U S A, 1992. **89**(23): p. 11194-8.
71. Tanaka, N., et al., *A novel human tyrosine kinase gene inducible in T cells by interleukin 2.* FEBS Lett, 1993. **324**(1): p. 1-5.
72. Deak, M., et al., *Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB.* Embo J, 1998. **17**(15): p. 4426-41.
73. Li, J., et al., *Novel NEMO/IkappaB kinase and NF-kappa B target genes at the pre-B to immature B cell transition.* J Biol Chem, 2001. **276**(21): p. 18579-90.
74. Baytel, D., et al., *The human Pim-2 proto-oncogene and its testicular expression.* Biochim Biophys Acta, 1998. **1442**(2-3): p. 274-85.
75. Wang, D., et al., *Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II.* J Biol Chem, 2000. **275**(42): p. 32592-7.
76. Nada, S., et al., *Constitutive activation of Src family kinases in mouse embryos that lack Csk.* Cell, 1993. **73**(6): p. 1125-35.

【００７５】
【外６】

77. Imamoto, A. and P. Soriano, *Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice.* Cell, 1993. 73(6): p. 1117-24.
78. Rothwarf, D.M., et al., *IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex.* Nature, 1998. 395(6699): p. 297-300.
79. Millward, T.A., et al., *Calcium regulation of Ndr protein kinase mediated by S100 calcium-binding proteins.* Embo J, 1998. 17(20): p. 5913-22.
80. Millward, T., P. Cron, and B.A. Hemmings, *Molecular cloning and characterization of a conserved nuclear serine(threonine) protein kinase.* Proc Natl Acad Sci U S A, 1995. 92(11): p. 5022-6.
81. Cheng, M., T.G. Boulton, and M.H. Cobb, *ERK3 is a constitutively nuclear protein kinase.* J Biol Chem, 1996. 271(15): p. 8951-8.
82. Boulton, T.G., et al., *ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF.* Cell, 1991. 65(4): p. 663-75.
83. Hande, K.R., *Clinical applications of anticancer drugs targeted to topoisomerase II.* Biochim Biophys Acta, 1998. 1400(1-3): p. 173-84.
84. Wo, Y.Y., et al., *Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphotyrosyl protein phosphatase.* J Biol Chem, 1992. 267(15): p. 10856-65.
85. Haystead, T.A., et al., *Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism.* Nature, 1989. 337(6202): p. 78-81.
86. Kishikawa, K., et al., *Phosphatidic acid is a potent and selective inhibitor of protein phosphatase 1 and an inhibitor of ceramide-mediated responses.* J Biol Chem, 1999. 274(30): p. 21335-41.
87. Warbrick, E., *The puzzle of PCNA's many partners.* Bioessays, 2000. 22(11): p. 997-1006.
88. Tiganis, T., B.E. Kemp, and N.K. Tonks, *The protein-tyrosine phosphatase TCPTP regulates epidermal growth factor receptor-mediated and phosphatidylinositol 3-kinase-dependent signaling.* J Biol Chem, 1999. 274(39): p. 27768-75.
89. Tiganis, T., et al., *Epidermal growth factor receptor and the adaptor protein p52Shc are specific substrates of T-cell protein tyrosine phosphatase.* Mol Cell Biol, 1998. 18(3): p. 1622-34.
90. Sanchez, Y., et al., *Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25.* Science, 1997. 277(5331): p. 1497-501.
91. Furnari, B., N. Rhind, and P. Russell, *Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase.* Science, 1997. 277(5331): p. 1495-7.
92. Mailand, N., et al., *Rapid destruction of human Cdc25A in response to DNA damage.* Science, 2000. 288(5470): p. 1425-9.

【0076】
【外7】

ves of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. J Biol Chem, 1995. **270**(29): p.

cation of the human YVH1 protein-tyrosine phosphatase orthologue reveals a novel zinc binding domain tion. J Biol Chem, 1999. **274**(34): p. 23991-5.
(+)-like gene in human cells. Nature, 1991. **353**(6339): p. 80-3.
' and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. Cell, 1991. **64**(6): p. 1111-22.
Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. Cell, 1987. **49**(4): p. 559-

ular masking of nuclear import signal on NF-AT4 by casein kinase I and MEKK1. Cell, 1998. **93**(5): p. 851-61.
n kinase I transduces Wnt signals. Nature, 1999. **401**(6751): p. 345-50.
gg, Identification of 21 novel human protein kinases, including 3 members of a family related to the cell cycle yillus nidulans. Cell Growth Differ, 1993. **4**(10): p. 821-30.
21 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. Cell): p. 197-208.
Identification and characterization of a human protein kinase related to budding yeast Cdc7p. Proc Natl Acad p. 14320-5.
ovel SR-protein-specific kinase, SRPK2, disassembles nuclear speckles. Biochem Biophys Res Commun, 1998.

2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of rs in mammalian cells. J Cell Biol, 1998. **140**(4): p. 737-50.
n of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways.): p. 90-4.
ovel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J Biol Chem, 1996.

ion of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. , 1997. **94**(18): p. 9687-92.
d M.W. Kilimann, *Structure of the human gene encoding the phosphorylase kinase beta subunit (PHKB).* Eur J). 374-80.

【００７７】
【外８】

109. Hobert, O., B. Jallal, and A. Ullrich, *Interaction of Vav with ENX-1, a putative transcriptional regulator of homeobox gene expression.* Mol Cell Biol, 1996. **16**(6): p. 3066-73.
110. Hobert, O., et al., *Isolation and developmental expression analysis of Enx-1, a novel mouse Polycomb group gene.* Mech Dev, 1996. **55**(2): p. 171-84.
111. Alexandru, G., et al., *Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage.* Embo J, 1999. **18**(10): p. 2707-21.
112. Cahill, D.P., et al., *Mutations of mitotic checkpoint genes in human cancers.* Nature, 1998. **392**(6673): p. 300-3.
113. Schultz, S.J., et al., *Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of Aspergillus nidulans.* Cell Growth Differ, 1994. **5**(6): p. 625-35.
114. Fry, A.M., P. Meraldi, and E.A. Nigg, *A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators.* Embo J, 1998. **17**(2): p. 470-81.
115. Zhao, Y., et al., *Cloning and chromosomal location of a novel member of the myotonic dystrophy family of protein kinases.* J Biol Chem, 1997. **272**(15): p. 10013-20.
116. Tung, R.M. and J. Blenis, *A novel human SPS1/STE20 homologue, KHS, activates Jun N-terminal kinase.* Oncogene, 1997. **14**(6): p. 653-9.
117. Yuan, Y., D.M. Li, and H. Sun, *PIR1, a novel phosphatase that exhibits high affinity to RNA . ribonucleoprotein complexes.* J Biol Chem, 1998. **273**(32): p. 20347-53.
118. Deshpande, T., et al., *Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5'-triphosphatase and diphosphatase activities.* J Biol Chem, 1999. **274**(23): p. 16590-4.
119. Bastians, H. and H. Ponstingl, *The novel human protein serine/threonine phosphatase 6 is a functional homologue of budding yeast Sit4p and fission yeast ppe1, which are involved in cell cycle regulation.* J Cell Sci, 1996. **109**(Pt 12): p. 2865-74.
120. Becker, W., et al., *Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases.* J Biol Chem, 1998. **273**(40): p. 25893-902.
121. Guimera, J., et al., *A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region.* Hum Mol Genet, 1996. **5**(9): p. 1305-10.
122. Lopez-Borges, S. and P.A. Lazo, *The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2 binding site of the p53 tumour suppressor protein.* Oncogene, 2000. **19**(32): p. 3656-64.
123. Nezu, J., et al., *Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase.* Genomics, 1997. **45**(2): p. 327-31.

【００７８】
【外９】

124. Lapidot-Lifson, Y., et al., *Cloning and antisense oligodeoxynucleotide inhibition of a human homolog of cdc2 required in hematopoiesis.* Proc Natl Acad Sci U S A, 1992. **89**(2): p. 579-83.
125. Mills, G.B., et al., *Expression of TTK, a novel human protein kinase, is associated with cell proliferation.* J Biol Chem, 1992. **267**(22): p. 16000-6.
126. Schmandt, R., et al., *IL-2-induced expression of TTK, a serine, threonine, tyrosine kinase, correlates with cell cycle progression.* J Immunol, 1994. **152**(1): p. 96-105.
127. Ruvolo, P.P., et al., *Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A.* J Biol Chem, 1999. **274**(29): p. 20296-300.
128. Wadzinski, B.E., et al., *Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation.* Mol Cell Biol, 1993. **13**(5): p. 2822-34.
129. Lee, M.G. and P. Nurse, *Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.* Nature, 1987. **327**(6117): p. 31-5.
130. Riabowol, K., et al., *The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells.* Cell, 1989. **57**(3): p. 393-401.
131. Zeng, Q., et al., *Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome.* J Biol Chem, 2000. **275**(28): p. 21444-52.
132. Diamond, R.H., et al., *PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth.* Mol Cell Biol, 1994. **14**(6): p. 3752-62.
133. Peng, Y., et al., *The gene encoding human nuclear protein tyrosine phosphatase, PRL-1. Cloning, chromosomal localization, and identification of an intron enhancer.* J Biol Chem, 1998. **273**(27): p. 17286-95.
134. Edwards, M.C., C. Wong, and S.J. Elledge, *Human cyclin K, a novel RNA polymerase II-associated cyclin possessing both carboxy-terminal domain kinase and Cdk-activating kinase activity.* Mol Cell Biol, 1998. **18**(7): p. 4291-300.
135. van den Berg, I.E., et al., *X-linked liver phosphorylase kinase deficiency is associated with mutations in the human liver phosphorylase kinase alpha subunit.* Am J Hum Genet, 1995. **56**(2): p. 381-7.
136. Grove, J.R., et al., *Cloning and expression of two human p70 S6 kinase polypeptides differing only at their amino termini.* Mol Cell Biol, 1991. **11**(11): p. 5541-50.
137. Bancrjee, P., et al., *Molecular structure of a major insulin/mitogen-activated 70-kDa S6 protein kinase.* Proc Natl Acad Sci U S A, 1990. **87**(21): p. 8550-4.
138. Weng, Q.P., et al., *Phosphatidylinositol 3-kinase signals activation of p70 S6 kinase in situ through site-specific p70 phosphorylation.* Proc Natl Acad Sci U S A, 1995. **92**(12): p. 5744-8.
139. Kornbluth, S., M. Sudol, and H. Hanafusa, *Association of the polyomavirus middle-T antigen with c-yes protein.* Nature, 1987. **325**(7000): p. 171-3.

【００７９】
【外１０】

140. Kitamura, N., et al., *Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus.* Nature, 1982. **297**(5863): p. 205-8.
141. Tobimatsu, T. and H. Fujisawa, *Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs.* J Biol Chem, 1989. **264**(30): p. 17907-12.
142. Hovens, C.M., et al., *RYK, a receptor tyrosine kinase-related molecule with unusual kinase domain motifs.* Proc Natl Acad Sci U S A, 1992. **89**(24): p. 11818-22.
143. Halford, M.M., et al., *Ryk-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk.* Nat Genet, 2000. **25**(4): p. 414-8.
144. Barker, H.M., et al., *Sequence of human protein serine/threonine phosphatase 1 gamma and localization of the gene (PPP1CC) encoding it to chromosome bands 12q24.1-q24.2.* Biochim Biophys Acta, 1993. 1178(2): p. 228-33.

【００８０】
【表１】

第１表

抗アポトーシス機能に連係していなかったが、アポトーシス耐性クローンに誘導される遺伝子

遺伝子	アクセッション番号	発現が増大された発現についてのカットオフを上回るクローンの数	カットオフを上回る％
チロシンキナーゼ			
Hck	M16591	4	46,5
TrkC	U05012	6	28,0
Hyl	X77278	11	24,5
Rse	U05682	10	22,5
RON	X70040	14	21,0
KIAA0641	AB014541	5	19,5
EphA2	M59371	12	19,5
Csk	X59932	8	18,0
EphB3	X75208	6	16,0
EphB4	U07695	5	12,5
Pyk-2	U33284	4	11,0
未知のホスファターゼ			
PB-26	AB040904	5	30,5
PB-28	AB040904	4	18,0
未知のキナーゼ			
UK19	AA292588	5	25,0
UK10	R52045	4	24,5
UK11	H39075	8	9,5
セリン/トレオニンキナーゼ			
GRK6	L16862	16	69,0
Dyrk4	Y09305	5	55,0
IRAK-2	AF026273	13	54,5
LIMK-1	D26309	5	45,5
MLK3	U07747	9	44,0
AMPK-β	AJ224538	4	40,0
MAPKKK6	AF100318	14	39,0
MAST205	6678957	14	37,5
DAPK	X76104	11	37,0
MAPKK3	4506098	11	36,0
PLK-1	L19559	12	34,5
PKN-H4	D26181	13	34,5
Bcr	X02596	10	32,0
MSK2	AF074715	8	30,0
Rac-α	M63167	16	28,0
MST-3	AF024636	7	28,0
PSK-H1	M14504	5	26,5
PCTAIRE1	X66363	6	23,0

【００８１】

【表２】

lok	AB015718	4	22,0
HsGAK	D88435	6	21,0
MAPKAPK3	U09578	7	20,5
JNKK2α	AF022805	8	19,5
FAST	X86779	5	19,0
MKK7	AF013588	8	17,5
MAPKK5	U25265	6	16,5
PAK1-関連キナーゼ	AF005046	11	16,0
ARK2	AF008552	4	16,0
MSSK1	U82808	4	15,0
PHK-γT	M31606	6	14,5
CDC42-結合プロテインキナーゼβ	AF128625	3	13,5
KIAA0151	D63485	9	11,0
KIAA0537	AB011109	6	11,5
STE20-様プロテインキナーゼ3	X99325	4	6,0
Ste-20	AF083420	3	5,5
アダプタータンパク質			
Grb-2	M96995	7	18,0
SHC	Y09847	6	17,5
SHB	X75342	10	15,0
ホスファターゼ			
PYST1	X93920	5	85,5
B23	U15932	5	33,0
PCP-2	X97198	10	31,5
PTP-J	U73727	9	30,0
PTP-Meg2	M83738	9	29,5
PP5	X89416	7	18,5
CDC25B	M81934	10	17,0
PTP-SL	Z30313	5	17,0
PP2B-R	M30773	6	17,0
PP1-Cα	M63960	6	16,0
PP2A-Rb55	M64930	5	15,5
PTPζ	X54135	4	13,0
Shp-1	X62055	4	7,0
PP2A-Ra65	J02902	6	7,0
PTPmu	X58288	3	6,5
メタロプロテアーゼ			
MMP-15	Z48482	19	71,0
ADAM12	X05232	8	59,0
MMP-3	J03209	14	34,5
ADAM15	NM003815	12	28,0
ADAM8	XM005675	13	27,0
G-タンパク質			
alphai2		16	44,0
GPIR-3		4	8,0

【００８２】

【表３】

その他			
p91/ISGF-3	M97935	7	104,0
90k	50318862	15	57,5
MHC-1	M11886	17	53,0
EF-2	X51466	16	44,0
α-チューブリン	NM_006082	9	43,5
KIF-1c	NM006612	6	28,0
フリン	X17094	8	27,0
rS9	4506744	13	23,0
GPDH	M33197	9	23,0
β-アクチン	X00351	11	21,5
ビメンチン	X56134	15	19,0
ニューロレクチン	K03515	14	17,5
チモシンβ	S54005	7	11,5
ヒストン3.3	M11354	6	7,0
PHB-4-PC	L14273	5	3,5

【００８３】
【表４】

第２表
アポトーシス耐性クローンに誘導される公知の抗アポトーシス性機能を有する遺伝子

遺伝子	アクセッション番号	発現が増大された発現についてのカットオフを上回るクローンの数	カットオフを上回る%
チロシンキナーゼ			
PDGFR α	M22734	4	39,0
HER2	M11730	4	38,5
EGFR	X00588	6	21,0
FGFR-3	M58051	5	20,5
HER4	L07868	5	32,5
Jak-2	AF058925	6	34,0
Tyk-2	X54637	8	18,5
セリン/トレオニンキナーゼ			
RSK	L07597	11	43,5
MAPKK2	L11285	11	33,5
PIM-2h	U77735	8	29,5
IKK1	AF012890	4	28,0
CKII-β	M30448	7	28,0
ALK-4	Z22536	6	27,0
ERK1	X60188	11	25,5
IKK γ	AF074382	12	23,5
AKT2	M95936	10	17,0
CKII-α	J02853	8	17,5
CaM-KII γ	L07044	7	16,0
MAPKAPK2	NM004759	6	15,0
ILK	U40282	7	14,0
CKI-δ	U29171	6	11,5
SGK	Y10032	4	11,5
CKII-β	M30448	5	9,0
A-Raf-1	X04790	5	9,0
ALK-1	L17075	5	6,5
ホスファターゼ			
PPX	X70218	16	26,0
リガンド			
TGF α	XM002732	11	63,0
IL1-β	NM000576	8	54,5
IL1-α	X02531	4	34,5
VEGF	NM003376	9	27,0
その他			
Bcl-x	Z23115	14	34,5
IL-4Stat	U16031	9	19,5
TIMP-1	X03124	17	108,5
myc	X00364	8	15,5
TIMP-2	S48568	13	36,5

【００８４】
【表５】

アポトーシス耐性クローンにおけるクラスター

太字：扁平上皮細胞癌における１つの共通のクラスタ中にも見出される

クラスター１

相関係数 0,71	
Bcr	**Rac-α**
JNKK2α	**IKKγ**
MKK7	**PPX**
PTP-SL	**ADAM15**
KIF-1c	**ADAM8**
SHB	**ERK1**
IRAK	**Rse**
PLK-1	**αi2S48C**
α-チューブリン	**MAPKKK6**
RSK	**90k**
GRK6	**pBTUB**
GPIR-3	
MAPKAPK3	
PKN-H4	
MAPKK2	
PKA-RIβ	
p130CAS	
LIMK-1	
MAST205	
PIM-2h	
Csk	
MAPKK3	
PCTAIRE1	
MST-3	

第３表

【００８５】
【表６】

クラスター 2

相関係数	0,73
Bcl-x	
AKT2	
EF-2	
PITALRE	
TIMP-2	
FGFR-2	
CDC25B	
CKI-δ	
EphA2	
PP2C	
Rac-α	
RON	
EphB4	

クラスター 3

相関係数	0,74
PDGFRα	
PB-28	
PHK-γT	
Tyk-2	
A-Raf-1	
GPDH	
HK-18B	
Erk6	
Cyto18	
HK-18B	
サイトケラチン8	
CK-8	
CKII-β	
MLK3	
MMP-11	

クラスター 4

相関係数	0,67
PP2A-Ra65	
HER2	
CAMKK	

クラスター 5

相関係数	0,71
PKC-ε	
MAPKK5	
PTP-Meg2	
PP2B-Cβ	
MKP-5	
Jak-2	
Shp-2	
IKK2	
PHK-αL	
JNK1	

クラスター 6

相関係数	0,61
Pyk-2	
Shp-1	
PP2B-Cγ	
lok	
CaM-KIIγ	
DRP-1	
CKI-γ2	
PSK-H1	
GPIR-1	
Chk2	
Axl	
IL-4Stat	
PCP-2	
DAPK	
PKA-Cα2	
PP5	
Abl	
Raf	
PTPζ	
IGF1-R	
pHE-A1	
PTP-1B	
MSTH1	
PKA-Cα	
PLCγ	
H5GAK	
VHR	
TESK1	
PRK	

クラスター 7

相関係数	0,83
MMP-15	
TIMP-1	

【００８６】
【表７】

扁平上皮細胞癌細胞系統におけるクラスター

SCaBER
UMSSC-17B
UMSSC-17A
UMSSC-22A
UMSSC-22B
UMSSC-10A
HlaC78
HlaC79
FaDu

太字：アポトーシス耐性クローンにおける１つの共通のクラスターにも見出される

クラスター**1**

相関係数 0,7
PRL-3_2_23
Dyrk2_2_16
MAPKK2_1_5
SHC_1_3
ERK3_1_4
RSK

クラスター**2**

相関係数 0,8
GSK-3α
PTP-SL
MAPKAPK3
GSK-3β
hPAK1
PKC-δ
PB-32
αq
KIF-1c
SHB
KIAA0687Nck-相互作用キナーゼ

第４表

【００８７】
【表８】

クラスター 3

相関係数	0,67
PB-32	
PB-38	
hPAK2	
TIMP-2	
PB-5	
CDK6	
Bcl-x	
H11	
Axl	
MMP-14	
hSLK	
MSTH1	
αq	
ADAM17	
PIR1	
ALK-2	

クラスター 4

相関係数	0,71
Tyk-2	
TIMP-1	
GPDH	
HPRT	
Dyrk4	
MMP-15	
Jak-1	
Myt1	
GPIR-3	
PCNA	
Chk2	
PK38	
α-チューブリン	
CDK4	
PKN-H4	
hPTK	
GPIR-2	
Bmx	
MMP-11	
PKU-α	

【００８８】
【表９】

第５表

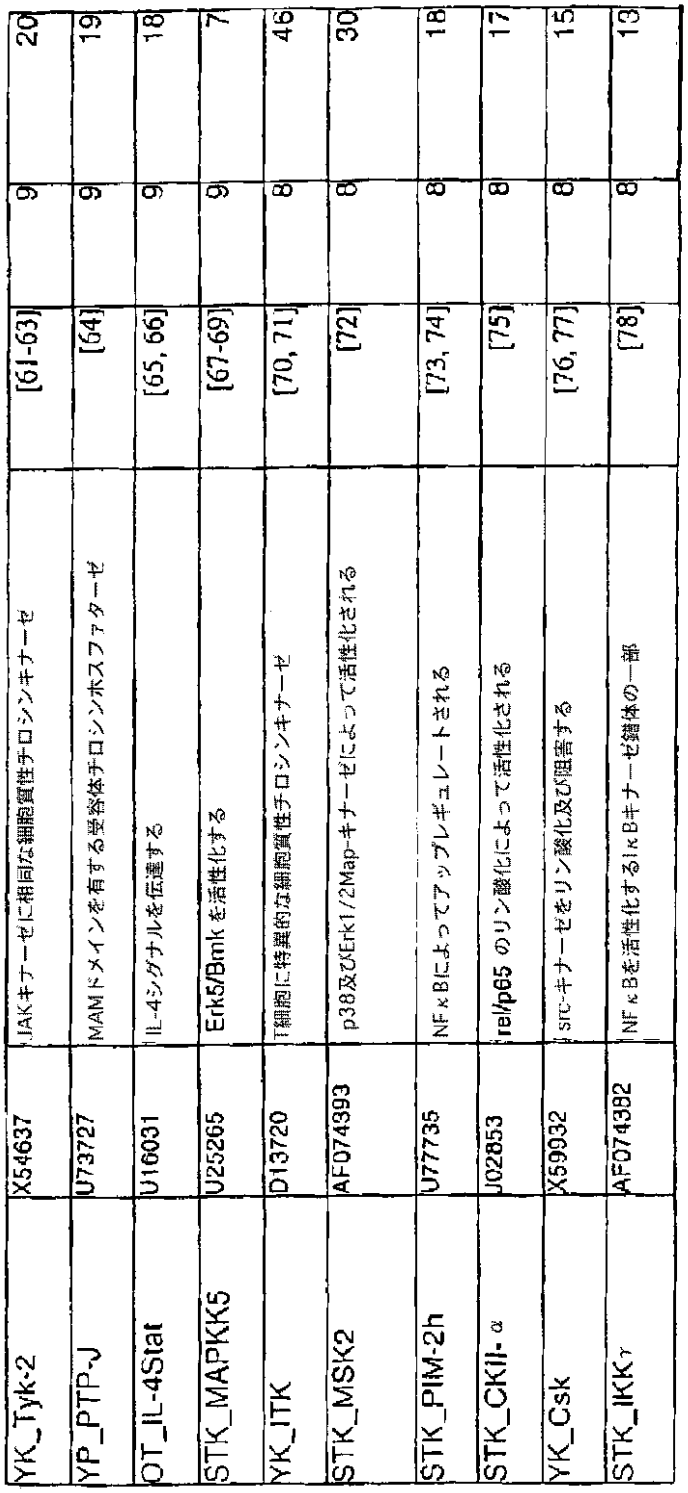
遺伝子	ジーンバンク番号	記載	参考文献	増大された発現を有するクローンの数	増大された発現についてのカットオフを上回る%
MP_MMP-15	Z48482	膜貫通メタロプロテイナーゼ、恐らくMMP-2をプロセシングする	[1, 2]	20	82
STP_PPX	X70218	セントロソームに局在する核は脱リン酸化によってNFκBを活性化する	[3]	17	18
OT_MHC-1	M11886	細胞表面上に抗原を提示する	[4]	16	44
OT_EF-2	XM031904	翻訳延長因子-2	[5]	15	30
OT_ビメンチン	X56134	中間フィラメント	[6]	15	14
GP_αi2	NM002070	ヘテロ三量体G-タンパク質のαサブユニットはアデニレートシクラーゼを阻害し、かつMAP-キナーゼを活性化する	[7-10]	14	42
STK_PKNα	D26181	PKCに関連し、Rho、脂肪酸及びカスパーゼ分解によって活性化される	[11-15]	14	34
STK_Bcr	X02596	セリン/トレオニンキナーゼ活性及びp21racについてのGAP活性をプロセシングする	[16, 17]	13	32
STYP_CDC25B	M81934	二重特異性、cdc-2の脱リン酸化によってG2からMへの細胞周期進行を誘導する	[18-21]	13	30
STP_PP5_1_3	X89416	核、グルココルチコイド受容体に結合し、かつこの受容体によって成長阻害を阻害する	[22-24]	13	22
STK_MAST205	6678957	微小管及びβ2-シントロフィンに結合する	[25, 26]	13	20
MP_ADAM8	NM001109	TNF-αによって誘導される	[27, 28]	13	18
MP_TIMP-2	S48568	MMP-14との錯体においてプロゼラチナーゼの活性化に寄与し、マイトジェン性に作用しうる	[29-31]	12	37

【００８９】

【表１０】

STK_MAPKKK6	U39657	MAPKKK5/ASK1を結合する	[32]	12	33
MP_ADAM15	NM003815	インテグリンに結合する	[33, 34]	12	32
STK_PLK-1	L19559	扁平上皮細胞癌についての、マイトシスの前期のために必須の、DNA損傷後に活性化される予想マーカー	[35-37]	12	21
YK_EphA2	M59371	胚形成の際に神経細胞の相反の役割を担い、PDGF及びEGFによってMAPK活性化を阻害する	[38-40]	12	16
AD_SHB	NM003028	アダプタータンパク質を含有するSH2ドメイン	[41]	12	15
STK_PKNβ	AB019692	PKNαに相同であり、成人の健康的な組織では発現されないが、癌においては発現される	[42]	11	49
YP_PTP-Meg2	M83738	細胞質性チロシンホスファターゼ	[43]	11	42
OT_VHL	NM_000551	ユビキチンリガーゼと錯形成する腫瘍サプレッサー	[44-46]	11	40
YK_Hyl	X77278	CSKにホモロジーを有する細胞質性チロシンキナーゼ	[47]	11	18
STK_MAPKAPK2	NM032960	p38MAPキナーゼにより活性化、AktにPDK2として作用しうる	[48, 49]	11	10
OT_ニューロレクチン	K03515	神経栄養性リガンド、全mRNAはホスホヘキソース-イソメラーゼもコードする	[50-53]	11	10
YP_PCP-2	X97198	MAMドメインを有する受容体チロシンホスファターゼ	[54]	10	30
OT_rS9	4506744	リボソームタンパク質9	[55]	10	16
YP_PTPσ	U35234	脳の胚形成に関連するLARファミリーの受容体チロシンホスファターゼ	[56, 57]	9	109
STK_KIAA0135	D50925	推定セリン/トレオニンキナーゼ	[58]	9	33
STK_β-ARK-1	X61157	β-アドレナリン作動性受容体をリン酸化し、かつ脱感受性化する	[59, 60]	9	25

【００９０】
【表１１】



YK_Tyk-2	X54637	JAKキナーゼに相同な細胞質性チロシンキナーゼ	[61-63]	9	20
YP_PTP-J	U73727	MAMドメインを有する受容体チロシンホスファターゼ	[64]	9	19
OT_IL-4Stat	U16031	IL-4シグナルを伝達する	[65, 66]	9	18
STK_MAPKK5	U25265	Erk5/Bmkを活性化する	[67-69]	9	7
YK_ITK	D13720	T細胞に特異的な細胞質性チロシンキナーゼ	[70, 71]	8	46
STK_MSK2	AF074393	p38及びErk1/2Map-キナーゼによって活性化される	[72]	8	30
STK_PIM-2h	U77735	NFκBによってアップレギュレートされる	[73, 74]	8	18
STK_CKII-α	J02853	rel/p65 のリン酸化によって活性化される	[75]	8	17
YK_Csk	X59032	src-キナーゼをリン酸化及び阻害する	[76, 77]	8	15
STK_IKKγ	AF074382	NFκBを活性化するIκBキナーゼ錯体の一部	[78]	8	13

【００９１】
【表１２】

遺伝子	ジーンバンク番号	記載	参考文献	低減された発現を有するクローンの数	低減された発現についてのカットオフを下回る※
STK_Ndr	Z35102	カルシウムによって活性化される核ホスファターゼ	[79, 80]	20	59
STK_ERK3	X80692	構成的な核MAP-キナーゼ	[81, 82]	19	159
OT_トポイソメラーゼ2	NM001068	トポイソメラーゼ-2インヒビターは癌に対する化学療法剤として使用される	[83]	19	143
YP_AZP-IISred酸ホスファターゼ	M63653	細胞質性ホスホチロシンタンパク質ホスファターゼ	[84]	19	92
STP_PP1-Cβ	X80910	セラミドによって活性化されるPP1の触媒サブユニット	[85, 86]	19	45
OT_PCNA	4505640	増殖性細胞核抗原	[87]	19	27
YP_TC-PTP	M25393	ER及び核に局在化され、EGF刺激後にPI3Kシグナルを阻害する	[88, 89]	19	155
STK_CHK1	AF016582	細胞周期のために必要なDNA損傷後にG2/Mで保持する、wee1及びcdc25をリン酸化する	[90-92]	18	104
STK_AMP-活性化プロテインキナーゼα1サブユニット	AF100763	アセチル-CoAカルボキシラーゼをリン酸化及び不活性化する	[93]	18	81
PP_YVH1	AF119226	二重特異性ホスファターゼ	[94]	18	72
STK_WEE1	X62048	G2/M進行を阻害し、cdcd2をリン酸化し、かつ阻害する	[95-97]	18	73
STK_CKI-alpha	X80693	Wnt経路の一部、NF-AT4の核輸送をリン酸化し、かつ阻害する	[98, 99]	18	49

【００９２】

【表１３】

STK_NEK3	Z29067	G2/M進行を担うアスペルギルス ニデュランスのNIMAキナーゼに相同	[100]	17	234
STK_MAD-3様PK	AF068760			17	104
STK_TAK1	U64205	cdc25関連キナーゼ、Cdc25cをリン酸化する	[101]	17	53
UP_PB-32	W30715	未知のホスファターゼ		16	178
STK_HsCdc7	AF015592	G1/S進行のために重要	[102]	16	138
STK_SRPK-2	U88666	SR-スプライシング因子をリン酸化する	[103, 104]	16	70
STK_MAPKK6	U39657	アポトーシスを誘導するAsk-1 a MAPKKKによって活性化されるp38 MAPキナーゼを活性化する	[105, 106]	16	54
STK_GCK	U07349	S.セレビシエ Ste20に相同、JNKを活性化する	[107]	16	324
STK_KIAA0619	AB014519	未知のキナーゼ		16	239
STK_PHK-β	X84908	グリコゲン-ホスホリラーゼをリン酸化する	[108]	15	246
OT_33a_Enx-1	AF070418	ホメオボックス遺伝子の発現を調節する	[109, 110]	15	187
STK_Bub1	AF046078	染色分体分離をコントロールする、癌における突然変異は増大された突然変異率をもたらす	[111, 112]	15	121
STK_NEK2	U11050	セントロソームに関連する	[113, 114]	15	37
STK_PK428	U59305	筋緊張性ジストロフィーキナーゼのファミリーに関連する	[115]	15	237
STK_KHS	U77129	S.セレビシエ Ste20に相同、JNKを活性化する	[116]	15	121
PP_PIR1	AF023917	二重特異性、核性、RNAを脱リン酸化させる、スペックルに関連する	[117, 118]	15	51

【００９３】
【表１４】

STP_PP6	X92972	細胞周期を調節するS.セレビシエSit4p及びS.ポンベppe1に相同	[119]	15		30
STK_MNB	U52373	二重特異性、DYRKキナーゼに相同、ダウン症候群で増幅される第21染色体の領域に局在する	[120, 121]	14		90
STK_VRK1	AB000449	ワクシニアウイルスキナーゼに相同、核性	[122, 123]	14		286
STK_CHED	M80629	cdc-2のホモログ	[124]	14		75
STK_TTK	M86699	二重特異性、発現は細胞周期と相関する	[125, 126]	13		465
UK_PB-11	AF061944	未知のキナーゼ		13		168
STP_PP2A-Cβ	X12656	核性、Bcl-2を脱リン酸化する	[127, 128]	13		33
UK_UK20	NM_016507	未知のキナーゼ		13		282
STK_GLK	AF000145	S.セレビシエSte20に相同、JNKを活性化する	[107]	13		140
STK_26b_CDC2_1_4	X05360	G2/M進行に必須	[96, 129, 130]	12		168
YP_Pri-1	U48297	細胞成長に影響しうる、核性、また細胞質膜及びエンドソームにも局在する	[131-133]	12		74
STK_サイクリンK	AF060515	cdk活性及びRNAポリメラーゼIIによる転写を調節できる	[134]	12		31
STK_PHK-αL	X80497	ホスホリラーゼキナーゼのサブユニット	[135]	12		24
STK_p70S6K	M60724	PI3キナーゼを介して活性化される	[136-138]	11		824
YK_Yes	4885660	srcキナーゼのファミリーに属する	[139, 140]	11		661
STK_CaM-KIIδ	U73504	脳内で高度に発現される	[141]	11		216

【００９４】
【表１５】

YK_Ryk	X69970	受容体チロシンキナーゼ、いかなる公知の受容体チロシンキナーゼのファミリーに属さない、恐らくEphシグナルに関連する	[142, 143]	11	166
YP_PRL-3	AF041434	PRL-1に相同	[131]	11	74
STP_PP1-Cγ	X74008	PP1の触媒サブユニット	[144]	11	59

【図面の簡単な説明】

【図１】

図１はアップレギュレートされたキナーゼの阻害を示している。

【図２】

図２は優性阻害型突然変異体及びアンチセンス構築物によるｐｙｋ－２の阻害を示している。

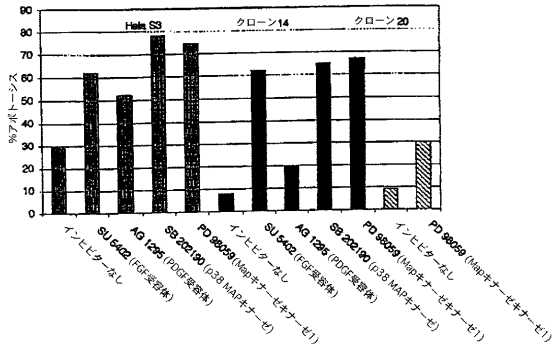
【図３】

図３はクローンのアポトーシス感受性を示している。

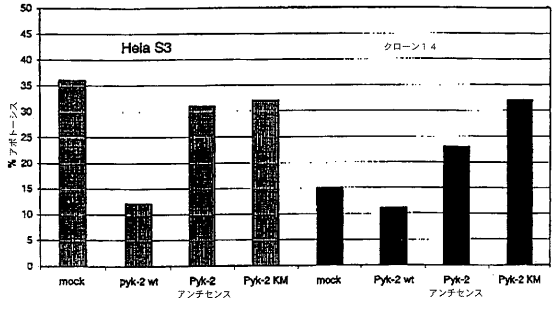
【図４】

図４は他のアポトーシスインデューサーとのアポトーシス感受性を示している。

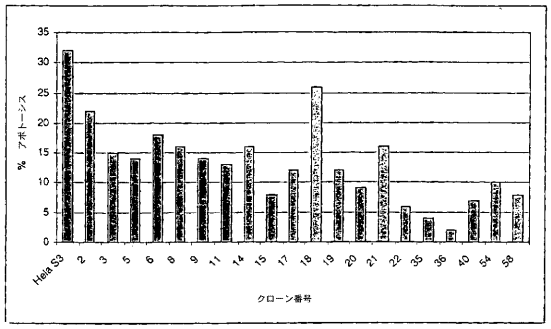
【図１】


Hela S3
クローン14
クローン20
%アポトーシス
インヒビターなし
SU 5402 (FGF受容体)
AG 1295 (PDGF受容体)
SB 202190 (p38 MAPキナーゼ)
PD 98059 (Mapキナーゼキナーゼ1)

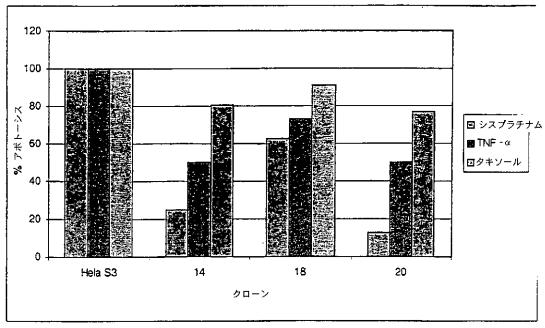
【図２】


Hela S3
クローン14
% アポトーシス
mock
pyk-2 wt
Pyk-2 アンチセンス
Pyk-2 KM
Pyk-2 wt
Pyk-2 アンチセンス
Pyk-2 KM

【図３】


% アポトーシス
Hela S3
クローン番号

【図４】


% アポトーシス
Hela S3
14
18
20
クローン
シスプラチナム
TNF-α
タキソール

【国際公開パンフレット】

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(54) Title: METHOD FOR IDENTIFYING FUNCTIONAL NUCLEIC ACIDS

(57) Abstract: The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

Method for identifying functional nucleic acids

Description

The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

A lot of information has been gathered about the execution apparatus of apoptosis (Hengartner, Nature 407 (2000), 770-776). But data on signals that control the initiation of apoptosis have only recently begun to be accumulated (Rich et al., Nature 407 (2000), 777-783). Previous methods for identifying apoptosis-associated genes or genes associated with other specific phenotypes are tedious. For example, Hudziak et al. (Cell Growth and Differentiaton 129 (1990), 129-134) describe a selection procedure for transformation and met protoonco gene amplification in NIH 3T3 fibroblasts using tumor necrosis factor-α. It is suggested that this method may be used for identifying other gene products, including other tyrosine kinases, associated with aggressive tumor growth. A fast or reliable procedure for identifying such genes is, however, not provided.

According to the present invention a novel method for identifying functional nucleic acid molecules is provided. This method is based on a genome evolution concept and therefore involves mutagenesis and/or genome arrangement steps followed by selection of cell clones displaying the desired phenotype. Subsequent transcriptome analysis in conjunction with bioinformatics-directed gene sorting allows not only comprehensive identification of genes that are critical for the selected cell characteristic, but even entire signalling pathways that govern a given cellular phenotype. This method can be employed towards a wide variety of cell characteristics for which a selection procedure is available.

Thus, a subject matter of the present invention is a method for identifying nucleic acid molecules functionally associated with a desired phenotype comprising the steps:

(a) providing a population of parental cells wherein said cell population substantially lacks the desired phenotype,

(b) optionally subjecting said cell population to a procedure resulting in a rearrangement and/or mutation of the cell genome,

(c) subjecting said cell population from (b) to a selection procedure for the desired phenotype,

(d) identifying and optionally characterizing cells exhibiting said desired phenotype,

(e) obtaining protein and/or mRNA from cells exhibiting said desired phenotype,

(f) determining gene expression in cells exhibiting said desired phenotype and

(g) comparing gene expression in cells exhibiting said desired phenotype with gene expression in cells substantially lacking the desired phenotype.

In the method of the invention essentially any type of parental cells (e.g. cell lines or primary cells) can be used. Most important the cells should lack the desired selection characteristic or display it only weakly. Preferred examples of starting cells are eukaryotic cells, e.g. mammalian cells, particularly human cells.

In order to generate cells, preferably cell clones exhibiting the desired phenotype, the parental cell may be subjected to a procedure resulting in an arrangement and/or mutation of the cell genome. This step is an evolution procedure comprising an induction of the parental cell to undergo genomic rearrangements and/or mutagenesis. In case of transformed cells, e.g. tumor cells such as Hela or normal cells having a low threshold to instability, e.g. immortalized cells such as NIH 3T3 cells, no special

induction is necessary, since these cells are continuously in a process of genome rearrangement and mutagenesis. It is sufficient to expose the parental cell culture to selection conditions either in form of clones or subdivided cultures preferably in multiple well plates, e.g. 96 well microtiter plates, or when the selection involves lethal conditions, exposure of cell monolayers. It should be noted, however, that also parental cells may be used which have a substantially stable genome. These cells, however, require a specific induction in order to obtain the desired genomic rearragement and/or mutagenesis.

In a preferred embodiment step (b) of the method comprises a mutagenesis procedure. This mutagenesis procedure may be selected from irradiation, e.g. by UV or γ-irradiation, chemical mutagenesis, e.g. by treatment with N-methyl maleimide or ethyl maleimide, or combinations thereof.

After the rearrangement and/or mutation of the cell genome has been achieved, the cell population is subjected to a selection procedure for the desired phenotype. After selection, cells, e.g. individual cell clones exhibiting the desired phenotype are identified and optionally characterized. The identification may comprise a morphological determination and/or a cell sorting procedure, e.g. by a Fluorescence Activated Cell Sorting procedure (FACS). The cells may be expanded and subsequently the desired phenotype/property may be verified and/or quantified.

Subsequently, protein and/or mRNA from cells exhibiting the desired phenotype is obtained. This material may be used for determining gene expression in cells exhibiting the desired phenotype and comparing gene expression in said cells with gene expression in cells substantially lacking the desired phenotype.

In a preferred embodiment, mRNA from cells exhibiting the desired phenotype is obtained. The mRNA may be extracted from the selected

genetically modified cell clones and either used directly, or after conversion into another nucleic acid, e.g. cDNA or cRNA as a probe for hybridization with a nucleic acid array. The nucleic acid, e.g. mRNA, cDNA or cRNA, used for hybridization with the array will usually be labelled in order to determine site-specific hybridization on the array. The array may be a solid carrier, e.g. a filter, chip, slide etc. having immobilized thereto a plurality of different nucleic acid molecules on specified locations on the carrier. The nucleic acid array may be selected from genomic DNA arrays, cDNA arrays and oligonucleotide arrays. Preferably, an array is used which preferentially comprises nucleic acids encoding functional cellular polypeptides or portions thereof, more preferably selected from kinases, phosphatases, enzymes and receptors. Hybridization on the array as a measure of gene expression in the selected cell clones may be determined according to known methods, e.g. by image analyis using a phosphor imager. In some cases, the desired new property of the cell may be determined by a large scale high throughput assay analysis of e.g. the conditioned media of subdivided cultures.

In addition or alternatively to expression profiling by mRNA analysis a proteomics approach determining the differences in protein content of the identified clones compared to the parental cell line and the identified clones or their supernatants may be carried out by suitable methods, e.g. by 2D gel electrophoresis. Proteins that differ in their concentration in the parental cell line and the identified clones will show a differently stained spot in the 2D gel. Furthermore, protein modifications like phosphorylations can be detected by this method. Once can also perform a separation of the cellular proteins prior to the analysis step, in order to reduce the complexity of the protein mixture. For instance, column chromatographic steps could be carried out that purify kinases (by affinity chromatography using an ATP column) or glycosylated proteins (using a lectin column) which then can be further separated by 2D gel electrophoresis. Any other method for

analyzing differences on the protein level (protein chips, mass spectrometry) may also be utilized.

The gene expression results in cells exhibiting the desired phenotype will be compared with gene expression in cells substantially lacking the desired phenotype, preferably in the parental cells. Further, the gene expression results may be analyzed by a cluster detection program. This analysis will yield a plurality of possible changes in the expression of genes that confer the desired cell phenotype.

The application of the method of the invention is very broad and includes essentially all cell characteristics that can be selected for and/or which can be determined with an assay. For example, the desired phenotype may be selected from cancer cell properties such as invasiveness, metastasis, loss of contact inhibition, loss of extracellular matrix requirement, growth factor independence, angiogenesis induction, immuno defense evasion, anti-apoptosis and/or increased levels of tumor markers.

In an especially preferred embodiment the desired phenotype is anti-apoptosis. Another application is the elucidation of cancer related genes by sorting cancer cells for a known tumor marker. Often tumor markers are a consequence and not a cause of the tumorigenicity of cells and are therefore not amenable as drug targets. But since the correlation of the marker with a cancer phenotype is established, sorting cells for increased marker expression will also sort for the genes that are linked to the marker and cause the cancer phenotype. These genes can be identified by comparing the expression profiles in the parental cell line and the sorted cells and are potential drug targets.

Alternatively, the desired phenotype may be selected from other properties such as production of secreted protein, e.g. insulin, growth hormone, interferons etc., susceptibility or resistance to pathogens, e.g. viruses such

as HCV, HBV or other pathogens, senescence and regulation of cell functions, i.e. the identification of genes that regulate certain cell functions e.g. identification of negative regulators of insulin receptor activity comprising a screen for cell clones with upregulated insulin receptor activity.

A further preferred embodiment is the identification of components of signal transduction pathways in general, e.g. to sort for cells that are better capable of transmitting the respective signal. For instance, the identification of components of a signal transduction pathway of a Receptor Tyrosine Kinase (RTK), particularly of a receptor of the EGF-receptor family, such as EGFR, HER2 and HER3, can be carried out by generating a cell line that expresses a suitable reporter protein, such as Green Fluorescent Protein (GFP) under the control of a promoter that is responsive to stimulation by a ligand of the respective receptor (e.g. c-fos promoter for EGF stimulation etc.). Stimulation of the receptor by the ligand will then lead to transcription of GFP and an increased green fluorescence that can be detected, e.g. by a FACS machine. Sorting the cells that show the highest fluorescence induction will enrich for cells that respond stronger to a ligand-indicated signal than the parental cell population. Analyzing the expression patterns of both cell populations will identify the genes whose varying expressions are responsible for the different reaction to the signal and hence influence the signal transduction pathway. This strategy can be applied to any signal for which a fluorescent output can be generated.

In the following, the invention is described in more detail with reference to the identification of anti-apoptotic nucleic acids using a cDNA array. It should be noted, however, that this embodiment is only illustrative for the method of the invention and should not be construed as limitation.

In order to identify nucleic acids which are associated with the regulation of apoptosis the method of the invention was used for the identification of genes, which are differentially expressed in apoptosis-sensitive and apoptosis-resistant cells.

Apoptosis was induced in the human cervix carcinoma cell line Hela S3 by Fas activation. Activation of Fas results in an autocatalytic activation of caspase-8 and thus to apoptosis. For Fas activation the parental cells were incubated with an anti-Fas antibody.

After the selection procedure only a low amount of living cells were present. These cells had a higher resistance against apoptosis than the parental cell line. The surviving cells were clonally expanded. mRNA was isolated from the clones and the parental cell line, which was subsequently reversed, transcribed into cDNA. Then cDNA arrays were hybridized with the cDNA from the clones and the parental cell line and thus the gene expression on the array determined. The sequences on the arrays were derived from about 1000 genes which preferentially encode kinases and phosphatases. By means of a comparison between the expression and the parental cell line and the expression and the clones, about 200 genes were identified which exhibited enhanced expression (an increase by more than the factor 2) in at least 10% of the clones. These are nucleic acids which are associated with the apoptosis resistance of the clones (Tables 1 and 2). Table 1 is a listing of genes which are induced in the apoptosis-resistant clones and have not yet been linked to an anti-apoptosis function. Table 2 is a listing of genes that are induced in apoptosis-resistant clones with previously known anti-apoptotic function.

An improved method for the identification of genes, which are differentially expressed in the parental cell line, e.g. Hela S3, and the clones having a desired phenotype, e.g. apoptosis-resistant clones, an evaluation procedure as described in Example 2, may be applied. For each nucleic acid analysed

in the parental cell line, a plurality of measured values is determined from which an average value and a standard deviation may be calculated. For example, RNA may be isolated at least twice from the parental cell line in at least two independent preparations. Material from each preparation is used for hybridization with at least two nucleic acid arrays. The average of those values for a given spot on the array is calculated and the standard deviation determined. Material from the desired clone is hybridized with one nucleic acid array. A gene is considered to be differentially expressed in the desired clone when its value exceeds a predetermined cut-off. The cut-off for upregulated genes is preferably the average of the respective values of the parental cell line plus two times standard deviation. The cut-off for down-regulated genes is preferably the average of the respective parental cell line values minus two times standard deviations. Using this procedure it is possible to correct errors inherent in the experimental procedure. Since those errors made during the preparation of the nucleic acid arrays will determine the standard deviation, any value of the desired clone that lies outside the standard deviation marks a differentially expressed gene. Therefore, it is possible to detect also small differences in gene expression that may not be detected by using an arbitrary cut-off. The values obtained by this improved evaluation procedure are depicted in Table 5.

Thus, a subject matter of the present invention is the use of nucleic acids as depicted in Table 1, Table 2, and Table 5 preferably in Table 1 and Table 5, and polypeptides encoded by these nucleic acids as "targets" for diagnostic and therapeutic applications, particularly for disorders which are associated with dysfunctions of apoptotic processes such as tumors. Further, the nucleic acids and the gene products are suitable as targets in screening procedures for identifying novel modulators of apoptotic/anti-apoptotic procedures, particularly drugs. The drugs may be biomolecules such as antibodies directed against the gene products, enzyme inhibitors or low molecular non-biological drugs. Methods of drug screening comprise

cellular based systems wherein usually a cell overexpressing the target nucleic acid of interest is used or molecular based systems wherein the polypeptide of interest in used in a partially purified or substantially purified and isolated form. Particular screening methods are known to the skilled person and need not be described in detail here. It should be noted, however, that also high throughput screening assays may be used.

Further, several groups or clusters of genes were identified whose expression patterns across the cell lines are similar. Clusters of apoptosis-resistant clones are depicted in Table 3. Clusters in squamous cell carcinoma cell lines are depicted in Table 4. The identification of such clusters allows the use of specific combinations of active agents in diagnostic and/or therapeutical applications as well as in screening methods. Thus, according to a preferred embodiment of the invention combinations of agents capable of modulating the presence and/or activity of several targets within a cluster may be used in order to multiply the efficacy.

Furthermore, the method of the present invention allows the generation of expression profiles of genes and particularly gene clusters associated with a desired phenotype. These expression profiles may be compared with the expression profile in a specific biological sample, which may be a body fluid or a tissue sample derived from a patient, e.g. a human, particularly a tumor patient. The comparison of the expression profile obtained by the method of the present invention with the expression profile in the biological samples allows the development of improved diagnostic, monitoring and/or therapeutic strategies which are specifically adapted to the individual patient.

In experiments it was demonstrated that an inhibition of the catalytic activity of proteins having an increased expression in the clones resulted in an enhanced increase of apoptosis. Also in the parental cell line the

inhibition resulted in an increased apoptosis. This outlines the importance of the identified nucleic acids and proteins for the apoptosis resistance of the clones and demonstrates the inhibition specifity.

Further, the invention is described in more detail in the following examples and figures.

Figure 1 shows the inhibition of upregulated kinases.

Cells were grown in Ham's F12 medium without FCS and treated with 100 ng/ml anti-Fas antibody CH-11 with and without inhibitors. Apoptosis was measured by FACS analysis as described in the examples. SU 5402: 10 μM, AG 1295: 1 μM, SB 203580: 10 μM, PD 98059: 25 μM.

Figure 2 shows the inhibition of pyk-2 by a dominant negative mutant and an antisense construct.

Figure 3 shows the apoptosis sensitivity of clones. 70% confluent cells were starved for 24h in medium without FCS and subsequently 100 ng/ml CH-11 was added. After a 16h incubation the cell nuclei were stained in hypotonic buffer and analysed by FACS. The percentage of the sub-G1-peak was deduced. The apoptotic rate without FCS was subtracted from the rate with FCS.

Figure 4 shows the apoptosis sensitivity with other apoptosis inducers. 70% confluent cells were starved for 24 h in medium without FCS and subsequently 10 μg/ml Cisplatinum or TNF-α plus 0.1 μg/ml Cycloheximide was added to the cells. After 16 h the cell nuclei were stained with propidium iodide and analysed by FACS. 50 nM Taxol was added to the cells for 3 h and the medium subsequently replaced by fresh medium with 10% FCS. 2 days later the percentage of sub-G1 cells was deduced. The

apoptotic rate without FCS was subtracted from the rate with FCS. The values are expressed as the percentage of the respective Hela S3 value.

Viral supernatant was produced using Phoenix A packaging cell line and the respective cloned constructs (expressing pyk-2 wild-type or pyk-2 KM mutant) cloned in the vector pLXSN. Hela S3 and clone 14 were infected over night. Medium was changed the next day and two days later cells were starved for 24 hours in medium without FCS before adding 100 ng/ml CH-11 over night. Apoptosis was measured as described in Fig. 1.

Example 1

1. Materials and Methods

1.1 Selection of Apoptosis-Resistant Clones

The cervix carcinoma cell line Hela S3 (ATCC CCL-2.2) was plated on 10 cm cell culture dishes (10^5 cells) in Ham's F12 growth medium containing 10% FCS. On the next day the medium was exchanged against medium without FCS supplemented with 100 ng/ml apoptosis activating anti-Fas antibody CH-11 (Coulter Immunotech). After 3 days when most of the cells were dead, the medium was exchanged once more against the medium containing 10% FCS without antibody. The surviving cells were clonally cultivated for 3 weeks. The clones were picked and expanded.

1.2 Apoptosis Assay

50000 cells per well obtained from the parental cell line Hela S3 or from the clones, respectively, were grown in a 12 well cell culture dish for 2 days in 2 ml Ham's F12 medium containing 10% FCS. On the third day the cells were washed twice with 1 ml Ham's F12 medium and then the medium exchanged against 1 ml Ham's F12 medium. On the next day the medium was supplemented with the respective inhibitors and 100 to 200 ng/ml CH-11. On the next day the medium was decanted and transferred

to an Eppendorf tube. The cells were washed once with 200 µl PBS, the PBS was transferred to the respective Eppendorf tube. Then the remaining cells were also transferred to the respective Eppendorf tube after treatment with EDTA/trypsin in PBS. The cells were pelleted by centrifugation, suspended in 500 µl hypotonic buffer (0.1% sodium citrate, 0.1% Triton-X100, 20 µg/ml propidium iodide) and incubated for 2-24 hours at 4°C. The resutling cell nuclei were analyzed by FACS.

1.3 FACS (Fluorescence Activated Cell Sorting) - Analysis for Determining Apoptotic Nuclei

The propidium iodide fluorescence of single nuclei was determined using a FACSCalibur (Becton Dickinson) cytometer. The forward scatter light (FSC) and the side scatter light (SSC) were recorded simultaneously. The FSC peak was adjusted at channel 500 in a 1024 channel linear scale and the red fluorescence peak at channel 200 of a logarithmic scale. The FSC cut-off value was determined by gating to 95% of the greatest nuclei of a negative control without supplements. Nuclei were classified as apoptotic when a subdiploid signal between the G1/G0 peak and channel 10 was present.

1.4 Preparation of cDNA

Total RNA was isolated by lysing of cells with guanidinium isothiocyanate and subsequent extraction with acid phenol (Current Protocols in Molecular Biology). mRNA was isolated by binding to oligo-dT cellulose according to standard methods (Current Protocols in Molecular Biology).

cDNA was synthesized from mRNA by reverse transcription using Cap-finder primer K1 and K2 (Clontech Inc., USA) and AMV-reverse transcriptase (Roche Diagnostics) and purified using the PCR purification kit (Qiagen). From 3 µg mRNA 50 µl cDNA consisting of one strand DNA and one strand RNA were obtained.

1.5 Preparation of cDNA Arrays

cDNAs cloned in p-Bluescript were spotted with a BioGrid spotter (BioRobotics, UK) on nylon membranes. 250 ng DNA were used per spot. For about one half of the genes two or more probes were used and each probe was spotted twice. The following designations were used:

YK = tyrosine kinase
STK = serin/threonin kinase
PP = phosphatase
Lig = ligand
UK = unknown kinase
UP = unknown phosphatase
OT = other

Example:
YK_1b_Abl_2 = tyrosine kinase 1, probe b, spot 2

1.6 Radioactive Labelling of cDNA

5 μl cDNA were labelled with 50 μ Ci α^{33}P-ATP using the Megaprime Labelling Kit (Amersham Pharmacia) and purified using the PCR purification kit (Qiagen). The thus obtained cDNA was hybridized with COT-DNA (Roche Diagnostics) in order to block repetitive sequences which might bind unspecifically to the cDNA array.

1.7 Hybridization of cDNA Arrays

The cDNA arrays were prehybridized for 4 hours or over night at 68°C in prehybridization solution (50 x Denhardt, 10 x SSC, 0.25 M Na_3PO_4, pH 6.8, 50 mM $Na_4P_2O_7$, 0.1 mg/ml tRNA (bakers's yeast, Roche Diagnostics)).

Subsequently the cDNA arrays were hybridized for 16 hours with the labelled cDNA in hybridization buffer (5 x SSC, 0.1% SDS, 0.1 mg/ml tRNA). The cDNA arrays were washed as follows:

2 x 20 min W1 (2 x SSC, 0.1% SDS) at 42°C
1 x 20 min W2 (0.2 x SSC, 0.1% SDS) at 42°C
1 x 60 min W2 at 65°C

The cDNA arrays were exposed for 48 hours on Phosphoimager plates (Fujifilm) and subsequently analyzed on a Phosphoimager (Bas-2500, Fujifilm).

1.8 Analysis of cDNA Arrays

The spot volume on the filter was determined using ArrayVision software (V 5.1, Imaging Research Inc.). All further calculations were carried out in Excel (Microsoft Corp.).

For better internal comparison of the cDNA arrays a normalization procedure was carried out as follows: From each spot on the array the background (average of p-Bluescript values of an array) was subtracted and divided by the sum of all spot volumina in the array. The thus obtained value was multiplied by 10000.

For the identification of genes which are differentially expressed in the parental cell line Hela S3 and the apoptosis-resistant clones, the quotient from the values of the clones and the average value of the different arrays of the parental cell line (reference arrays) was calculated. All normalized values smaller than 0.1 were set to 0.1 for the calculation. 90% of all values different from 0 were above this value. The respective gene was defined as differentially expressed, if the percentage differs by at least 100%. Only such genes were analyzed wherein the deviation of the values

on the reference arrays for the respective spot on the array was sufficiently small. The following filters were used for sorting out these genes:

If the values of the reference arrays and of the respective clone for a spot were smaller than 2.5, the deviation of the reference arrays from each other must be in the range from 0.2 to 5.

If the values of the reference arrays were smaller than 2.5 and that of the clone greater than 2.5 or vice versa, the deviation of the reference arrays from each other has to be in the range from 0.3 to 3.

If both the values of the reference arrays and of the clone were greater than 2.5, the deviation of the reference arrays from each other has to be in the range from 0.5 to 2.

1.9 Gene Clustering

For gene clustering the Program Cluster (Michael Eisen, Stanford University) was used. The quotients from the values of the clones and the average value of the respective arrays of the parental cell lines were used. Spots exhibiting high deviations in the values on the reference arrays were excluded. For this purpose the filters were used which had already been applied in the identification of induced genes. From 1922 spots 1451 remained. These values were logarithmically transferred to clusters and further filtered on spots wherein the value of at least 80% of the clones was different from 0. The thus resulting 520 spots were analyzed via an hierarchical cluster algorithm.

The overall similarity of the expression patterns and the cluster mirrors in the correlation coefficient which has a value between 1 and -1. A correlation coefficient of 1 means the expression patterns are identical, 0 means that they are completely independent and -1 the opposite of each other.

2. Results

2.1 Apoptosis-Resistant Clones are Obtained by Selection of Hela S3 Using CH-11 Antibody

40 clones were obtained after selection with CH-11 antibody. 20 of these clones were tested in view of their sensitivity to CH-11. The degree to which the clones are resistant differs between individual clones, but none of them is completely resistant to apoptosis suggesting that the apoptosis machinery is functional. The clones are also refractive to apoptosis induced by TNF-α and cisplatin.

2.2 Numerous Genes Show Enhanced Expression in Apoptosis-Resistant Clones

Tables 1 and 2 show listings of genes which show enhanced expression in apoptosis-resistant clones. Further, the Genbank Accession numbers of the respective clones, the number of clones in which expression exceeds cut-off for increased expression and the average percentage over cut-off is given.

Most of the analyzed genes encode protein phosphatases and kinases, i.e. enzymes which are important for cell regulation.

From the thus determined induced clones several have not yet been associated with apoptosis and/or tumorogenesis (Table 1). Other genes such as CAMKK (calmodulin dependent kinase kinase), EGFR (epidermal growth factor receptor), Bcr (breakpoint cluster region), FGFR-1 (fibroblast growth factor receptor 1), Nik (NFκB-interacting kinase) and DAPK (death-associated protein kinase) are already known as apoptosis-associated genes.

2.3 Gene Clustering Shows Groups of Genes Which are Commonly Regulated

By clustering of expression data groups of genes were found which are commonly up- or downregulated. The common regulation suggests a common function of the genes. Thus not only single apoptosis-modulating genes, but also signal transduction cascades consisting of a plurality of genes are found. The clusters identified in apoptosis-resistant clones are shown in Table 3. The clustering of the genes allows to group the upregulated genes and deduce different anti-apoptotic signalling pathways instead of single genes only. The clusters that were found in the apoptosis-resistant clones could also be partially found in expression data of squamous cell carcinoma cell lines (Table 4). That suggests that by the screen physiologically relevant apoptosis clusters can be found that are important for tumor development and hence could serve as drug targets.

Cluster 1 contains some genes induced in many clones such as CAMKK, UK11 (unknown kinase 11), PTP α (protein tyrosine phosphatase α) and PRK (proliferation related kinase).

Cluster 2 contains 3 genes exhibiting a highly correlated expression, namely serin/threonin phosphatase VH2, TIMP (tissue inhibitor of metalloproteinase 1) and MMP-15 (matrix metalloproteinase 15). Interestingly, an enzyme (MMP-15) and a potential inhibitor (TIMP-1) are commonly regulated.

Cluster 3 comprises inter alia the membrane bound tyrosine phosphatase Lar and the proapoptotic serin/theronin kinase DAP kinase.

In cluster 4 BCR, a potential inhibitor of p38 and the JNK signal pathways, and an activator of p38, namely MAPKK-3 (mitogen activated kinase kinase 3) are commonly regulated.

2.4 Inhibition of the Induced Genes Enhances Apoptosis

In order to show that the induced genes are in fact modulators of apoptosis selected enzymes were inhibited by specific inhibitors and apoptosis was induced. Inhibitors for the following enzymes were used:

- SU 5402 inhibits FGF receptors, but is not specific for a defined FGF receptor
- AG 1295 inhibits the PDGF receptor
- SB 203580 inhibits the p38 MAP kinase

PD 98059 inhibits the MAP kinase kinase 1, which in turn activates the MAP kinases ERK1 and ERK2. This inhibitor was used as control for SB 203580, because SB 203580 also partially inhibits ERK1 and ERK2. Furthermore, ERK2 shows an enhanced expression in the clones. The results for Hela S3, clone 14 and clone 20 (partially) are shown in Fig. 1.

It was found that an inhibition of FGF receptors in Hela S3 cells leads to an increase in apoptosis of about 50%. In clones 14 and 5 SU 5402 leads to an increase of nearly 300% or 50%, respectively. Thus, in a clone having an increased expression of two FGF receptors (clone 14, FGFR-1 and FGFR-3) an inhibition of FGF receptors leads to an enhanced increase of apoptosis. In clone 5, which does not show any enhanced expression of FGF receptors, the increase in apoptosis is comparable to the parental cell line Hela S3.

An inhibition of the PDGF receptor leads to an increase of about 30% in Hela S3. In clone 14, which shows enhanced expression of PDGF receptor, the inhibition results nearly in a doubling of the number of apoptotic cells. In contrast thereto, clone 5, which does not contain any detectable PDGF receptor, exhibits only 30% increase in apoptosis after treatment with AG 1295.

The p38 MAP kinase was inhibited because BCR, an inhibitor of the p38 MAP kinase signal pathway, and MAPKK-3 (MEK-3), which is a p38 activator, exhibited an enhanced expression in the clones. Further, both genes are grouped in a cluster.

p38 inhibition in Hela S3 results in a 25% increase of apoptosis. In clone 14 exhibiting an enhanced MEK-3 expression, an inhibition of p38 leads to a 60% increase of apoptosis. In contrast thereto, an inhibition of MEK-1 results in a doubling of the apoptosis rate. The increase in apoptosis after inhibition of p38 compared to Hela S3 and the constant apoptosis after inhibition of MEK-1 might be explained by inhibition of ERK1/2 and additional inhibition of p38.

In clone 20, which expresses MEK-3 on a similar level as Hela S3, treatment with SB 203580 only leads to a slight increase of apoptosis. In contrast thereto, treatment with PD 98059 triples the apoptosis rate. Thus, SB 203580 acts specifically in this system and the differences in the increase of apoptosis after inhibition of p38 correlate with the expression of the p38 activator MEK-3.

These inhibition experiments demonstrate conclusively that the method of the invention for identifying apoptosis-associated genes is efficient.

2.5 Inhibition by Introducing a Dominant Negative Mutant or an Antisense Strand

The respective enzymes upregulated in apoptosis-resistant clones can also be inhibited by introducing a dominant negative mutant or the antisense strand. Figure 2 shows that - as as example - the wild-type pyk-2 confers increased resistance when introduced in Hela S3. In clone 14 with a higher expression of pyk-2 introduction of the wild-type enzyme has no effect but the mutant with the lysine mutated to methionine (pyk-2 KM) in the

reactive center of the enzyme reverts the phenotype of the clone. The antisense construct has a corresponding but weaker effect.

Example 2

The experimental procedure was carried out as described in Example 1.

For the identification of genes differentially expressed in the parental cell line Hela S3 and the apoptosis resistant clones, the following evaluation procedure was applied. For each spot on the cDNA arrays of the parental cell line Hela S3 four values were determined in the following manner. RNA was isolated twice from Hela S3 in two independent preparations. Each RNA preparation was used to synthesize cDNA and each cDNA was hybridized with two cDNA arrays. The average of those 4 values for a given spot on the cDNA array was calculated and the standard deviation determined. The cDNA of each apoptosis resistant clone was hybridized with one cDNA array. A gene was considered to be differentially expressed in the apoptosis resistant clones when its value exceeded the following cut offs. The cut off for upregulated genes was the average of the respective Hela S3 values plus two times standard deviation. Accordingly, the cut off for downregulated genes was the average of the respective Hela S3 values minus two times standard deviation. The magnitude of the up-or downregulation was expressed as percent over/under the cut off. For example, a value of 100% over the cut off for upregulated genes means a 2-fold induction compared to the cut off, and a value of 100% under the cut off for downregulated genes means a bisection of that value in the resistant clones.

For gene clustering the program Cluster (Michael Eisen, Stanford University) may be used. The normalized values of the four reference arrays and the array of the 20 apoptosis resistant clones were used. Genes with a value greater than 1 in at least 20 of the 24 investigated arrays were filtered out and employed for the following calculations. The cut off

For gene clustering the program Cluster (Michael Eisen, Stanford University) may be used. The normalized values of the four reference arrays and the array of the 20 apoptosis resistant clones were used. Genes with a value greater than 1 in at least 20 of the 24 investigated arrays were filtered out and employed for the following calculations. The cut off of 1 was utilized in order to avoid clustering of genes whose value was so close to the background that a clustering would be unreliable. Thus, out of 2400 spots, 520 remained that were analysed via a hierarchical cluster algorithm.

The results are shown in Table 5.

References:

1. Will, H. and B. Hinzmann, *cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment.* Eur J Biochem, 1995. **231**(3): p. 602-8.
2. Butler, G.S., et al., *Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases.* Eur J Biochem, 1997. **244**(2): p. 653-7.
3. Hu, M.C., et al., *Protein phosphatase X interacts with c-Rel and stimulates c-Rel/nuclear factor kappaB activity.* J Biol Chem, 1998. **273**(50): p. 33561-5.
4. Kersh, G.J. and P.M. Allen, *Essential flexibility in the T-cell recognition of antigen.* Nature, 1996. **380**(6574): p. 495-8.
5. Kohno, K., et al., *Amino acid sequence of mammalian elongation factor 2 deduced from the cDNA sequence: homology with GTP-binding proteins.* Proc Natl Acad Sci U S A, 1986. **83**(14): p. 4978-82.
6. Geiger, B., *Intermediate filaments. Looking for a function.* Nature, 1987. **329**(6138): p. 392-3.
7. Didsbury, J.R., Y.S. Ho, and R. Snyderman, *Human Gi protein alpha-subunit: deduction of amino acid structure from a cloned cDNA.* FEBS Lett, 1987. **211**(2): p. 160-4.
8. Bokoch, G.M. and A.G. Gilman, *Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin.* Cell, 1984. **39**(2 Pt 1): p. 301-8.
9. Sullivan, K.A., et al., *Inhibitory and stimulatory G proteins of adenylate cyclase: cDNA and amino acid sequences of the alpha chains.* Proc Natl Acad Sci U S A, 1986. **83**(18): p. 6687-91.

10. Williams, N.G., H. Zhong, and K.P. Minneman, *Differential coupling of alpha1-, alpha2-, and beta-adrenergic receptors to mitogen-activated protein kinase pathways and differentiation in transfected PC12 cells.* J Biol Chem, 1998. **273**(38): p. 24624-32.
11. Mukai, H. and Y. Ono, *A novel protein kinase with leucine zipper-like sequences: its catalytic domain is highly homologous to that of protein kinase C.* Biochem Biophys Res Commun, 1994. **199**(2): p. 897-904.
12. Wick, M.J., et al., *Mechanism of phosphorylation of protein kinase B/Akt by a constitutively active 3-phosphoinositide-dependent protein kinase-1.* J Biol Chem, 2000. **275**(51): p. 40400-6.
13. Takahashi, M., et al., *Proteolytic activation of PKN by caspase-3 or related protease during apoptosis.* Proc Natl Acad Sci U S A, 1998. **95**(20): p. 11566-71.
14. Watanabe, G., et al., *Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho.* Science, 1996. **271**(5249): p. 645-8.
15. Mukai, H., et al., *Activation of PKN, a novel 120-kDa protein kinase with leucine zipper-like sequences, by unsaturated fatty acids and by limited proteolysis.* Biochem Biophys Res Commun, 1994. **204**(1): p. 348-56.
16. Maru, Y. and O.N. Witte, *The BCR gene encodes a novel serine/threonine kinase activity within a single exon.* Cell, 1991. **67**(3): p. 459-68.
17. Diekmann, D., et al., *Bcr encodes a GTPase-activating protein for p21rac.* Nature, 1991. **351**(6325): p. 400-2.
18. Galaktionov, K. and D. Beach, *Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins.* Cell, 1991. **67**(6): p. 1181-94.
19. Honda, R., et al., *Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase.* FEBS Lett, 1993. **318**(3): p. 331-4.
20. Sebastian, B., A. Kakizuka, and T. Hunter, *Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15.* Proc Natl Acad Sci U S A, 1993. **90**(8): p. 3521-4.
21. Miyata, H., et al., *Overexpression of CDC25B overrides radiation-induced G2-M arrest and results in increased apoptosis in esophageal cancer cells.* Cancer Res, 2001. **61**(7): p. 3188-93.
22. Zuo, Z., et al., *Ser/Thr protein phosphatase type 5 (PP5) is a negative regulator of glucocorticoid receptor-mediated growth arrest.* Biochemistry, 1999. **38**(28): p. 8849-57.
23. Silverstein, A.M., et al., *Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin.* J Biol Chem, 1997. **272**(26): p. 16224-30.
24. Chen, M.X., et al., *A novel human protein serine/threonine phosphatase, which possesses four tetratricopeptide repeat motifs and localizes to the nucleus.* Embo J, 1994. **13**(18): p. 4278-90.

25. Lumeng, C., et al., *Interactions between beta 2-syntrophin and a family of microtubule-associated serine/threonine kinases.* Nat Neurosci, 1999. **2**(7): p. 611-7.
26. Walden, P.D. and N.J. Cowan, *A novel 205-kilodalton testis-specific serine/threonine protein kinase associated with microtubules of the spermatid manchette.* Mol Cell Biol, 1993. **13**(12): p. 7625-35.
27. Yoshiyama, K., et al., *CD156 (human ADAM8): expression, primary amino acid sequence, and gene location.* Genomics, 1997. **41**(1): p. 56-62.
28. Schlomann, U., et al., *Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration.* J Neurosci, 2000. **20**(21): p. 7964-71.
29. Wang, Z., R. Juttermann, and P.D. Soloway, *TIMP-2 is required for efficient activation of proMMP-2 in vivo.* J Biol Chem, 2000. **275**(34): p. 26411-5.
30. Zucker, S., et al., *Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP).* J Biol Chem, 1998. **273**(2): p. 1216-22.
31. Hayakawa, T., et al., *Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2).* J Cell Sci, 1994. **107**(Pt 9): p. 2373-9.
32. Wang, X.S., et al., *MAPKKK6, a novel mitogen-activated protein kinase kinase kinase, that associates with MAPKKK5.* Biochem Biophys Res Commun, 1998. **253**(1): p. 33-7.
33. Kratzschmar, J., L. Lum, and C.P. Blobel, *Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence.* J Biol Chem, 1996. **271**(9): p. 4593-6.
34. Nath, D., et al., *Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells.* J Cell Sci, 1999. **112**(Pt 4): p. 579-87.
35. Knecht, R., et al., *Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck.* Cancer Res, 1999. **59**(12): p. 2794-7.
36. Smits, V.A., et al., *Polo-like kinase-1 is a target of the DNA damage checkpoint.* Nat Cell Biol, 2000. **2**(9): p. 672-6.
37. Toyoshima-Morimoto, F., et al., *Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase.* Nature, 2001. **410**(6825): p. 215-20.
38. Schmucker, D. and S.L. Zipursky, *Signaling downstream of eph receptors and ephrin ligands.* Cell, 2001. **105**(6): p. 701-4.
39. Miao, H., et al., *Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway.* Nat Cell Biol, 2001. **3**(5): p. 527-30.
40. Miao, H., et al., *Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation.* Nat Cell Biol, 2000. **2**(2): p. 62-9.
41. Welsh, M., et al., *Sib is a ubiquitously expressed Src homology 2 protein.* Oncogene, 1994. **9**(1): p. 19-27.

42. Oishi, K., et al., *Identification and characterization of PKNbeta, a novel isoform of protein kinase PKN: expression and arachidonic acid dependency are different from those of PKNalpha.* Biochem Biophys Res Commun, 1999. **261**(3): p. 808-14.
43. Gu, M., I. Warshawsky, and P.W. Majerus, *Cloning and expression of a cytosolic megakaryocyte protein-tyrosine-phosphatase with sequence homology to retinaldehyde-binding protein and yeast SEC14p.* Proc Natl Acad Sci U S A, 1992. **89**(7): p. 2980-4.
44. Stebbins, C.E., W.G. Kaelin, Jr., and N.P. Pavletich, *Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function.* Science, 1999. **284**(5413): p. 455-61.
45. Maxwell, P.H., et al., *The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis.* Nature, 1999. **399**(6733): p. 271-5.
46. Kibel, A., et al., *Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C.* Science, 1995. **269**(5229): p. 1444-6.
47. Sakano, S., et al., *Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase).* Oncogene, 1994. **9**(4): p. 1155-61.
48. Rane, M.J., et al., *p38 Kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils.* J Biol Chem, 2001. **276**(5): p. 3517-23.
49. Rouse, J., et al., *A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins.* Cell, 1994. **78**(6): p. 1027-37.
50. Gurney, M.E., et al., *Neuroleukin: a lymphokine product of lectin-stimulated T cells.* Science, 1986. **234**(4776): p. 574-81.
51. Falk, P., et al., *Mouse glucose-6 phosphate isomerase and neuroleukin have identical 3' sequences.* Nature, 1988. **332**(6163): p. 455-7.
52. Chaput, M., et al., *The neurotrophic factor neuroleukin is 90% homologous with phosphohexose isomerase.* Nature, 1988. **332**(6163): p. 454-5.
53. Gurney, M.E., et al., *Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and sensory neurons.* Science, 1986. **234**(4776): p. 566-74.
54. Wang, H., et al., *Characterization of PCP-2, a novel receptor protein tyrosine phosphatase of the MAM domain family.* Oncogene, 1996. **12**(12): p. 2555-62.
55. Frigerio, J.M., J.C. Dagorn, and J.L. Iovanna, *Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs.* Biochim Biophys Acta, 1995. **1262**(1): p. 64-8.
56. Yan, H., et al., *A novel receptor tyrosine phosphatase-sigma that is highly expressed in the nervous system.* J Biol Chem, 1993. **268**(33): p. 24880-6.
57. Elchebly, M., et al., *Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma.* Nat Genet, 1999. **21**(3): p. 330-3.
58. Nagase, T., et al., *Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121-KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1.* DNA Res, 1995. **2**(4): p. 167-74, 199-210.

59. Benovic, J.L., et al., *Beta-adrenergic receptor kinase: primary structure delineates a multigene family.* Science, 1989. **246**(4927): p. 235-40.
60. Benovic, J.L., et al., *Purification and characterization of the beta-adrenergic receptor kinase.* J Biol Chem, 1987. **262**(19): p. 9026-32.
61. Colamonici, O.R., et al., *p135tyk2, an interferon-alpha-activated tyrosine kinase, is physically associated with an interferon-alpha receptor.* J Biol Chem, 1994. **269**(5): p. 3518-22.
62. Stahl, N., et al., *Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components.* Science, 1994. **263**(5143): p. 92-5.
63. Bernards, A., *Predicted tyk2 protein contains two tandem protein kinase domains.* Oncogene, 1991. **6**(7): p. 1185-7.
64. Wang, B., et al., *Molecular cloning and characterization of a novel human receptor protein tyrosine phosphatase gene, hPTP-J: down-regulation of gene expression by PMA and calcium ionophore in Jurkat T lymphoma cells.* Biochem Biophys Res Commun, 1997. **231**(1): p. 77-81.
65. Takeda, K., et al., *Essential role of Stat6 in IL-4 signalling.* Nature, 1996. **380**(6575): p. 627-30.
66. Masuda, A., et al., *Interleukin-15 Prevents Mouse Mast Cell Apoptosis through STAT6-mediated Bcl-xL Expression.* J Biol Chem, 2001. **276**(28): p. 26107-13.
67. Kato, Y., et al., *BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C.* Embo J, 1997. **16**(23): p. 7054-66.
68. Kato, Y., et al., *Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor.* Nature, 1998. **395**(6703): p. 713-6.
69. Zhou, G., Z.Q. Bao, and J.E. Dixon, *Components of a new human protein kinase signal transduction pathway.* J Biol Chem, 1995. **270**(21): p. 12665-9.
70. Siliciano, J.D., T.A. Morrow, and S.V. Desiderio, *itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2.* Proc Natl Acad Sci U S A, 1992. **89**(23): p. 11194-8.
71. Tanaka, N., et al., *A novel human tyrosine kinase gene inducible in T cells by interleukin 2.* FEBS Lett, 1993. **324**(1): p. 1-5.
72. Deak, M., et al., *Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB.* Embo J, 1998. **17**(15): p. 4426-41.
73. Li, J., et al., *Novel NEMO/IkappaB kinase and NF-kappa B target genes at the pre-B to immature B cell transition.* J Biol Chem, 2001. **276**(2): p. 18579-90.
74. Baytel, D., et al., *The human Pim-2 proto-oncogene and its testicular expression.* Biochim Biophys Acta, 1998. **1442**(2-3): p. 274-85.
75. Wang, D., et al., *Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II.* J Biol Chem, 2000. **275**(42): p. 32592-7.
76. Nada, S., et al., *Constitutive activation of Src family kinases in mouse embryos that lack Csk.* Cell, 1993. **73**(6): p. 1125-35.

77. Imamoto, A. and P. Soriano, *Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice.* Cell, 1993. **73**(6): p. 1117-24.
78. Rothwarf, D.M., et al., *IKK-gamma is an essential regulatory subunit of the Ikappab kinase complex.* Nature, 1998. **395**(6699): p. 297-300.
79. Millward, T.A., et al., *Calcium regulation of Ndr protein kinase mediated by S100 calcium-binding proteins.* Embo J, 1998. **17**(20): p. 5913-22.
80. Millward, T., P. Cron, and B.A. Hemmings, *Molecular cloning and characterization of a conserved nuclear serine(threonine) protein kinase.* Proc Natl Acad Sci U S A, 1995. **92**(11): p. 5022-6.
81. Cheng, M., T.G. Boulton, and M.H. Cobb, *ERK3 is a constitutively nuclear protein kinase.* J Biol Chem, 1996. **271**(15): p. 8951-8.
82. Boulton, T.G., et al., *ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF.* Cell, 1991. **65**(4): p. 663-75.
83. Hande, K.R., *Clinical applications of anticancer drugs targeted to topoisomerase II.* Biochim Biophys Acta, 1998. **1400**(1-3): p. 173-84.
84. Wo, Y.Y., et al., *Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphotyrosyl protein phosphatase.* J Biol Chem, 1992. **267**(15): p. 10856-65.
85. Haystead, T.A., et al., *Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism.* Nature, 1989. **337**(6202): p. 78-81.
86. Kishikawa, K., et al., *Phosphatidic acid is a potent and selective inhibitor of protein phosphatase 1 and an inhibitor of ceramide-mediated responses.* J Biol Chem, 1999. **274**(30): p. 21335-41.
87. Warbrick, E., *The puzzle of PCNA's many partners.* Bioessays, 2000. **22**(11): p. 997-1006.
88. Tiganis, T., B.E. Kemp, and N.K. Tonks, *The protein-tyrosine phosphatase TCPTP regulates epidermal growth factor receptor-mediated and phosphatidylinositol 3-kinase-dependent signaling.* J Biol Chem, 1999. **274**(39): p. 27768-75.
89. Tiganis, T., et al., *Epidermal growth factor receptor and the adaptor protein p52Shc are specific substrates of T-cell protein tyrosine phosphatase.* Mol Cell Biol, 1998. **18**(3): p. 1622-34.
90. Sanchez, Y., et al., *Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25.* Science, 1997. **277**(5331): p. 1497-501.
91. Furnari, B., N. Rhind, and P. Russell, *Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase.* Science, 1997. **277**(5331): p. 1495-7.
92. Mailand, N., et al., *Rapid destruction of human Cdc25A in response to DNA damage.* Science, 2000. **288**(5470): p. 1425-9.

'es of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. J Biol Chem, 1995. **270**(29): p.

:ation of the human YVH1 protein-tyrosine phosphatase orthologue reveals a novel zinc binding domain *'ion.* J Biol Chem, 1999. **274**(34): p. 23991-5.
(+)-like gene in human cells. Nature, 1991. **353**(6339): p. 80-3.
' and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. Cell, 1991. **64**(6): p. 1111-22.
Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. Cell, 1987. **49**(4): p. 559-

ular masking of nuclear import signal on NF-AT4 by casein kinase I and MEKK1. Cell, 1998. **93**(5): p. 851-61.
n kinase I transduces Wnt signals. Nature, 1999. **401**(6751): p. 345-50.
gg, Identification of 21 novel human protein kinases, including 3 members of a family related to the cell cycle *gillus nidulans.* Cell Growth Differ, 1993. **4**(10): p. 821-30.
'1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. Cell *):* p. 197-208.
Identification and characterization of a human protein kinase related to budding yeast Cdc7p. Proc Natl Acad p. 14320-5.
ovel SR-protein-specific kinase, SRPK2, disassembles nuclear speckles. Biochem Biophys Res Commun, 1998.

'2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of rs in mammalian cells. J Cell Biol, 1998. **140**(4): p. 737-50.
n of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways.): p. 90-4.
ovel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J Biol Chem, 1996.

ion of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase.), 1997. **94**(18): p. 9687-92.
) M.W. Killimann, *Structure of the human gene encoding the phosphorylase kinase beta subunit (PHKB).* Eur J), 374-80.

109. Hobert, O., B. Jallal, and A. Ullrich, *Interaction of Vav with ENX-1, a putative transcriptional regulator of homeobox gene expression.* Mol Cell Biol, 1996. **16**(6): p. 3066-73.
110. Hobert, O., et al., *Isolation and developmental expression analysis of Enx-1, a novel mouse Polycomb group gene.* Mech Dev, 1996. **55**(2): p. 171-84.
111. Alexandru, G., et al., *Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage.* Embo J, 1999. **18**(10): p. 2707-21.
112. Cahill, D.P., et al., *Mutations of mitotic checkpoint genes in human cancers.* Nature, 1998. **392**(6673): p. 300-3.
113. Schultz, S.J., et al., *Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of Aspergillus nidulans.* Cell Growth Differ, 1994. **5**(6): p. 625-35.
114. Fry, A.M., P. Meraldi, and E.A. Nigg, *A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators.* Embo J, 1998. **17**(2): p. 470-81.
115. Zhao, Y., et al., *Cloning and chromosomal location of a novel member of the myotonic dystrophy family of protein kinases.* J Biol Chem, 1997. **272**(15): p. 10013-20.
116. Tung, R.M. and J. Blenis, *A novel human SPS1/STE20 homologue, KHS, activates Jun N-terminal kinase.* Oncogene, 1997. **14**(6): p. 653-9.
117. Yuan, Y., D.M. Li, and H. Sun, *PIR1, a novel phosphatase that exhibits high affinity to RNA . ribonucleoprotein complexes.* J Biol Chem, 1998. **273**(32): p. 20347-53.
118. Deshpande, T., et al., *Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5'-triphosphatase and diphosphatase activities.* J Biol Chem, 1999. **274**(23): p. 16590-4.
119. Bastians, H. and H. Ponstingl, *The novel human protein serine/threonine phosphatase 6 is a functional homologue of budding yeast Sit4p and fission yeast ppe1, which are involved in cell cycle regulation.* J Cell Sci, 1996. **109**(Pt 12): p. 2865-74.
120. Becker, W., et al., *Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases.* J Biol Chem, 1998. **273**(40): p. 25893-902.
121. Guimera, J., et al., *A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region.* Hum Mol Genet, 1996. **5**(9): p. 1305-10.
122. Lopez-Borges, S. and P.A. Lazo, *The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2 binding site of the p53 tumour suppressor protein.* Oncogene, 2000. **19**(32): p. 3656-64.
123. Nezu, J., et al., *Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase.* Genomics, 1997. **45**(2): p. 327-31.

124. Lapidot-Lifson, Y., et al., *Cloning and antisense oligodeoxynucleotide inhibition of a human homolog of cdc2 required in hematopoiesis.* Proc Natl Acad Sci U S A, 1992. **89**(2): p. 579-83.
125. Mills, G.B., et al., *Expression of TTK, a novel human protein kinase, is associated with cell proliferation.* J Biol Chem, 1992. **267**(22): p. 16000-6.
126. Schmandt, R., et al., *IL-2-induced expression of TTK, a serine, threonine, tyrosine kinase, correlates with cell cycle progression.* J Immunol, 1994. **152**(1): p. 96-105.
127. Ruvolo, P.P., et al., *Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A.* J Biol Chem, 1999. **274**(29): p. 20296-300.
128. Wadzinski, B.E., et al., *Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation.* Mol Cell Biol, 1993. **13**(5): p. 2822-34.
129. Lee, M.G. and P. Nurse, *Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.* Nature, 1987. **327**(6117): p. 31-5.
130. Riabowol, K., et al., *The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells.* Cell, 1989. **57**(3): p. 393-401.
131. Zeng, Q., et al., *Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome.* J Biol Chem, 2000. **275**(28): p. 21444-52.
132. Diamond, R.H., et al., *PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth.* Mol Cell Biol, 1994. **14**(6): p. 3752-62.
133. Peng, Y., et al., *The gene encoding human nuclear protein tyrosine phosphatase, PRL-1. Cloning, chromosomal localization, and identification of an intron enhancer.* J Biol Chem, 1998. **273**(27): p. 17286-95.
134. Edwards, M.C., C. Wong, and S.J. Elledge, *Human cyclin K, a novel RNA polymerase II-associated cyclin possessing both carboxy-terminal domain kinase and Cdk-activating kinase activity.* Mol Cell Biol, 1998. **18**(7): p. 4291-300.
135. van den Berg, I.E., et al., *X-linked liver phosphorylase kinase deficiency is associated with mutations in the human liver phosphorylase kinase alpha subunit.* Am J Hum Genet, 1995. **56**(2): p. 381-7.
136. Grove, J.R., et al., *Cloning and expression of two human p70 S6 kinase polypeptides differing only at their amino termini.* Mol Cell Biol, 1991. **11**(11): p. 5541-50.
137. Banerjee, P., et al., *Molecular structure of a major insulin/mitogen-activated 70-kDa S6 protein kinase.* Proc Natl Acad Sci U S A, 1990. **87**(21): p. 8550-4.
138. Weng, Q.P., et al., *Phosphatidylinositol 3-kinase signals activation of p70 S6 kinase in situ through site-specific p70 phosphorylation.* Proc Natl Acad Sci U S A, 1995. **92**(12): p. 5744-8.
139. Kornbluth, S., M. Sudol, and H. Hanafusa, *Association of the polyomavirus middle-T antigen with c-yes protein.* Nature, 1987. **325**(7000): p. 171-3.

140. Kitamura, N., et al., *Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus* Nature, 1982. **297**(5863): p. 205-8.
141. Tobimatsu, T. and H. Fujisawa, *Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs.* J Biol Chem, 1989. **264**(30): p. 17907-12.
142. Hovens, C.M., et al., *RYK, a receptor tyrosine kinase-related molecule with unusual kinase domain motifs.* Proc Natl Acad Sci U S A, 1992. **89**(24): p. 11818-22.
143. Halford, M.M., et al., *Ryk-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk.* Nat Genet, 2000. **25**(4): p. 414-8.
144. Barker, H.M., et al., *Sequence of human protein serine/threonine phosphatase 1 gamma and localization of the gene (PPP1CC) encoding it to chromosome bands 12q24.1-q24.2.* Biochim Biophys Acta, 1993. **1178**(2): p. 228-33.

Table 1

Genes that have not been linked to an antiapoptotic function before and are induced in the apoptosis resistant clones

Gene	Accession number	Number of clones in which expression exceeds cut off for increased expression	% over cutoff
Tyrosine Kinases			
Hck	M16591	4	46,5
TrkC	U05012	6	28,0
Hyl	X77278	11	24,5
Rse	U05682	10	22,5
RON	X70040	14	21,0
KIAA0641	AB014541	5	19,5
EphA2	M59371	12	19,5
Csk	X59932	8	18,0
EphB3	X75208	6	16,0
EphB4	U07695	5	12,5
Pyk-2	U33284	4	11,0
Unknown Phosphatases			
PB-26	AB040904	5	30,5
PB-28	AB040904	4	18,0
Unknown Kinases			
UK19	AA292586	5	25,0
UK10	R52045	4	24,5
UK11	H39075	8	9,5
Serine/Threonine Kinases			
GRK6	L16862	16	69,0
Dyrk4	Y09305	5	55,0
IRAK-2	AF026273	13	54,5
LIMK-1	D26309	5	45,5
MLK3	U07747	9	44,0
AMPK-beta	AJ224538	4	40,0
MAPKKK6	AF100318	14	39,0
MAST205	6678957	14	37,5
DAPK	X76104	11	37,0
MAPKK3	4506098	11	36,0
PLK-1	L19559	12	34,5
PKN-H4	D26181	13	34,5
Bcr	X02596	10	32,0
MSK2	AF074715	8	30,0
Rac-alpha	M63167	16	28,0
MST-3	AF024636	7	28,0
PSK-H1	M14504	5	26,5
PCTAIRE1	X66363	6	23,0

lok	AB015718	4	22,0
HsGAK	D88435	6	21,0
MAPKAPK3	U09578	7	20,5
JNKK2alpha	AF022805	8	19,5
FAST	X86779	5	19,0
MKK7	AF013588	8	17,5
MAPKK5	U25265	6	16,5
PAK1-relatedkinase	AF005046	11	16,0
ARK2	AF008552	4	16,0
MSSK1	U82808	4	15,0
PHK-gammaT	M31606	6	14,5
CDC42-bindingproteinkinasebeta	AF128625	3	13,5
KIAA0151	D63485	9	11,0
KIAA0537	AB011109	6	11,5
STE20-like	X99325	4	6,0
Ste-20likeproteinkinase3	AF083420	3	5,5
Adapter Proteins			
Grb-2	M96995	7	18,0
SHC	Y09847	6	17,5
SHB	X75342	10	15,0
Phosphatases			
PYST1	X83820	5	85,5
B23	U15932	5	33,0
PCP-2	X97198	10	31,5
PTP-J	U73727	9	30,0
PTP-Meg2	M83738	9	29,5
PP5	X89416	7	18,5
CDC25B	M81934	10	17,0
PTP-SL	Z30313	5	17,0
PP2B-R	M30773	6	17,0
PP1-Calpha	M63960	6	16,0
PP2A-Rb55	M64930	5	15,5
PTPzeta	X54135	4	13,0
Shp-1	X62055	4	7,0
PP2A-Ra65	J02902	6	7,0
PTPmu	X58288	3	6,5
Metalloproteases			
MMP-15	Z48482	19	71,0
ADAM12	X05232	8	59,0
MMP-3	J03209	14	34,5
ADAM15	NM003815	12	28,0
ADAM8	XM005675	13	27,0
G-proteins			
alphai2		16	44,0
GPIR-3		4	8,0

Table 1 (continued)

Other			
p91/ISGF-3	M97935	7	104,0
90k	50318862	15	57,5
MHC-1	M11886	17	53,0
EF-2	X51466	16	44,0
alpha-tubulin	NM_006082	9	43,5
KIF-1c	NM006612	6	28,0
Furin	X17094	8	27,0
rS9	4506744	13	23,0
GPDH	M33197	9	23,0
beta-Aktin	X00351	11	21,5
Vimentin	X56134	15	19,0
neuroiektin	K03515	14	17,5
Thymosinbeta	S54005	7	11,5
Histon3.3	M11354	6	7,0
PHB-4-PC	L14273	5	3,5

Table 1 (continued)

Table 2

Genes with known antiapoptotic function that are induced in the apoptosis resistant clones

Gene	Accession number	Number of clones in which expression exceeds cut off for increased expression	% over cut off
Tyrosine Kinases			
PDGFRalpha	M22734	4	39,0
HER2	M11730	4	38,5
EGFR	X00588	6	21,0
FGFR-3	M58051	5	20,5
HER4	L07868	5	32,5
Jak-2	AF058925	6	34,0
Tyk-2	X54637	8	18,5
Serine/Threonine Kinases			
RSK	L07597	11	43,5
MAPKK2	L11285	11	33,5
PIM-2h	U77735	8	29,5
IKK1	AF012890	4	28,0
CKII-beta	M30448	7	28,0
ALK-4	Z22536	6	27,0
ERK1	X80188	11	25,5
IKKgamma	AF074382	12	23,5
AKT2	M95936	10	17,0
CKII-alpha	J02853	8	17,5
CaM-KIIgamma	L07044	7	16,0
MAPKAPK2	NM004759	6	15,0
ILK	U40282	7	14,0
CKI-delta	U29171	6	11,5
SGK	Y10032	4	11,5
CKII-beta	M30448	5	9,0
A-Raf-1	X04790	5	9,0
ALK-1	L17075	5	6,5
Phosphatases			
PPX	X70218	16	26,0
Ligands			
TGFalpha	XM002732	11	63,0
IL1-beta	NM000576	8	54,5
IL1-alpha	X02531	4	34,5
VEGF	NM003376	9	27,0
Other			
Bcl-x	Z23115	14	34,5
IL-4Stat	U16031	9	19,5
TIMP-1	X03124	17	108,5
myc	X00364	8	15,5
TIMP-2	S48568	13	36,5

Clusters in the apoptosis resistant clones

Bold: also found in one common cluster in squamous cell carcinoma

Cluster 1

Correlation factor 0,71	
Bcr	Rac-alpha
JNKK2alpha	IKKgamma
MKK7	PPX
PTP-SL	ADAM15
KIF-1c	ADAM8
SHB	ERK1
IRAK	Rse
PLK-1	alphai2S48C
alpha-tubulin	MAPKKK6
RSK	90k
GRK6	pBTUB
GPIR-3	
MAPKAPK3	
PKN-H4	
MAPKK2	
PKA-RIbeta	
p130CAS	
LIMK-1	
MAST205	
PIM-2h	
Csk	
MAPKK3	
PCTAIRE1	
MST-3	

Table 3

Cluster 2

Correlation factor 0,73
Bcl-x
AKT2
EF-2
PITALRE
TIMP-2
FGFR-2
CDC25B
CKI-delta
EphA2
PP2C
Rac-alpha
RON
EphB4

Cluster 3

Correlation factor 0,74
PDGFRalpha
PB-28
PHK-gammaT
Tyk-2
A-Raf-1
GPDH
HK-18B
Erk6
Cyto18
HK-18B
cytokeratin8
CK-8
CKII-beta
MLK3
MMP-11

Cluster 4

Correlation factor 0,67
PP2A-Ra65
HER2
CAMKK

Cluster 5

Correlation factor 0,71
PKC-epsilon
MAPKK5
PTP-Meg2
PP2B-Cbeta
MKP-5
Jak-2
Shp-2
IKK2
PHK-alphaL
JNK1

Cluster 6

Correlation factor 0,61
Pyk-2
Shp-1
PP2B-Cgamma
lck
CaM-KIIgamma
DRP-1
CKI-gamma2
PSK-H1
GPIR-1
Chk2
Axl
IL-4Stat
PCP-2
DAPK
PKA-Calpha2
PP5
Abl
Raf
PTPzeta
IGF1-R
pHE-A1
PTP-1B
MSTH1
PKA-Calpha
PLCgamma
HsGAK
VHR
TESK1
PRK

Cluster 7

Correlation factor 0,83
MMP-15
TIMP-1

Table 3 (continued)

Clusters in squamous cell carcinoma cell lines

SCaBER
UMSSC-17B
UMSSC-17A
UMSSC-22A
UMSSC-22B
UMSSC-10A
HlaC78
HlaC79
FaDu

Bold: also found in one common cluster in apoptosis resistant clones

Cluster 1
Correlation factor 0,7
PRL-3_2_23
Dyrk2_2_16
MAPKK2_1_5
SHC_1_3
ERK3_1_4
RSK

Cluster 2
Correlation factor 0,8
GSK-3alpha
PTP-SL
MAPKAPK3
GSK-3beta
hPAK1
PKC-delta
PB-32
alphaq
KIF-1c
SHB
KIAA0687Nck-interactingkinase

Table 4

Cluster 3

Correlation factor 0,67
PB-32
PB-38
hPAK2
TIMP-2
PB-5
CDK6
Bcl-x
H11
Axl
MMP-14
hSLK
MSTH1
alphaq
ADAM17
PIR1
ALK-2

Cluster 4

Correlation factor 0,71
Tyk-2
TIMP-1
GPDH
HPRT
Dyrk4
MMP-15
Jak-1
Myt1
GPIR-3
PCNA
Chk2
PK38
alpha-tubulin
CDK4
PKN-H4
hPTK
GPIR-2
Bmx
MMP-11
PKU-alpha

Table 4 (continued)

TABLE 5 (1/7)

Gene	Genbank Nr.	Description	Reference	Number of clones with increased expression	% over cut off for increased expression
MP_MMP-15	Z48482	transmembrane metalloprotease, probably processes MMP-2	[1, 2]	20	82
STP_PPX	X70218	nuclear, localized to centrosomes, activates NFκB by dephosphorylation	[3]	17	18
OT_MHC-1	M11886	presents antigens on the cell surface	[4]	16	44
OT_EF-2	XM031904	translation-elongation-factor-2	[5]	15	30
OT_Vimentin	X56134	intermediate filament	[6]	15	14
GP_alphai2	NM002070	alpha subunit of heterotrimeric G-proteins, can inhibit adenylate cyclase and activate MAP-kinase	[7-10]	14	42
STK_PKNalpha	D26181	related to PKC, activated by Rho, fatty acids and caspase cleavage	[11-15]	14	34
STK_Bcr	X02596	possesses serine/threonine kinase activity and GAP activity for p21rac	[16, 17]	13	32
STYP_CDC25B	M81934	dual specific, induces cell cycle progression form G2 to M by dephosphorylation of cdc-2	[18-21]	13	30
STP_PP5_1_3	X89416	nuclear, binds to the glucocorticoid receptor and inhibits growth inhibition by this receptor	[22-24]	13	22
STK_MAST205	6678957	binds to microtubuli and β2-syntrophin	[25, 26]	13	20
MP_ADAM8	NM001109	induced by TNF-α	[27, 28]	13	18
MP_TIMP-2	S48568	contributes to the activation of pro-Gelatinase A in complex with MMP-14, may act milogenically	[29-31]	12	37

TABLE 5 (2/7)

STK_MAPKKK6	U39657	binds MAPKKK5/ASK1	[32]	12		33
MP_ADAM15	NM003815	binds to Integrins	[33, 34]	12		32
STK_PLK-1	L19559	prognostic marker for squamous cell carcinoma, essential for Pro-phase of mitosis, activated after DNA damage	[35-37]	12		21
YK_EphA2	M59371	plays a role in repulsion of nerve cells during embryogenesis, inhibits MAPK-activation by PDGF and EGF	[38-40]	12		16
AD_SHB	NM003028	SH2 domain containing adaptor protein	[41]	12		15
STK_PKNbeta	AB019692	homologues to PKNalpha, not expressed in adult healthy tissues but in cancer	[42]	11		49
YP_PTP-Meg2	M83738	cytosolic tyrosine phosphatase	[43]	11		42
OT_VHL	NM_000551	tumor suppressor that forms a complex with ubiquitin ligase	[44-46]	11		40
YK_Hyl	X77278	cytoplasmic tyrosine kinase with homology to CSK	[47]	11		18
STK_MAPKAPK2	NM032960	activated by p38 MAP kinase, can act as PDK2 for Akt	[48, 49]	11		10
OT_neuroleukin	K03515	neurotrophic ligand, entire mRNA also codes for Phosphohexose-Isomerase	[50-53]	11		10
YP_PCP-2	X97198	receptor tyrosine phosphatase with MAM domain	[54]	10		30
OT_rS9	4506744	ribosomal protein 9	[55]	10		16
YP_PTPsigma	U35234	receptor tyrosine phosphatase of the LAR family, involved in brain embryogenisis	[56, 57]	9		109
STK_KIAA0135	D50925	putative Serin/Threonin-Kinase	[58]	9		33
STK_beta-ARK-1	X61157	phosphorylates and desensitizes β-adrenergic receptor	[59, 60]	9		25

TABLE 5 (3/7)

YK_Tyk-2	X54637	cytoplasmic tyrosine kinase, homologues to JAK-kinases	[61-63]	9	20
YP_PTP-J	U73727	receptor tyrosine phosphatase with MAM domain	[64]	9	19
OT_IL-4Stat	U16031	transcuces IL-4 signals	[65, 66]	9	18
STK_MAPKK5	U25265	activates Erk5/Bmk	[67-69]	9	7
YK_ITK	D13720	cytoplasmic tyrosine kinase, specific for T-cells	[70, 71]	8	46
STK_MSK2	AF074393	activated by p38 and Erk1/2 Map-Kinases	[72]	8	30
STK_PIM-2h	U77735	upregulated by NFκB	[73, 74]	8	18
STK_CKII-alpha	J02853	activated by phosphorylation of relA/p65	[75]	8	17
YK_Csk	X59932	phosphorylates and inhibits src-kinases	[76, 77]	8	15
STK_IKKgamma	AF074382	part of the IkappaB-Kinase complex that activates NFκB	[78]	8	13

TABLE 5 (4/7)

Gene	Genbank Nr.	Description	Reference	Number of Clones with reduced expression	% under cut off for reduced expression
STK_Ndr	Z35102	nuclear phosphatase, activated by Calcium	[79, 80]	20	59
STK_ERK3	X60692	constitutively nuclear MAP-Kinase	[81, 82]	19	159
OT_Topoisomerase2	NM001068	topoisomerase-2 inhibitors are used as chemotherapeutica against cancer	[83]	19	143
YP_AZP-ISredacidphosphatase	M83653	cytoplasmic phosphotyrosyl protein phosphatase	[84]	19	92
STP_PP1-Cbeta	X80910	catalytic subunit of PP1, activated by ceramid	[85, 86]	19	45
OT_PCNA	4505640	Proliferating Cellular Nuclear Antigen	[87]	19	27
YP_TC-PTP	M25393	localized to ER and nucleus, inhibits PI3K signals after EGF stimulation	[88, 89]	19	155
STK_CHK1	AF016582	after DNA damage necessary for cell cycle halt at G2/M, phosphorylates wee1 and cdc25	[90-92]	18	104
STK_AMP-activatedproteinkinasealpha1subunit	AF100763	phosphorylates and deactivates Acetyl-CoA Carboxylase	[93]	18	81
PP_YVH1	AF119226	dual-specific phosphatase	[94]	18	72
STK_WEE1	X62048	inhibits G2/M progression, phosphorylates and inhibits cdc2 2	[95-97]	18	73
STK_CKI-alpha	X80693	part of the Wnt pathway, phosphorylates and inhibits nuclear transport of NF-AT4	[98, 99]	18	49

TABLE 5 (5/7)

STK_NEK3	Z29057	Homologous to NIMA kinase of *Aspergillus Nidulans*, which is responsible for G2/M progression	[100]	17		234
STK_MAD-3likePK	AF068760			17		104
STK_TAK1	U64205	cdc25 associated kinase, phosphorylates Cdc25c	[101]	17		53
UP_PB-32	W30715	unknown phosphatase		16		178
STK_HsCdc7	AF015592	important for G1/S progression	[102]	16		138
STK_SRPK-2	U88666	phosphorylates SR-Splice-factors	[103, 104]	16		70
STK_MAPKK6	U39657	activates p38 MAP-kinase, activated by Ask-1 a MAPKKK that induces apoptosis	[105, 106]	16		54
STK_GCK	U07349	homologous to *S. cerevisiae* Ste20, activates JNK	[107]	16		324
STK_KIAA0619	AB014519	unknown kinase		16		239
STK_PHK-beta	X84908	phosphorylates Glycogen-Phosphorylase	[108]	15		246
OT_33a_Enx-1	AF070418	regulates expression of Homeobox-genes	[109, 110]	15		187
STK_Bub1	AF046078	controls segregation of chromatids, mutation in cancer causes increased mutation rate	[111, 112]	15		121
STK_NEK2	U11050	associates with centrosomes	[113, 114]	15		37
STK_PK428	U59305	related to family of myotonic dystrophy kinases	[115]	15		237
STK_KHS	U77129	homologous to *S. cerevisiae* Ste20, activates JNK	[116]	15		121
PP_PIR1	AF023917	dual specific, nuclear, dephosphorylates RNA, associated with speckles	[117, 118]	15		51

TABLE 5 (6/7)

STP_PP6	X92972	homologous to S. carevisiae Sit4p and S. pombe ppe1, which regulate the cell cycle	[119]	15		30
STK_MNB	U52373	dual specific, homologous to DYRK kinase, located in region of chromosome 21 that is amplified in Down-Syndrom	[120, 121]	14		90
STK_VRK1	AB000449	homologous to Vaccinia Virus Kinase, nuclear	[122, 123]	14		286
STK_CHED	M80629	Homolog of cdc-2	[124]	14		75
STK_TTK	M86699	dual specific, expression correlates with cell cycle	[125, 126]	13		465
UK_PB-11	AF061944	unknown kinase		13		168
STP_PP2A-Cbeta	X12656	nuclear, dephosphorylates Bcl-2	[127, 128]	13		33
UK_UK20	NM_016507	unknown kinase		13		282
STK_GLK	AF000145	homologous to *S. cerevisiae* Ste20, activates JNK	[107]	13		140
STK_26b_CDC2_1_4	X05360	essential for G2/M progression	[96, 129, 130]	12		168
YP_Pri-1	U48297	may influence cell growth, nuclear but also associated with plasma membranes and endosomes	[131-133]	12		74
STK_cyclinK	AF060515	can regulate cdk-activity and transcription by RNA-polymeerase II	[134]	12		31
STK_PHK-alphaL	X80497	subunit of phosphorylase kinase	[135]	12		24
STK_p70S6K	M60724	activated via PI3Kinase	[136-138]	11		824
YK_Yes	4885660	belongs to family of src-kinases	[139, 140]	11		661
STK_CaM-KIIdelta	U73504	highly expressed in brain	[141]	11		216

TABLE 5 (7/7)

YK_Ryk	X69970	receptor tyrosine kinase, doesn't belong to any known family of receptor tyrosine kineses, probably involved in Eph-signalling	[142, 143]	11	166
YP_PRL-3	AF041434	homologous to PRL-1	[131]	11	74
STP_PP1-Cgamma	X74008	catalytic subunit of PP1	[144]	11	59

Claims

1. A method for identifying nucleic acid molecules functionally associated with a desired phenotype comprising the steps:
   (a) providing a population of parental cells wherein said cell population substantially lacks the desired phenotype,
   (b) optionally subjecting said cell population to a procedure resulting in a rearrangement and/or mutation of the cell genome,
   (c) subjecting said cell population from (b) to a selection procedure for the desired phenotype,
   (d) identifying and optionally characterizing cells exhibiting said desired phenotype,
   (e) obtaining protein and/or mRNA from cells exhibiting said desired phenotype,
   (f) determining gene expression in cells exhibiting said desired phenotype and
   (g) comparing gene expression in cells exhibiting said desired phenotype with gene expression in cells substantially lacking the desired phenotype.

2. The method of claim 1 wherein the desired phenotype is selected from cancer cell properties.

3. The method of claim 2 wherein the cancer cell properties are selected from invasiveness, metastasis, loss of contact inhibition, loss of extracellular matrix requirement, growth factor independence, angiogenesis induction, immuno defense evasion, anti-apoptosis and/or increased levels of tumor markers.

4. The method of claim 2 wherein the desired phenotype is anti-apoptosis.

5. The method of claim 1 wherein the desired phenotpye is selected from production of secreted protein, susceptibility or resistance to pathogens, senescene, regulation of cell functions and modification of signal transduction pathways.

6. The method of any one of claims 1-5 wherein a parental cell is selected which is continuously in a process of genome rearrangement and mutagenesis.

7. The method of claim 6 wherein the parental cell is an immortalized or transformed cell.

8. The method of any one of claims 1-5 wherein a parental cell is selected which has a substantially stable genome.

9. The method of claim 8 wherein step (b) comprises a mutagenesis procedure.

10. The method of claim 9 wherein said mutagenesis procedure is selected from irradiation, chemical mutagenesis and combinations thereof.

11. The method of any one of claims 1-10 wherein step (d) comprises a cell sorting procedure.

12. The method of claim 11 wherein said cell sorting procedure is a Fluorescence Activated Cell Sorting Procedure (FACS).

13. The method of any one of claims 1-12 comprising obtaining mRNA in step (e) and hybridizing said mRNA or a nucleic acid made therefrom with a nucleic acid array.

14. The method of claim 13 wherein the nucleic acid made from mRNA is selected from the group consisting of cDNA and cRNA.

15. The method of any one of claims 13-14 wherein said nucleic acid array comprises a solid carrier having immobilized thereto a plurality of different nucleic acid molecules.

16. The method of any one of claims 13-15 wherein said nucleic acid array is selected from arrays of genomic DNA arrays, cDNA arrays and oligonucleotide arrays.

17. The method of any one of claims 13-16 wherein said nucleic acid array comprises nucleic acids encoding functional cellular polypeptides or portions thereof selected from kinases, phosphatases, enzymes and receptors.

18. The method of any one of claims 1-12 comprising obtaining protein in step (e) and analyzing the protein content in cells exhibiting the desired phenotype.

19. The method of claim 18 wherein said analyzing comprises 2D gel electrophoresis, mass spectrometry and/or binding to protein arrays.

20. The method of claim 18 or 19 wherein before analyzing a pretreatment step in order to reduce the complexity of the protein mixture is carried out.

21. The method of any one of claims 1-20 further comprising the identification of a plurality of genes (gene cluster) which is associated with the desired phenotype.

22. The method of any one of claims 1-21 further comprising a validation step wherein the association of a defined gene or gene cluster with the desired phenotype is determined.

23. The method of claim 22 wherein the validation step comprises generating of dominant-negative mutants.

24. The method of any one of claims 1-23 further comprising a screening procedure wherein the activity of a test substance for a defined gene or gene cluster associated with the desired phenotype is determined.

25. Use of the method of any one of claims 1-24 for generating expression profiles of genes or gene clusters associated with a desired phenotype.

26. The use of claim 25 wherein the expression profile is compared with the expression profile in a biological sample.

27. The use of claim 26 wherein the sample is derived from a human patient.

28. Use of the nucleic acid shown in Table 1, Table 2 and Table 5 or fragments thereof or peptides or polypeptides encoded by said nucleic acids or fragments as targets.

29. The use of claim 28 for diagnostic applications.

30. The use of claim 28 for therapeutic applications.

31. The use of claim 28 for a screening procedure to identify novel drugs.

Figure 1

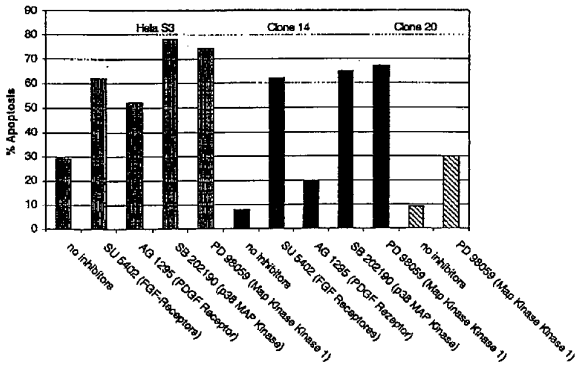

% Apoptosis
Hela S3
Clone 14
Clone 20
no inhibitore
SU 5402 (FGF-Receptore)
AG 1295 (PDGF-Receptor)
SB 202190 (p38 MAP Kinase)
PD 98059 (Map Kinase Kinase 1)
no inhibitore
SU 5402 (FGF Receptores)
AG 1295 (PDGF Rezeptor)
SB 202190 (p38 MAP Kinase)
PD 98059 (Map Kinase Kinase 1)
no inhibitore
PD 98059 (Map Kinase Kinase 1)

Figure 2

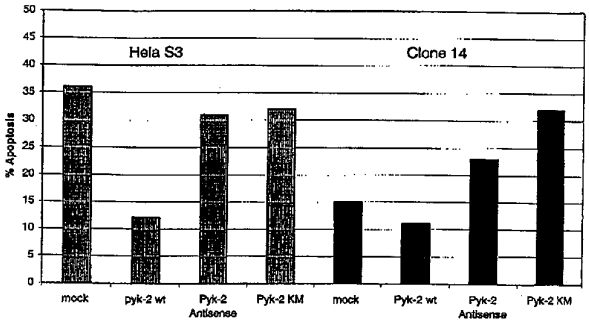

Hela S3
Clone 14
% Apoptosis
0
5
10
15
20
25
30
35
40
45
50
mock
pyk-2 wt
Pyk-2 Antisense
Pyk-2 KM
mock
Pyk-2 wt
Pyk-2 Antisense
Pyk-2 KM

FIGURE 3

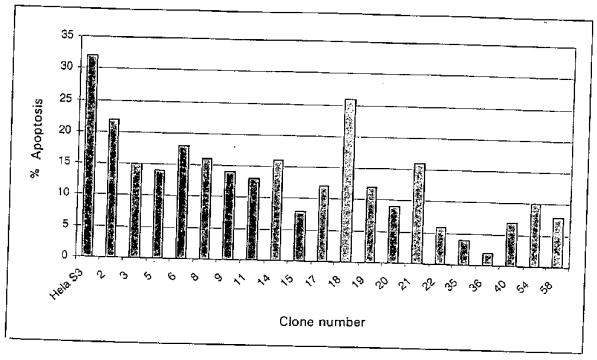
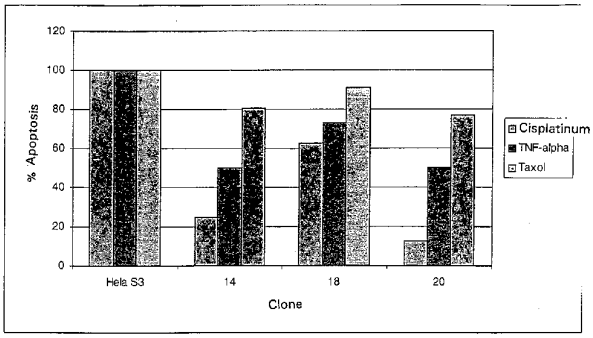

% Apoptosis
35
30
25
20
15
10
5
0
Hela S3
2
3
5
6
8
9
11
14
15
17
18
19
20
21
22
35
36
40
54
58
Clone number

FIGURE 4


120
100
80
60
40
20
0
% Apoptosis
Hela S3
14
18
20
Clone
Cisplatinum
TNF-alpha
Taxol

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(54) Title: METHOD FOR IDENTIFYING FUNCTIONAL NUCLEIC ACIDS

(57) Abstract: The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

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(54) Title: METHOD FOR IDENTIFYING FUNCTIONAL NUCLEIC ACIDS

(57) Abstract: The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

Method for identifying functional nucleic acids

Description

The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

A lot of information has been gathered about the execution apparatus of apoptosis (Hengartner, Nature 407 (2000), 770-776). But data on signals that control the initiation of apoptosis have only recently begun to be accumulated (Rich et al., Nature 407 (2000), 777-783). Previous methods for identifying apoptosis-associated genes or genes associated with other specific phenotypes are tedious. For example, Hudziak et al. (Cell Growth and Differentiaton 129 (1990), 129-134) describe a selection procedure for transformation and met protoonco gene amplification in NIH 3T3 fibroblasts using tumor necrosis factor-α. It is suggested that this method may be used for identifying other gene products, including other tyrosine kinases, associated with aggressive tumor growth. A fast or reliable procedure for identifying such genes is, however, not provided.

According to the present invention a novel method for identifying functional nucleic acid molecules is provided. This method is based on a genome evolution concept and therefore involves mutagenesis and/or genome arrangement steps followed by selection of cell clones displaying the desired phenotype. Subsequent transcriptome analysis in conjunction with bioinformatics-directed gene sorting allows not only comprehensive identification of genes that are critical for the selected cell characteristic, but even entire signalling pathways that govern a given cellular phenotype. This method can be employed towards a wide variety of cell characteristics for which a selection procedure is available.

Thus, a subject matter of the present invention is a method for identifying nucleic acid molecules functionally associated with a desired phenotype comprising the steps:

(a) providing a population of parental cells wherein said cell population substantially lacks the desired phenotype,

(b) optionally subjecting said cell population to a procedure resulting in a rearrangement and/or mutation of the cell genome,

(c) subjecting said cell population from (b) to a selection procedure for the desired phenotype,

(d) identifying and optionally characterizing cells exhibiting said desired phenotype,

(e) obtaining protein and/or mRNA from cells exhibiting said desired phenotype,

(f) determining gene expression in cells exhibiting said desired phenotype and

(g) comparing gene expression in cells exhibiting said desired phenotype with gene expression in cells substantially lacking the desired phenotype.

In the method of the invention essentially any type of parental cells (e.g. cell lines or primary cells) can be used. Most important the cells should lack the desired selection characteristic or display it only weakly. Preferred examples of starting cells are eukaryotic cells, e.g. mammalian cells, particularly human cells.

In order to generate cells, preferably cell clones exhibiting the desired phenotype, the parental cell may be subjected to a procedure resulting in an arrangement and/or mutation of the cell genome. This step is an evolution procedure comprising an induction of the parental cell to undergo genomic rearrangements and/or mutagenesis. In case of transformed cells, e.g. tumor cells such as Hela or normal cells having a low threshold to instability, e.g. immortalized cells such as NIH 3T3 cells, no special

induction is necessary, since these cells are continuously in a process of genome rearrangement and mutagenesis. It is sufficient to expose the parental cell culture to selection conditions either in form of clones or subdivided cultures preferably in multiple well plates, e.g. 96 well microtiter plates, or when the selection involves lethal conditions, exposure of cell monolayers. It should be noted, however, that also parental cells may be used which have a substantially stable genome. These cells, however, require a specific induction in order to obtain the desired genomic rearragement and/or mutagenesis.

In a preferred embodiment step (b) of the method comprises a mutagenesis procedure. This mutagenesis procedure may be selected from irradiation, e.g. by UV or γ-irradiation, chemical mutagenesis, e.g. by treatment with N-methyl maleimide or ethyl maleimide, or combinations thereof.

After the rearrangement and/or mutation of the cell genome has been achieved, the cell population is subjected to a selection procedure for the desired phenotype. After selection, cells, e.g. individual cell clones exhibiting the desired phenotype are identified and optionally characterized. The identification may comprise a morphological determination and/or a cell sorting procedure, e.g. by a Fluorescence Activated Cell Sorting procedure (FACS). The cells may be expanded and subsequently the desired phenotype/property may be verified and/or quantified.

Subsequently, protein and/or mRNA from cells exhibiting the desired phenotype is obtained. This material may be used for determining gene expression in cells exhibiting the desired phenotype and comparing gene expression in said cells with gene expression in cells substantially lacking the desired phenotype.

In a preferred embodiment, mRNA from cells exhibiting the desired phenotype is obtained. The mRNA may be extracted from the selected

genetically modified cell clones and either used directly, or after conversion into another nucleic acid, e.g. cDNA or cRNA as a probe for hybridization with a nucleic acid array. The nucleic acid, e.g. mRNA, cDNA or cRNA, used for hybridization with the array will usually be labelled in order to determine site-specific hybridization on the array. The array may be a solid carrier, e.g. a filter, chip, slide etc. having immobilized thereto a plurality of different nucleic acid molecules on specified locations on the carrier. The nucleic acid array may be selected from genomic DNA arrays, cDNA arrays and oligonucleotide arrays. Preferably, an array is used which preferentially comprises nucleic acids encoding functional cellular polypeptides or portions thereof, more preferably selected from kinases, phosphatases, enzymes and receptors. Hybridization on the array as a measure of gene expression in the selected cell clones may be determined according to known methods, e.g. by image analyis using a phosphor imager. In some cases, the desired new property of the cell may be determined by a large scale high throughput assay analysis of e.g. the conditioned media of subdivided cultures.

In addition or alternatively to expression profiling by mRNA analysis a proteomics approach determining the differences in protein content of the identified clones compared to the parental cell line and the identified clones or their supernatants may be carried out by suitable methods, e.g. by 2D gel electrophoresis. Proteins that differ in their concentration in the parental cell line and the identified clones will show a differently stained spot in the 2D gel. Furthermore, protein modifications like phosphorylations can be detected by this method. Once can also perform a separation of the cellular proteins prior to the analysis step, in order to reduce the complexity of the protein mixture. For instance, column chromatographic steps could be carried out that purify kinases (by affinity chromatography using an ATP column) or glycosylated proteins (using a lectin column) which then can be further separated by 2D gel electrophoresis. Any other method for

6

analyzing differences on the protein level (protein chips, mass spectrometry) may also be utilized.

The gene expression results in cells exhibiting the desired phenotype will be compared with gene expression in cells substantially lacking the desired phenotype, preferably in the parental cells. Further, the gene expression results may be analyzed by a cluster detection program. This analysis will yield a plurality of possible changes in the expression of genes that confer the desired cell phenotype.

The application of the method of the invention is very broad and includes essentially all cell characteristics that can be selected for and/or which can be determined with an assay. For example, the desired phenotype may be selected from cancer cell properties such as invasiveness, metastasis, loss of contact inhibition, loss of extracellular matrix requirement, growth factor independence, angiogenesis induction, immuno defense evasion, anti-apoptosis and/or increased levels of tumor markers.

In an especially preferred embodiment the desired phenotype is anti-apoptosis. Another application is the elucidation of cancer related genes by sorting cancer cells for a known tumor marker. Often tumor markers are a consequence and not a cause of the tumorigenicity of cells and are therefore not amenable as drug targets. But since the correlation of the marker with a cancer phenotype is established, sorting cells for increased marker expression will also sort for the genes that are linked to the marker and cause the cancer phenotype. These genes can be identified by comparing the expression profiles in the parental cell line and the sorted cells and are potential drug targets.

Alternatively, the desired phenotype may be selected from other properties such as production of secreted protein, e.g. insulin, growth hormone, interferons etc., susceptibility or resistance to pathogens, e.g. viruses such

as HCV, HBV or other pathogens, senescence and regulation of cell functions, i.e. the identification of genes that regulate certain cell functions e.g. identification of negative regulators of insulin receptor activity comprising a screen for cell clones with upregulated insulin receptor activity.

A further preferred embodiment is the identification of components of signal transduction pathways in general, e.g. to sort for cells that are better capable of transmitting the respective signal. For instance, the identification of components of a signal transduction pathway of a Receptor Tyrosine Kinase (RTK), particularly of a receptor of the EGF-receptor family, such as EGFR, HER2 and HER3, can be carried out by generating a cell line that expresses a suitable reporter protein, such as Green Fluorescent Protein (GFP) under the control of a promoter that is responsive to stimulation by a ligand of the respective receptor (e.g. c-fos promoter for EGF stimulation etc.). Stimulation of the receptor by the ligand will then lead to transcription of GFP and an increased green fluorescence that can be detected, e.g. by a FACS machine. Sorting the cells that show the highest fluorescence induction will enrich for cells that respond stronger to a ligand-indicated signal than the parental cell population. Analyzing the expression patterns of both cell populations will identify the genes whose varying expressions are responsible for the different reaction to the signal and hence influence the signal transduction pathway. This strategy can be applied to any signal for which a fluorescent output can be generated.

In the following, the invention is described in more detail with reference to the identification of anti-apoptotic nucleic acids using a cDNA array. It should be noted, however, that this embodiment is only illustrative for the method of the invention and should not be construed as limitation.

In order to identify nucleic acids which are associated with the regulation of apoptosis the method of the invention was used for the identification of genes, which are differentially expressed in apoptosis-sensitive and apoptosis-resistant cells.

Apoptosis was induced in the human cervix carcinoma cell line Hela S3 by Fas activation. Activation of Fas results in an autocatalytic activation of caspase-8 and thus to apoptosis. For Fas activation the parental cells were incubated with an anti-Fas antibody.

After the selection procedure only a low amount of living cells were present. These cells had a higher resistance against apoptosis than the parental cell line. The surviving cells were clonally expanded. mRNA was isolated from the clones and the parental cell line, which was subsequently reversed, transcribed into cDNA. Then cDNA arrays were hybridized with the cDNA from the clones and the parental cell line and thus the gene expression on the array determined. The sequences on the arrays were derived from about 1000 genes which preferentially encode kinases and phosphatases. By means of a comparison between the expression and the parental cell line and the expression and the clones, about 200 genes were identified which exhibited enhanced expression (an increase by more than the factor 2) in at least 10% of the clones. These are nucleic acids which are associated with the apoptosis resistance of the clones (Tables 1 and 2). Table 1 is a listing of genes which are induced in the apoptosis-resistant clones and have not yet been linked to an anti-apoptosis function. Table 2 is a listing of genes that are induced in apoptosis-resistant clones with previously known anti-apoptotic function.

An improved method for the identification of genes, which are differentially expressed in the parental cell line, e.g. Hela S3, and the clones having a desired phenotype, e.g. apoptosis-resistant clones, an evaluation procedure as described in Example 2, may be applied. For each nucleic acid analysed

in the parental cell line, a plurality of measured values is determined from which an average value and a standard deviation may be calculated. For example, RNA may be isolated at least twice from the parental cell line in at least two independent preparations. Material from each preparation is used for hybridization with at least two nucleic acid arrays. The average of those values for a given spot on the array is calculated and the standard deviation determined. Material from the desired clone is hybridized with one nucleic acid array. A gene is considered to be differentially expressed in the desired clone when its value exceeds a predetermined cut-off. The cut-off for upregulated genes is preferably the average of the respective values of the parental cell line plus two times standard deviation. The cut-off for down-regulated genes is preferably the average of the respective parental cell line values minus two times standard deviations. Using this procedure it is possible to correct errors inherent in the experimental procedure. Since those errors made during the preparation of the nucleic acid arrays will determine the standard deviation, any value of the desired clone that lies outside the standard deviation marks a differentially expressed gene. Therefore, it is possible to detect also small differences in gene expression that may not be detected by using an arbitrary cut-off. The values obtained by this improved evaluation procedure are depicted in Table 5.

Thus, a subject matter of the present invention is the use of nucleic acids as depicted in Table 1, Table 2, and Table 5 preferably in Table 1 and Table 5, and polypeptides encoded by these nucleic acids as "targets" for diagnostic and therapeutic applications, particularly for disorders which are associated with dysfunctions of apoptotic processes such as tumors. Further, the nucleic acids and the gene products are suitable as targets in screening procedures for identifying novel modulators of apoptotic/anti-apoptotic procedures, particularly drugs. The drugs may be biomolecules such as antibodies directed against the gene products, enzyme inhibitors or low molecular non-biological drugs. Methods of drug screening comprise

cellular based systems wherein usually a cell overexpressing the target nucleic acid of interest is used or molecular based systems wherein the polypeptide of interest in used in a partially purified or substantially purified and isolated form. Particular screening methods are known to the skilled person and need not be described in detail here. It should be noted, however, that also high throughput screening assays may be used.

Further, several groups or clusters of genes were identified whose expression patterns across the cell lines are similar. Clusters of apoptosis-resistant clones are depicted in Table 3. Clusters in squamous cell carcinoma cell lines are depicted in Table 4. The identification of such clusters allows the use of specific combinations of active agents in diagnostic and/or therapeutical applications as well as in screening methods. Thus, according to a preferred embodiment of the invention combinations of agents capable of modulating the presence and/or activity of several targets within a cluster may be used in order to multiply the efficacy.

Furthermore, the method of the present invention allows the generation of expression profiles of genes and particularly gene clusters associated with a desired phenotype. These expression profiles may be compared with the expression profile in a specific biological sample, which may be a body fluid or a tissue sample derived from a patient, e.g. a human, particularly a tumor patient. The comparison of the expression profile obtained by the method of the present invention with the expression profile in the biological samples allows the development of improved diagnostic, monitoring and/or therapeutic strategies which are specifically adapted to the individual patient.

In experiments it was demonstrated that an inhibition of the catalytic activity of proteins having an increased expression in the clones resulted in an enhanced increase of apoptosis. Also in the parental cell line the

inhibition resulted in an increased apoptosis. This outlines the importance of the identified nucleic acids and proteins for the apoptosis resistance of the clones and demonstrates the inhibition specifity.

Further, the invention is described in more detail in the following examples and figures.

Figure 1 shows the inhibition of upregulated kinases.

Cells were grown in Ham's F12 medium without FCS and treated with 100 ng/ml anti-Fas antibody CH-11 with and without inhibitors. Apoptosis was measured by FACS analysis as described in the examples. SU 5402: 10 μM, AG 1295: 1 μM, SB 203580: 10 μM, PD 98059: 25 μM.

Figure 2 shows the inhibition of pyk-2 by a dominant negative mutant and an antisense construct.

Figure 3 shows the apoptosis sensitivity of clones. 70% confluent cells were starved for 24h in medium without FCS and subsequently 100 ng/ml CH-11 was added. After a 16h incubation the cell nuclei were stained in hypotonic buffer and analysed by FACS. The percentage of the sub-G1-peak was deduced. The apoptotic rate without FCS was subtracted from the rate with FCS.

Figure 4 shows the apoptosis sensitivity with other apoptosis inducers. 70% confluent cells were starved for 24 h in medium without FCS and subsequently 10 μg/ml Cisplatinum or TNF-α plus 0.1 μg/ml Cycloheximide was added to the cells. After 16 h the cell nuclei were stained with propidium iodide and analysed by FACS. 50 nM Taxol was added to the cells for 3 h and the medium subsequently replaced by fresh medium with 10% FCS. 2 days later the percentage of sub-G1 cells was deduced. The

apoptotic rate without FCS was subtracted from the rate with FCS. The values are expressed as the percentage of the respective Hela S3 value.

Viral supernatant was produced using Phoenix A packaging cell line and the respective cloned constructs (expressing pyk-2 wild-type or pyk-2 KM mutant) cloned in the vector pLXSN. Hela S3 and clone 14 were infected over night. Medium was changed the next day and two days later cells were starved for 24 hours in medium without FCS before adding 100 ng/ml CH-11 over night. Apoptosis was measured as described in Fig. 1.

Example 1

1. Materials and Methods

1.1 Selection of Apoptosis-Resistant Clones

The cervix carcinoma cell line Hela S3 (ATCC CCL-2.2) was plated on 10 cm cell culture dishes (10^6 cells) in Ham's F12 growth medium containing 10% FCS. On the next day the medium was exchanged against medium without FCS supplemented with 100 ng/ml apoptosis activating anti-Fas antibody CH-11 (Coulter Immunotech). After 3 days when most of the cells were dead, the medium was exchanged once more against the medium containing 10% FCS without antibody. The surviving cells were clonally cultivated for 3 weeks. The clones were picked and expanded.

1.2 Apoptosis Assay

50000 cells per well obtained from the parental cell line Hela S3 or from the clones, respectively, were grown in a 12 well cell culture dish for 2 days in 2 ml Ham's F12 medium containing 10% FCS. On the third day the cells were washed twice with 1 ml Ham's F12 medium and then the medium exchanged against 1 ml Ham's F12 medium. On the next day the medium was supplemented with the respective inhibitors and 100 to 200 ng/ml CH-11. On the next day the medium was decanted and transferred

to an Eppendorf tube. The cells were washed once with 200 µl PBS, the PBS was transferred to the respective Eppendorf tube. Then the remaining cells were also transferred to the respective Eppendorf tube after treatment with EDTA/trypsin in PBS. The cells were pelleted by centrifugation, suspended in 500 µl hypotonic buffer (0.1% sodium citrate, 0.1% Triton-X100, 20 µg/ml propidium iodide) and incubated for 2-24 hours at 4°C. The resutling cell nuclei were analyzed by FACS.

1.3 FACS (Fluorescence Activated Cell Sorting) - Analysis for Determining Apoptotic Nuclei

The propidium iodide fluorescence of single nuclei was determined using a FACSCalibur (Becton Dickinson) cytometer. The forward scatter light (FSC) and the side scatter light (SSC) were recorded simultaneously. The FSC peak was adjusted at channel 500 in a 1024 channel linear scale and the red fluorescence peak at channel 200 of a logarithmic scale. The FSC cut-off value was determined by gating to 95% of the greatest nuclei of a negative control without supplements. Nuclei were classified as apoptotic when a subdiploid signal between the G1/G0 peak and channel 10 was present.

1.4 Preparation of cDNA

Total RNA was isolated by lysing of cells with guanidinium isothiocyanate and subsequent extraction with acid phenol (Current Protocols in Molecular Biology). mRNA was isolated by binding to oligo-dT cellulose according to standard methods (Current Protocols in Molecular Biology).

cDNA was synthesized from mRNA by reverse transcription using Cap-finder primer K1 and K2 (Clontech Inc., USA) and AMV-reverse transcriptase (Roche Diagnostics) and purified using the PCR purification kit (Qiagen). From 3 µg mRNA 50 µl cDNA consisting of one strand DNA and one strand RNA were obtained.

1.5 Preparation of cDNA Arrays

cDNAs cloned in p-Bluescript were spotted with a BioGrid spotter (BioRobotics, UK) on nylon membranes. 250 ng DNA were used per spot. For about one half of the genes two or more probes were used and each probe was spotted twice. The following designations were used:

YK = tyrosine kinase
STK = serin/threonin kinase
PP = phosphatase
Lig = ligand
UK = unknown kinase
UP = unknown phosphatase
OT = other

Example:
YK_1b_Abl_2 = tyrosine kinase 1, probe b, spot 2

1.6 Radioactive Labelling of cDNA

5 µl cDNA were labelled with 50 µ Ci α^{33}P-ATP using the Megaprime Labelling Kit (Amersham Pharmacia) and purified using the PCR purification kit (Qiagen). The thus obtained cDNA was hybridized with COT-DNA (Roche Diagnostics) in order to block repetitive sequences which might bind unspecifically to the cDNA array.

1.7 Hybridization of cDNA Arrays

The cDNA arrays were prehybridized for 4 hours or over night at 68°C in prehybridization solution (50 x Denhardt, 10 x SSC, 0.25 M Na_3PO_4, pH 6.8, 50 mM $Na_4P_2O_7$, 0.1 mg/ml tRNA (bakers's yeast, Roche Diagnostics)).

Subsequently the cDNA arrays were hybridized for 16 hours with the labelled cDNA in hybridization buffer (5 x SSC, 0.1% SDS, 0.1 mg/ml tRNA). The cDNA arrays were washed as follows:

2 x 20 min W1 (2 x SSC, 0.1% SDS) at 42°C
1 x 20 min W2 (0.2 x SSC, 0.1% SDS) at 42°C
1 x 60 min W2 at 65°C

The cDNA arrays were exposed for 48 hours on Phosphoimager plates (Fujifilm) and subsequently analyzed on a Phosphoimager (Bas-2500, Fujifilm).

1.8 Analysis of cDNA Arrays

The spot volume on the filter was determined using ArrayVision software (V 5.1, Imaging Research Inc.). All further calculations were carried out in Excel (Microsoft Corp.).

For better internal comparison of the cDNA arrays a normalization procedure was carried out as follows: From each spot on the array the background (average of p-Bluescript values of an array) was subtracted and divided by the sum of all spot volumina in the array. The thus obtained value was multiplied by 10000.

For the identification of genes which are differentially expressed in the parental cell line Hela S3 and the apoptosis-resistant clones, the quotient from the values of the clones and the average value of the different arrays of the parental cell line (reference arrays) was calculated. All normalized values smaller than 0.1 were set to 0.1 for the calculation. 90% of all values different from 0 were above this value. The respective gene was defined as differentially expressed, if the percentage differs by at least 100%. Only such genes were analyzed wherein the deviation of the values

on the reference arrays for the respective spot on the array was sufficiently small. The following filters were used for sorting out these genes:

If the values of the reference arrays and of the respective clone for a spot were smaller than 2.5, the deviation of the reference arrays from each other must be in the range from 0.2 to 5.

If the values of the reference arrays were smaller than 2.5 and that of the clone greater than 2.5 or vice versa, the deviation of the reference arrays from each other has to be in the range from 0.3 to 3.

If both the values of the reference arrays and of the clone were greater than 2.5, the deviation of the reference arrays from each other has to be in the range from 0.5 to 2.

1.9 Gene Clustering

For gene clustering the Program Cluster (Michael Eisen, Stanford University) was used. The quotients from the values of the clones and the average value of the respective arrays of the parental cell lines were used. Spots exhibiting high deviations in the values on the reference arrays were excluded. For this purpose the filters were used which had already been applied in the identification of induced genes. From 1922 spots 1451 remained. These values were logarithmically transferred to clusters and further filtered on spots wherein the value of at least 80% of the clones was different from 0. The thus resulting 520 spots were analyzed via an hierarchical cluster algorithm.

The overall similarity of the expression patterns and the cluster mirrors in the correlation coefficient which has a value between 1 and -1. A correlation coefficient of 1 means the expression patterns are identical, 0 means that they are completely independent and -1 the opposite of each other.

2. Results

2.1 Apoptosis-Resistant Clones are Obtained by Selection of Hela S3 Using CH-11 Antibody

40 clones were obtained after selection with CH-11 antibody. 20 of these clones were tested in view of their sensitivity to CH-11. The degree to which the clones are resistant differs between individual clones, but none of them is completely resistant to apoptosis suggesting that the apoptosis machinery is functional. The clones are also refractive to apoptosis induced by TNF-α and cisplatin.

2.2 Numerous Genes Show Enhanced Expression in Apoptosis-Resistant Clones

Tables 1 and 2 show listings of genes which show enhanced expression in apoptosis-resistant clones. Further, the Genbank Accession numbers of the respective clones, the number of clones in which expression exceeds cut-off for increased expression and the average percentage over cut-off is given.

Most of the analyzed genes encode protein phosphatases and kinases, i.e. enzymes which are important for cell regulation.

From the thus determined induced clones several have not yet been associated with apoptosis and/or tumorogenesis (Table 1). Other genes such as CAMKK (calmodulin dependent kinase kinase), EGFR (epidermal growth factor receptor), Bcr (breakpoint cluster region), FGFR-1 (fibroblast growth factor receptor 1), Nik (NFκB-interacting kinase) and DAPK (death-associated protein kinase) are already known as apoptosis-associated genes.

2.3 Gene Clustering Shows Groups of Genes Which are Commonly Regulated

By clustering of expression data groups of genes were found which are commonly up- or downregulated. The common regulation suggests a common function of the genes. Thus not only single apoptosis-modulating genes, but also signal transduction cascades consisting of a plurality of genes are found. The clusters identified in apoptosis-resistant clones are shown in Table 3. The clustering of the genes allows to group the upregulated genes and deduce different anti-apoptotic signalling pathways instead of single genes only. The clusters that were found in the apoptosis-resistant clones could also be partially found in expression data of squamous cell carcinoma cell lines (Table 4). That suggests that by the screen physiologically relevant apoptosis clusters can be found that are important for tumor development and hence could serve as drug targets.

Cluster 1 contains some genes induced in many clones such as CAMKK, UK11 (unknown kinase 11), PTP α (protein tyrosine phosphatase α) and PRK (proliferation related kinase).

Cluster 2 contains 3 genes exhibiting a highly correlated expression, namely serin/threonin phosphatase VH2, TIMP (tissue inhibitor of metalloproteinase 1) and MMP-15 (matrix metalloproteinase 15). Interestingly, an enzyme (MMP-15) and a potential inhibitor (TIMP-1) are commonly regulated.

Cluster 3 comprises inter alia the membrane bound tyrosine phosphatase Lar and the proapoptotic serin/theronin kinase DAP kinase.

In cluster 4 BCR, a potential inhibitor of p38 and the JNK signal pathways, and an activator of p38, namely MAPKK-3 (mitogen activated kinase kinase 3) are commonly regulated.

2.4 Inhibition of the Induced Genes Enhances Apoptosis

In order to show that the induced genes are in fact modulators of apoptosis selected enzymes were inhibited by specific inhibitors and apoptosis was induced. Inhibitors for the following enzymes were used:

- SU 5402 inhibits FGF receptors, but is not specific for a defined FGF receptor
- AG 1295 inhibits the PDGF receptor
- SB 203580 inhibits the p38 MAP kinase

PD 98059 inhibits the MAP kinase kinase 1, which in turn activates the MAP kinases ERK1 and ERK2. This inhibitor was used as control for SB 203580, because SB 203580 also partially inhibits ERK1 and ERK2. Furthermore, ERK2 shows an enhanced expression in the clones. The results for Hela S3, clone 14 and clone 20 (partially) are shown in Fig. 1.

It was found that an inhibition of FGF receptors in Hela S3 cells leads to an increase in apoptosis of about 50%. In clones 14 and 5 SU 5402 leads to an increase of nearly 300% or 50%, respectively. Thus, in a clone having an increased expression of two FGF receptors (clone 14, FGFR-1 and FGFR-3) an inhibition of FGF receptors leads to an enhanced increase of apoptosis. In clone 5, which does not show any enhanced expression of FGF receptors, the increase in apoptosis is comparable to the parental cell line Hela S3.

An inhibition of the PDGF receptor leads to an increase of about 30% in Hela S3. In clone 14, which shows enhanced expression of PDGF receptor, the inhibition results nearly in a doubling of the number of apoptotic cells. In contrast thereto, clone 5, which does not contain any detectable PDGF receptor, exhibits only 30% increase in apoptosis after treatment with AG 1295.

The p38 MAP kinase was inhibited because BCR, an inhibitor of the p38 MAP kinase signal pathway, and MAPKK-3 (MEK-3), which is a p38 activator, exhibited an enhanced expression in the clones. Further, both genes are grouped in a cluster.

p38 inhibition in Hela S3 results in a 25% increase of apoptosis. In clone 14 exhibiting an enhanced MEK-3 expression, an inhibition of p38 leads to a 60% increase of apoptosis. In contrast thereto, an inhibition of MEK-1 results in a doubling of the apoptosis rate. The increase in apoptosis after inhibition of p38 compared to Hela S3 and the constant apoptosis after inhibition of MEK-1 might be explained by inhibition of ERK1/2 and additional inhibition of p38.

In clone 20, which expresses MEK-3 on a similar level as Hela S3, treatment with SB 203580 only leads to a slight increase of apoptosis. In contrast thereto, treatment with PD 98059 triples the apoptosis rate. Thus, SB 203580 acts specifically in this system and the differences in the increase of apoptosis after inhibition of p38 correlate with the expression of the p38 activator MEK-3.

These inhibition experiments demonstrate conclusively that the method of the invention for identifying apoptosis-associated genes is efficient.

2.5 Inhibition by Introducing a Dominant Negative Mutant or an Antisense Strand

The respective enzymes upregulated in apoptosis-resistant clones can also be inhibited by introducing a dominant negative mutant or the antisense strand. Figure 2 shows that - as as example - the wild-type pyk-2 confers increased resistance when introduced in Hela S3. In clone 14 with a higher expression of pyk-2 introduction of the wild-type enzyme has no effect but the mutant with the lysine mutated to methionine (pyk-2 KM) in the

reactive center of the enzyme reverts the phenotype of the clone. The antisense construct has a corresponding but weaker effect.

Example 2

The experimental procedure was carried out as described in Example 1.

For the identification of genes differentially expressed in the parental cell line Hela S3 and the apoptosis resistant clones, the following evaluation procedure was applied: For each spot on the cDNA arrays of the parental cell line Hela S3 four values were determined in the following manner. RNA was isolated twice from Hela S3 in two independent preparations. Each RNA preparation was used to synthesize cDNA and each cDNA was hybridized with two cDNA arrays. The average of those 4 values for a given spot on the cDNA array was calculated and the standard deviation determined. The cDNA of each apoptosis resistant clone was hybridized with one cDNA array. A gene was considered to be differentially expressed in the apoptosis resistant clones when its value exceeded the following cut offs. The cut off for upregulated genes was the average of the respective Hela S3 values plus two times standard deviation. Accordingly, the cut off for downregulated genes was the average of the respective Hela S3 values minus two times standard deviation. The magnitude of the up-or downregulation was expressed as percent over/under the cut off. For example, a value of 100% over the cut off for upregulated genes means a 2-fold induction compared to the cut off, and a value of 100% under the cut off for downregulated genes means a bisection of that value in the resistant clones.

For gene clustering the program Cluster (Michael Eisen, Stanford University) may be used. The normalized values of the four reference arrays and the array of the 20 apoptosis resistant clones were used. Genes with a value greater than 1 in at least 20 of the 24 investigated arrays were filtered out and employed for the following calculations. The cut off

For gene clustering the program Cluster (Michael Eisen, Stanford University) may be used. The normalized values of the four reference arrays and the array of the 20 apoptosis resistant clones were used. Genes with a value greater than 1 in at least 20 of the 24 investigated arrays were filtered out and employed for the following calculations. The cut off of 1 was utilized in order to avoid clustering of genes whose value was so close to the background that a clustering would be unreliable. Thus, out of 2400 spots, 520 remained that were analysed via a hierarchical cluster algorithm.

The results are shown in Table 5.

References:

1. Will, H. and B. Hinzmann, *cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment.* Eur J Biochem, 1995. 231(3): p. 602-8.
2. Butler, G.S., et al., *Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases.* Eur J Biochem, 1997. 244(2): p. 653-7.
3. Hu, M.C., et al., *Protein phosphatase X interacts with c-Rel and stimulates c-Rel/nuclear factor kappaB activity.* J Biol Chem, 1998. 273(50): p. 33561-5.
4. Kersh, G.J. and P.M. Allen, *Essential flexibility in the T-cell recognition of antigen.* Nature, 1996. 380(6574): p. 495-8.
5. Kohno, K., et al., *Amino acid sequence of mammalian elongation factor 2 deduced from the cDNA sequence: homology with GTP-binding proteins.* Proc Natl Acad Sci U S A, 1986. 83(14): p. 4978-82.
6. Geiger, B., *Intermediate filaments. Looking for a function.* Nature, 1987. 329(6138): p. 392-3.
7. Didsbury, J.R., Y.S. Ho, and R. Snyderman, *Human Gi protein alpha-subunit: deduction of amino acid structure from a cloned cDNA.* FEBS Lett, 1987. 211(2): p. 160-4.
8. Bokoch, G.M. and A.G. Gilman, *Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin.* Cell, 1984. 39(2 Pt 1): p. 301-8.
9. Sullivan, K.A., et al., *Inhibitory and stimulatory G proteins of adenylate cyclase: cDNA and amino acid sequences of the alpha chains.* Proc Natl Acad Sci U S A, 1986. 83(18): p. 6687-91.

10. Williams, N.G., H. Zhong, and K.P. Minneman, *Differential coupling of alpha1-, alpha2-, and beta-adrenergic receptors to mitogen-activated protein kinase pathways and differentiation in transfected PC12 cells.* J Biol Chem, 1998. 273(38): p. 24624-32.
11. Mukai, H. and Y. Ono, *A novel protein kinase with leucine zipper-like sequences: its catalytic domain is highly homologous to that of protein kinase C.* Biochem Biophys Res Commun, 1994. 199(2): p. 897-904.
12. Wick, M.J., et al., *Mechanism of phosphorylation of protein kinase BAkt by a constitutively active 3-phosphoinositide-dependent protein kinase-1.* J Biol Chem, 2000. 275(51): p. 40400-6.
13. Takahashi, M., et al., *Proteolytic activation of PKN by caspase-3 or related protease during apoptosis.* Proc Natl Acad Sci U S A, 1998. 95(20): p. 11566-71.
14. Watanabe, G., et al., *Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho.* Science, 1996. 271(5249): p. 645-8.
15. Mukai, H., et al., *Activation of PKN, a novel 120-kDa protein kinase with leucine zipper-like sequences, by unsaturated fatty acids and by limited proteolysis.* Biochem Biophys Res Commun, 1994. 204(1): p. 348-56.
16. Maru, Y. and O.N. Witte, *The BCR gene encodes a novel serine/threonine kinase activity within a single exon.* Cell, 1991. 67(3): p. 459-68.
17. Dieknann, D., et al., *Bcr encodes a GTPase-activating protein for p21rac.* Nature, 1991. 351(6325): p. 400-2.
18. Galaktionov, K. and D. Beach, *Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins.* Cell, 1991. 67(6): p. 1181-94.
19. Honda, R., et al., *Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase.* FEBS Lett, 1993. 318(3): p. 331-4.
20. Sebastian, B., A. Kakizuka, and T. Hunter, *Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15.* Proc Natl Acad Sci U S A, 1993. 90(8): p. 3521-4.
21. Miyata, H., et al., *Overexpression of CDC25B overrides radiation-induced G2-M arrest and results in increased apoptosis in esophageal cancer cells.* Cancer Res, 2001. 61(7): p. 3188-93.
22. Zuo, Z., et al., *Ser/Thr protein phosphatase type 5 (PP5) is a negative regulator of glucocorticoid receptor-mediated growth arrest.* Biochemistry, 1999. 38(28): p. 8849-57.
23. Silverstein, A.M., et al., *Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin.* J Biol Chem, 1997. 272(26): p. 16224-30.
24. Chen, M.X., et al., *A novel human protein serine/threonine phosphatase, which possesses four tetratricopeptide repeat motifs and localizes to the nucleus.* Embo J, 1994. 13(18): p. 4278-90.

25. Lumeng, C., et al., *Interactions between beta 2-syntrophin and a family of microtubule-associated serine/threonine kinases.* Nat Neurosci, 1999. 2(7): p. 611-7.
26. Walden, P.D. and N.J. Cowan, *A novel 205-kilodalton testis-specific serine/threonine protein kinase associated with microtubules of the spermatid manchette.* Mol Cell Biol, 1993. 13(12): p. 7625-35.
27. Yoshiyama, K., et al., *CD156 (human ADAM8): expression, primary amino acid sequence, and gene location.* Genomics, 1997. 41(1): p. 56-62.
28. Schlomann, U., et al., *Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration.* J Neurosci, 2000. 20(21): p. 7964-71.
29. Wang, Z., R. Juttermann, and P.D. Soloway, *TIMP-2 is required for efficient activation of proMMP-2 in vivo.* J Biol Chem, 2000. 275(34): p. 26411-5.
30. Zucker, S., et al., *Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP).* J Biol Chem, 1998. 273(2): p. 1216-22.
31. Hayakawa, T., et al., *Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2).* J Cell Sci, 1994. 107(Pt 9): p. 2373-9.
32. Wang, X.S., et al., *MAPKKK6, a novel mitogen-activated protein kinase kinase kinase, that associates with MAPKKK5.* Biochem Biophys Res Commun, 1998. 253(1): p. 33-7.
33. Kratzschmar, J., L. Lum, and C.P. Blobel, *Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence.* J Biol Chem, 1996. 271(9): p. 4593-6.
34. Nath, D., et al., *Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells.* J Cell Sci, 1999. 112(Pt 4): p. 579-87.
35. Knecht, R., et al., *Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck.* Cancer Res, 1999. 59(12): p. 2794-7.
36. Smits, V.A., et al., *Polo-like kinase-1 is a target of the DNA damage checkpoint.* Nat Cell Biol, 2000. 2(9): p. 672-6.
37. Toyoshima-Morimoto, F., et al., *Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase.* Nature, 2001. 410(6825): p. 215-20.
38. Schmucker, D. and S.L. Zipursky, *Signaling downstream of eph receptors and ephrin ligands.* Cell, 2001. 105(6): p. 701-4.
39. Miao, H., et al., *Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway.* Nat Cell Biol, 2001. 3(5): p. 527-30.
40. Miao, H., et al., *Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation.* Nat Cell Biol, 2000. 2(2): p. 62-9.
41. Welsh, M., et al., *Stb is a ubiquitously expressed Src homology 2 protein.* Oncogene, 1994. 9(1): p. 19-27.

42. Oishi, K., et al., *Identification and characterization of PKNbeta, a novel isoform of protein kinase PKN: expression and arachidonic acid dependency are different from those of PKNalpha.* Biochem Biophys Res Commun, 1999. 261(3): p. 808-14.
43. Gu, M., I. Warshawsky, and P.W. Majerus, *Cloning and expression of a cytosolic megakaryocyte protein-tyrosine-phosphatase with sequence homology to retinaldehyde-binding protein and yeast SEC14p.* Proc Natl Acad Sci U S A, 1992. 89(7): p. 2980-4.
44. Stebbins, C.E., W.G. Kaelin, Jr., and N.P. Pavletich, *Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function.* Science, 1999. 284(5413): p. 455-61.
45. Maxwell, P.H., et al., *The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis.* Nature, 1999. 399(6733): p. 271-5.
46. Kibel, A., et al., *Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C.* Science, 1995. 269(5229): p. 1444-6.
47. Sakano, S., et al., *Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase).* Oncogene, 1994. 9(4): p. 1155-61.
48. Rane, M.J., et al., *p38 Kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in human neutrophils.* J Biol Chem, 2001. 276(5): p. 3517-23.
49. Rouse, J., et al., *A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins.* Cell, 1994. 78(6): p. 1027-37.
50. Gurney, M.E., et al., *Neuroleukin: a lymphokine product of lectin-stimulated T cells.* Science, 1986. 234(4776): p. 574-81.
51. Faik, P., et al., *Mouse glucose-6-phosphate isomerase and neuroleukin have identical 3' sequences.* Nature, 1988. 332(6163): p. 455-7.
52. Chaput, M., et al., *The neurotrophic factor neuroleukin is 90% homologous with phosphohexose isomerase.* Nature, 1988. 332(6163): p. 454-5.
53. Gurney, M.E., et al., *Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and sensory neurons.* Science, 1986. 234(4776): p. 566-74.
54. Wang, H., et al., *Characterization of PCP-2, a novel receptor protein tyrosine phosphatase of the MAM domain family.* Oncogene, 1996. 12(12): p. 2555-62.
55. Frigerio, J.M., J.C. Dagorn, and J.L. Iovanna, *Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs.* Biochim Biophys Acta, 1995. 1262(1): p. 64-8.
56. Yan, H., et al., *A novel receptor tyrosine phosphatase-sigma that is highly expressed in the nervous system.* J Biol Chem, 1993. 268(33): p. 24880-6.
57. Elchebly, M., et al., *Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma.* Nat Genet, 1999. 21(3): p. 330-3.
58. Nagase, T., et al., *Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121-KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1.* DNA Res, 1995. 2(4): p. 167-74, 199-210.

59. Benovic, J.L., et al., *Beta-adrenergic receptor kinase: primary structure delineates a multigene family.* Science, 1989. 246(4927): p. 235-40.
60. Benovic, J.L., et al., *Purification and characterization of the beta-adrenergic receptor kinase.* J Biol Chem, 1987. 262(19): p. 9026-32.
61. Colamonici, O.R., et al., *p135tyk2, an interferon-alpha-activated tyrosine kinase, is physically associated with an interferon-alpha receptor.* J Biol Chem, 1994. 269(5): p. 3518-22.
62. Stahl, N., et al., *Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components.* Science, 1994. 263(5143): p. 92-5.
63. Bernards, A., *Predicted tyk2 protein contains two tandem protein kinase domains.* Oncogene, 1991. 6(7): p. 1185-7.
64. Wang, B., et al., *Molecular cloning and characterization of a novel human receptor protein tyrosine phosphatase gene, hPTP-J: down-regulation of gene expression by PMA and calcium ionophore in Jurkat T lymphoma cells.* Biochem Biophys Res Commun, 1997. 231(1): p. 77-81.
65. Takeda, K., et al., *Essential role of Stat6 in IL-4 signalling.* Nature, 1996. 380(6575): p. 627-30.
66. Masuda, A., et al., *Interleukin-15 Prevents Mouse Mast Cell Apoptosis through STAT6-mediated Bcl-xL Expression.* J Biol Chem, 2001. 276(28): p. 26107-13.
67. Kato, Y., et al., *BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C.* Embo J, 1997. 16(23): p. 7054-66.
68. Kato, Y., et al., *Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor.* Nature, 1998. 395(6703): p. 713-6.
69. Zhou, G., Z.Q. Bao, and J.E. Dixon, *Components of a new human protein kinase signal transduction pathway.* J Biol Chem, 1995. 270(21): p. 12665-9.
70. Siliciano, J.D., T.A. Morrow, and S.V. Desiderio, *itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2.* Proc Natl Acad Sci U S A, 1992. 89(23): p. 11194-8.
71. Tanaka, N., et al., *A novel human tyrosine kinase gene inducible in T cells by interleukin 2.* FEBS Lett, 1993. 324(1): p. 1-5.
72. Deak, M., et al., *Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB.* Embo J, 1998. 17(15): p. 4426-41.
73. Li, J., et al., *Novel NEMO/IkappaB kinase and NF-kappa B target genes at the pre-B to immature B cell transition.* J Biol Chem, 2001. 276(21): p. 18579-90.
74. Baytel, D., et al., *The human Pim-2 proto-oncogene and its testicular expression.* Biochim Biophys Acta, 1998. 1442(2-3): p. 274-85.
75. Wang, D., et al., *Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II.* J Biol Chem, 2000. 275(42): p. 32592-7.
76. Nada, S., et al., *Constitutive activation of Src family kinases in mouse embryos that lack Csk.* Cell, 1993. 73(6): p. 1125-35.

77. Inamoto, A. and P. Soriano, *Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice.* Cell, 1993. 73(6): p. 1117-24.
78. Rothwarf, D.M., et al., *IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex.* Nature, 1998. 395(6699): p. 297-300.
79. Millward, T.A., et al., *Calcium regulation of Ndr protein kinase mediated by S100 calcium-binding proteins.* Embo J, 1998. 17(20): p. 5913-22.
80. Millward, T., P. Cron, and B.A. Hemmings, *Molecular cloning and characterization of a conserved nuclear serine(threonine) protein kinase.* Proc Natl Acad Sci U S A, 1995. 92(11): p. 5022-6.
81. Cheng, M., T.G. Boulton, and M.H. Cobb, *ERK3 is a constitutively nuclear protein kinase.* J Biol Chem, 1996. 271(15): p. 8951-8.
82. Boulton, T.G., et al., *ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF.* Cell, 1991. 65(4): p. 663-75.
83. Hande, K.R., *Clinical applications of anticancer drugs targeted to topoisomerase II.* Biochim Biophys Acta, 1998. 1400(1-3): p. 173-84.
84. Wo, Y.Y., et al., *Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphoryrosyl protein phosphatase.* J Biol Chem, 1992. 267(15): p. 10856-65.
85. Haystead, T.A., et al., *Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism.* Nature, 1989. 337(6202): p. 78-81.
86. Kishikawa, K., et al., *Phosphatidic acid is a potent and selective inhibitor of protein phosphatase 1 and an inhibitor of ceramide-mediated responses.* J Biol Chem, 1999. 274(30): p. 21335-41.
87. Warbrick, E., *The puzzle of PCNA's many partners.* Bioessays, 2000. 22(11): p. 997-1006.
88. Tiganis, T., B.E. Kemp, and N.K. Tonks, *The protein-tyrosine phosphatase TCPTP regulates epidermal growth factor receptor-mediated and phosphatidylinositol 3-kinase-dependent signaling.* J Biol Chem, 1999. 274(39): p. 27768-75.
89. Tiganis, T., et al., *Epidermal growth factor receptor and the adaptor protein p52Shc are specific substrates of T-cell protein tyrosine phosphatase.* Mol Cell Biol, 1998. 18(3): p. 1622-34.
90. Sanchez, Y., et al., *Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25.* Science, 1997. 277(5331): p. 1497-501.
91. Furnari, B., N. Rhind, and P. Russell, *Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase.* Science, 1997. 277(5331): p. 1495-7.
92. Mailand, N., et al., *Rapid destruction of human Cdc25A in response to DNA damage.* Science, 2000. 288(5470): p. 1425-9.

es of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. J Biol Chem, 1995. 270(29): p.

cation of the human YVH1 protein-tyrosine phosphatase orthologue reveals a novel zinc binding domain tion. J Biol Chem, 1999. 274(34): p. 23991-5.
(+)-like gene in human cells. Nature, 1991. 353(6339): p. 80-3.
' and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. Cell, 1991. 64(6): p. 1111-22.
Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. Cell, 1987. 49(4): p. 559-

ular masking of nuclear import signal on NF-AT4 by casein kinase I and MEKK1. Cell, 1998. 93(5): p. 851-61.
n kinase I transduces Wnt signals. Nature, 1999. 401(6751): p. 345-50.
gg, Identification of 21 novel human protein kinases, including 3 members of a family related to the cell cycle pilus nidulans. Cell Growth Differ, 1993. 4(10): p. 821-30.
1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. Cell): p. 197-208.
Identification and characterization of a human protein kinase related to budding yeast Cdc7p. Proc Natl Acad p. 14320-5.
ovel SR-protein-specific kinase, SRPK2, disassembles nuclear speckles. Biochem Biophys Res Commun, 1998.

2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of rs in mammalian cells. J Cell Biol, 1998. 140(4): p. 737-50.
n of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways.): p. 90-4.
ovel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J Biol Chem, 1996.

ion of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. , 1997. 94(18): p. 9687-92.
d M.W. Kilimann, *Structure of the human gene encoding the phosphorylase kinase beta subunit (PHKB).* Eur J): 374-80.

109. Hobert, O., B. Jallal, and A. Ullrich, *Interaction of Vav with ENX-1, a putative transcriptional regulator of homeobox gene expression.* Mol Cell Biol, 1996. 16(6): p. 3066-73.
110. Hobert, O., et al., *Isolation and developmental expression analysis of Enx-1, a novel mouse Polycomb group gene.* Mech Dev, 1996. 55(2): p. 171-84.
111. Alexandru, G., et al., *Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage.* Embo J, 1999. 18(10): p. 2707-21.
112. Cahill, D.P., et al., *Mutations of mitotic checkpoint genes in human cancers.* Nature, 1998. 392(6673): p. 300-3.
113. Schultz, S.J., et al., *Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of Aspergillus nidulans.* Cell Growth Differ, 1994. 5(6): p. 625-35.
114. Fry, A.M., P. Meraldi, and E.A. Nigg, *A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators.* Embo J, 1998. 17(2): p. 470-81.
115. Zhao, Y., et al., *Cloning and chromosomal location of a novel member of the myotonic dystrophy family of protein kinases.* J Biol Chem, 1997. 272(15): p. 10013-20.
116. Tung, R.M. and J. Blenis, *A novel human SPS1/STE20 homologue, KHS, activates Jun N-terminal kinase.* Oncogene, 1997. 14(6): p. 653-9.
117. Yuan, Y., D.M. Li, and H. Sun, *PIR1, a novel phosphatase that exhibits high affinity to RNA . ribonucleoprotein complexes.* J Biol Chem, 1998. 273(32): p. 20347-53.
118. Deshpande, T., et al., *Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5'-triphosphatase and diphosphatase activities.* J Biol Chem, 1999. 274(23): p. 16590-4.
119. Bastians, H. and H. Ponstingl, *The novel human protein serine/threonine phosphatase 6 is a functional homologue of budding yeast Sit4p and fission yeast ppe1, which are involved in cell cycle regulation.* J Cell Sci, 1996. 109(Pt 12): p. 2865-74.
120. Becker, W., et al., *Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases.* J Biol Chem, 1998. 273(40): p. 25893-902.
121. Guimera, J., et al., *A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region.* Hum Mol Genet, 1996. 5(9): p. 1305-10.
122. Lopez-Borges, S. and P.A. Lazo, *The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2 binding site of the p53 tumour suppressor protein.* Oncogene, 2000. 19(32): p. 3656-64.
123. Nezu, J., et al., *Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase.* Genomics, 1997. 45(2): p. 327-31.

124. Lapidot-Lifson, Y., et al., *Cloning and antisense oligodeoxynucleotide inhibition of a human homolog of cdc2 required in hematopoiesis.* Proc Natl Acad Sci U S A, 1992. 89(2): p. 579-83.
125. Mills, G.B., et al., *Expression of TTK, a novel human protein kinase, is associated with cell proliferation.* J Biol Chem, 1992. 267(22): p. 16000-6.
126. Schmandt, R., et al., *IL-2-induced expression of TTK, a serine, threonine, tyrosine kinase, correlates with cell cycle progression.* J Immunol, 1994. 152(1): p. 96-105.
127. Ruvolo, P.P., et al., *Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A.* J Biol Chem, 1999. 274(29): p. 20296-300.
128. Wadzinski, B.E., et al., *Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation.* Mol Cell Biol, 1993. 13(5): p. 2822-34.
129. Lee, M.G. and P. Nurse, *Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.* Nature, 1987. 327(6117): p. 31-5.
130. Riabowol, K., et al., *The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells.* Cell, 1989. 57(3): p. 393-401.
131. Zeng, Q., et al., *Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome.* J Biol Chem, 2000. 275(28): p. 21444-52.
132. Diamond, R.H., et al., *PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth.* Mol Cell Biol, 1994. 14(6): p. 3752-62.
133. Peng, Y., et al., *The gene encoding human nuclear protein tyrosine phosphatase, PRL-1. Cloning, chromosomal localization, and identification of an intron enhancer.* J Biol Chem, 1998. 273(27): p. 17286-95.
134. Edwards, M.C., C. Wong, and S.J. Elledge, *Human cyclin K, a novel RNA polymerase II-associated cyclin possessing both carboxy-terminal domain kinase and Cdk-activating kinase activity.* Mol Cell Biol, 1998. 18(7): p. 4291-300.
135. van den Berg, I.E., et al., *X-linked liver phosphorylase kinase deficiency is associated with mutations in the human liver phosphorylase kinase alpha subunit.* Am J Hum Genet, 1995. 56(2): p. 381-7.
136. Grove, J.R., et al., *Cloning and expression of two human p70 S6 kinase polypeptides differing only at their amino termini.* Mol Cell Biol, 1991. 11(11): p. 5541-50.
137. Banerjee, P., et al., *Molecular structure of a major insulin/mitogen-activated 70-kDa S6 protein kinase.* Proc Natl Acad Sci U S A, 1990. 87(21): p. 8550-4.
138. Weng, Q.P., et al., *Phosphatidylinositol 3-kinase signals activation of p70 S6 kinase in situ through site-specific p70 phosphorylation.* Proc Natl Acad Sci U S A, 1995. 92(12): p. 5744-8.
139. Kornbluth, S., M. Sudol, and H. Hanafusa, *Association of the polyomavirus middle-T antigen with c-yes protein.* Nature, 1987. 325(7000): p. 171-3.

140. Kitamura, N., et al., *Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus.* Nature, 1982. 297(5863): p. 205-8.
141. Tobimatsu, T. and H. Fujisawa, *Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs.* J Biol Chem, 1989. 264(30): p. 17907-12.
142. Hovens, C.M., et al., *RYK, a receptor tyrosine kinase-related molecule with unusual kinase domain motifs.* Proc Natl Acad Sci U S A, 1992. 89(24): p. 11818-22.
143. Halford, M.M., et al., *Ryk-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk.* Nat Genet, 2000. 25(4): p. 414-8.
144. Barker, H.M., et al., *Sequence of human protein serine/threonine phosphatase 1 gamma and localization of the gene (PPP1CC) encoding it to chromosome bands 12q24.1-q24.2.* Biochim Biophys Acta, 1993. 1178(2): p. 228-33.

Table 1

Genes that have not been linked to an antiapoptotic function before and are induced in the apoptosis resistant clones

Gene	Accession number	Number of clones in which expression exceeds cut off for increased expression	% over cutoff
Tyrosine Kinases			
Hck	M16591	4	46,5
TrkC	U05012	6	28,0
Hyl	X77278	11	24,5
Rse	U05682	10	22,5
RON	X70040	14	21,0
KIAA0641	AB014541	5	19,5
EphA2	M59371	12	19,5
Csk	X59932	8	18,0
EphB3	X75208	6	16,0
EphB4	U07695	5	12,5
Pyk-2	U33284	4	11,0
Unknown Phosphatases			
PB-26	AB040904	5	30,5
PB-28	AB040904	4	18,0
Unknown Kinases			
UK19	AA292586	5	25,0
UK10	R52045	4	24,5
UK11	H39075	8	9,5
Serine/Threonine Kinases			
GRK6	L16862	16	69,0
Dyrk4	Y09305	5	55,0
IRAK-2	AF026273	13	54,5
LIMK-1	D26309	5	45,5
MLK3	U07747	9	44,0
AMPK-beta	AJ224538	4	40,0
MAPKKK6	AF100318	14	39,0
MAST205	6678957	14	37,5
DAPK	X76104	11	37,0
MAPKK3	4506098	11	36,0
PLK-1	L19559	12	34,5
PKN-H4	D26181	13	34,5
Bcr	X02596	10	32,0
MSK2	AF074715	8	30,0
Rac-alpha	M63167	16	28,0
MST-3	AF024636	7	28,0
PSK-H1	M14504	5	26,5
PCTAIRE1	X66363	6	23,0

lok	AB015718	4	22,0
HsGAK	D88435	6	21,0
MAPKAPK3	U09578	7	20,5
JNKK2alpha	AF022806	8	19,5
FAST	X86779	5	19,0
MKK7	AF013588	8	17,5
MAPKK5	U25265	6	16,5
PAK1-relatedkinase	AF005045	11	16,0
ARK2	AF008552	4	16,0
MSSK1	U82808	4	15,0
PHK-gammaT	M31606	6	14,5
CDC42-bindingproteinkinasebeta	AF128625	3	13,5
KIAA0151	D63485	9	11,0
KIAA0537	AB011109	6	11,5
STE20-like	X99325	4	6,0
Ste-20likeproteinkinase3	AF083420	3	5,5
Adapter Proteins			
Grb-2	M96995	7	18,0
SHC	Y09847	6	17,5
SHB	X75342	10	15,0
Phosphatases			
PYST1	X93920	5	65,5
B23	U15932	5	33,0
PCP-2	X97198	10	31,5
PTP-J	U73727	9	30,0
PTP-Meg2	M83738	9	29,5
PP5	X89416	7	18,5
CDC25B	M81934	10	17,0
PTP-SL	Z30313	5	17,0
PP2B-R	M30773	6	17,0
PP1-Calpha	M63960	6	16,0
PP2A-Rb55	M64930	5	15,5
PTPzeta	X54135	4	13,0
Shp-1	X62055	4	7,0
PP2A-Ra65	J02902	6	7,0
PTPmu	X58288	3	6,5
Metalloproteases			
MMP-15	Z48482	19	71,0
ADAM12	X05232	8	59,0
MMP-3	J03209	14	34,5
ADAM15	NM003815	12	28,0
ADAM8	XM005675	13	27,0
G-proteins			
alphai2		16	44,0
GPIR-3		4	8,0

Table 1 (continued)

Other			
p91/ISGF-3	M97935	7	104,0
90k	50318862	15	57,5
MHC-1	M11886	17	53,0
EF-2	X51466	16	44,0
alpha-tubulin	NM_006082	9	43,5
KIF-1c	NM006612	6	28,0
Furin	X17094	8	27,0
rS9	4506744	13	23,0
GPDH	M33197	9	23,0
beta-Aktin	X00351	11	21,5
Vimentin	X56134	15	19,0
neurolektin	K03515	14	17,5
Thymosinbeta	S54005	7	11,5
Histon3.3	M11354	6	7,0
PHB-4-PC	L14273	5	3,5

Table 1 (continued)

Table 2

Genes with known antiapoptotic function that are induced in the apoptosis resistant clones

Gene	Accession number	Number of clones in which expression exceeds cut off for increased expression	% over cut off
Tyrosine Kinases			
PDGFRalpha	M22734	4	39,0
HER2	M11730	4	38,5
EGFR	X00588	6	21,0
FGFR-3	M58051	5	20,5
HER4	L07868	5	32,5
Jak-2	AF058925	6	34,0
Tyk-2	X54637	8	18,5
Serine/Threonine Kinases			
RSK	L07597	11	43,5
MAPKK2	L11285	11	33,5
PIM-2h	U77735	8	29,5
IKK1	AF012890	4	28,0
CKII-beta	M30448	7	28,0
ALK-4	Z22536	6	27,0
ERK1	X80188	11	25,5
IKKgamma	AF074382	12	23,5
AKT2	M95936	10	17,0
CKII-alpha	J02853	8	17,5
CaM-Kitgamma	L07044	7	16,0
MAPKAPK2	NM004758	6	15,0
ILK	U40282	7	14,0
CKI-delta	U29171	6	11,5
SGK	Y10032	4	11,5
CKII-beta	M30448	5	9,0
A-Raf-1	X04790	5	9,0
ALK-1	L17075	5	6,5
Phosphatases			
PPX	X70218	16	26,0
Ligands			
TGFalpha	XM002732	11	63,0
IL1-beta	NM000576	8	54,5
IL1-alpha	X02531	4	34,5
VEGF	NM003376	9	27,0
Other			
Bcl-x	Z23115	14	34,5
IL-4Stat	U16031	9	19,5
TIMP-1	X03124	17	108,5
myc	X00364	8	15,5
TIMP-2	S48568	13	36,5

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Clusters in the apoptosis resistant clones

Bold: also found in one common cluster in squamous cell carcinoma

Cluster 1

Correlation factor 0,71	
Bcr	Rac-alpha
JNKK2alpha	IKKgamma
MKK7	PPX
PTP-SL	ADAM15
KIF-1c	ADAM8
SHB	ERK1
IRAK	Rse
PLK-1	alphai2548C
alpha-tubulin	MAPKKK6
RSK	90k
GRK6	pBTUB
GPIR-3	
MAPKAPK3	
PKN-H4	
MAPKK2	
PKA-RIbeta	
p130CAS	
LIMK-1	
MAST205	
PIM-2h	
Csk	
MAPKK3	
PCTAIRE1	
MST-3	

Table 3

Cluster 2

Correlation factor 0,73
Bcl-x
AKT2
EF-2
PITALRE
TIMP-2
FGFR-2
CDC25B
CKI-delta
EphA2
PP2C
Rac-alpha
RON
EphB4

Cluster 3

Correlation factor 0,74
PDGFRalpha
PB-28
PHK-gammaT
Tyk-2
A-Raf-1
GPDH
HK-18B
Erk6
Cyto18
HK-18B
cytokeratin8
CK-8
CKII-beta
MLK3
MMP-11

Cluster 4

Correlation factor 0,67
PP2A-Ra65
HER2
CAMKK

Cluster 5

Correlation factor 0,71
PKC-epsilon
MAPKK5
PTP-Meg2
PP2B-Cbeta
MKP-5
Jak-2
Shp-2
IKK2
PHK-alphaL
JNK1

Cluster 6

Correlation factor 0,61
Pyk-2
Shp-1
PP2B-Cgamma
lok
CaM-KIIgamma
DRP-1
CKI-gamma2
PSK-H1
GPIR-1
Chk2
Axl
IL-4Stat
PCP-2
DAPK
PKA-Calpha2
PP5
Abl
Raf
PTPzeta
IGF1-R
pHE-A1
PTP-1B
MSTH1
PKA-Calpha
PLCgamma
HsGAK
VHR
TESK1
PRK

Cluster 7

Correlation factor 0,83
MMP-15
TIMP-1

Table 3 (continued)

Clusters in squamous cell carcinoma cell lines

SCaBER
UMSSC-17B
UMSSC-17A
UMSSC-22A
UMSSC-22B
UMSSC-10A
HlaC78
HlaC79
FaDu

Bold: also found in one common cluster in apoptosis resistant clones

Cluster 1

Correlation factor 0,7
PRL-3_2_23
Dyrk2_2_16
MAPKK2_1_5
SHC_1_3
ERK3_1_4
RSK

Cluster 2

Correlation factor 0,8
GSK-3alpha
PTP-SL
MAPKAPK3
GSK-3beta
hPAK1
PKC-delta
PB-32
alphaq
KIF-1c
SHB
KIAA0687Nck-interactingkinase

Table 4

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Cluster 3

Correlation factor 0,67
PB-32
PB-38
hPAK2
TIMP-2
PB-5
CDK6
Bcl-x
H11
Axl
MMP-14
hSLK
MSTH1
alphaq
ADAM17
PIR1
ALK-2

Cluster 4

Correlation factor 0,71
Tyk-2
TIMP-1
GPDH
HPRT
Dyrk4
MMP-15
Jak-1
Myt1
GPIR-3
PCNA
Chk2
PK38
alpha-tubulin
CDK4
PKN-H4
hPTK
GPIR-2
Bmx
MMP-11
PKU-alpha

Table 4 (continued)

TABLE 5 (1/7)

Gene	Genbank Nr.	Description	Reference	Number of clones with increased expression	% over cut off for increased expression
MP_MMP-15	Z48482	transmembrane metalloprotease, probably processes MMP-2	[1, 2]	20	82
STP_PPX	X70218	nuclear, localized to centrosomes, activates NFκB by dephosphorylation	[3]	17	18
OT_MHC-1	M11886	presents antigens on the cell surface	[4]	16	44
OT_EF-2	XM031904	translation-elongation-factor-2	[5]	15	30
OT_Vimentin	X56134	intermediate filament	[6]	15	14
GP_alphai2	NM002070	alpha subunit of heterotrimeric G-proteins, can inhibit adenylate cyclase and activate MAP-kinase	[7-10]	14	42
STK_PKNalpha	D26181	related to PKC, activated by Rho, fatty acids and caspase cleavage	[11-15]	14	34
STK_Bcr	X02596	possesses serine/threonine kinase activity and GAP activity for p21rac	[16, 17]	13	32
STYP_CDC25B	M81934	dual specific, induces cell cycle progression form G2 to M by dephosphorylation of cdc-2	[18-21]	13	30
STP_PP5_1_3	X89416	nuclear, binds to the glucocorticoid receptor and inhibits growth inhibition by this receptor	[22-24]	13	22
STK_MAST205	6678957	binds to microtubuli and β1-syntrophin	[25, 26]	13	20
MP_ADAM8	NM001109	induced by TNF-α	[27, 28]	13	18
MP_TIMP-2	S48568	contributes to the activation of pro-Gelatinase A in complex with MMP-14, may act mitogenically	[29-31]	12	37

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TABLE 5 (2/7)

STK_MAPKKK6	U39657	binds MAPKKKS/ASK1	[32]	12		33
MP_ADAM15	NM003815	binds to Integrins	[33, 34]	12		32
STK_PLK-1	L19559	prognostic marker for squamous cell carcinoma, essential for Pro-phase of mitosis, acitvated after DNA damage	[35-37]	12		21
YK_EphA2	M59371	plays a role in repulsion of nerve cells during embryogenisis, inhibits MAPK-activation by PDGF and EGF	[38-40]	12		16
AD_SHB	NM003028	SH2 domain containing adaptor protein	[41]	12		15
STK_PKNbeta	AB019692	homologues to PKNalpha, not expressed in adult healthy tissues but in cancer	[42]	11		49
YP_PTP-Meg2	M83738	cytosolic tyrosine phosphatase	[43]	11		42
OT_VHL	NM_000551	tumor suppressor that forms a complex with ubiquitin ligase	[44-46]	11		40
YK_Hyl	X77278	cytoplasmic tyrosine kinase with homology to CSK	[47]	11		18
STK_MAPKAPK2	NM032960	activated by p38 MAP kinase, can act as PDK2 for Akt	[48, 49]	11		10
OT_neuroleukin	K03515	neurotrophic ligand, entire mRNA also codes for Phosphohexose-Isomerase	[50-53]	11		10
YP_PCP-2	X97198	receptor tyrosine phosphatase with MAM domain	[54]	10		30
OT_rS9	4506744	ribosomal protein 9	[55]	10		16
YP_PTPsigma	U35234	receptor tyrosine phosphatase of the LAR family, involved in brain embryogenisis	[56, 57]	9		109
STK_KIAA0135	O50925	putative Serin/Threonin-Kinase	[58]	9		33
STK_beta-ARK-1	X61157	phosphorylates and desensitizes β-adrenergic receptor	[59, 60]	9		25

TABLE 5 (3/7)

YK_Tyk-2	X54637	cytoplasmic tyrosine kinase, homologues to JAK-kinases	[61-63]	9	20
YP_PTP-J	U73727	receptor tyrosine phosphatase with MAM domain	[64]	9	19
OT_IL-4Stat	U16031	transduces IL-4 signals	[65, 66]	9	18
STK_MAPKK5	U25265	activates Erk5/Bmk	[67-69]	9	7
YK_ITK	D13720	cytoplasmic tyrosine kinase, specific for T-cells	[70, 71]	8	46
STK_MSK2	AF074393	activated by p38 and Erk1/2 Map-Kinases	[72]	8	30
STK_PIM-2h	U77735	upregulated by NFκB	[73, 74]	8	18
STK_CKII-alpha	J02853	activated by phosphorylation of rel/p65	[75]	8	17
YK_Csk	X59932	phosphorylates and inhibits src-Kinases	[76, 77]	8	15
STK_IKKgamma	AF074382	part of the Ikappa8-Kinase complex that activates NFκB	[78]	8	13

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TABLE 5 (4/7)

Gene	Genbank Nr.	Description	Reference	Number of Clones with reduced expression	% under cut off for reduced expression
STK_Ndr	Z35102	nuclear phosphatase, activated by Calcium	[79, 80]	20	59
STK_ERK3	X80692	constitutively nuclear MAP-Kinase	[81, 82]	19	159
OT_Topoisomerase2	NM001068	topoisomerase-2 inhibitors are used as chemotherapeutica against cancer	[83]	19	143
YP_AZP-ISredacidphosphatase	M83653	cytoplasmic phosphotyrosyl protein phosphatase	[84]	19	92
STP_PP1-Cbeta	X80910	catalytic subunit of PP1, activated by ceramid	[85, 86]	19	45
OT_PCNA	4505640	Proliferating Cellular Nuclear Antigen	[87]	19	27
YP_TC-PTP	M25393	localized to ER and nucleus, inhibits PI3K signals after EGF stimulation	[88, 89]	19	155
STK_CHK1	AF016582	after DNA damage necessary for cell cycle halt at G2/M, phosphorylates wee1 and cdc25	[90-92]	18	104
STK_AMP-activatedproteinkinasealpha1subunit	AF100763	phosphorylates and deactivates Acetyl-CoA Carboxylase	[93]	18	81
PP_YVH1	AF119226	dual-specific phosphatase	[94]	18	72
STK_WEE1	X62048	inhibits G2/M progression, phosphorylates and inhibits cdc2	[95-97]	18	73
STK_CKI-alpha	X80693	part of the Wnt pathway, phosphorylates and inhibits nuclear transport of NF-AT4	[98, 99]	18	49

TABLE 5 (5/7)

STK_NEK3	Z28067	Homologous to NIMA kinase of *Aspergillus Nidulans*, which is responsible for G2/M progression	[100]	17		234
STK_MAD-3likePK	AF068760			17		104
STK_TAK1	U64205	cdc25 associated kinase, phosphorylates Cdc25c	[101]	17		53
UP_PB-32	W30715	unknown phosphatase		16		178
STK_HsCdc7	AF015592	important for G1/S progression	[102]	16		138
STK_SRPK-2	U88666	phosphorylates SR-Splice-factors	[103, 104]	16		70
STK_MAPKK6	U39657	activates p38 MAP-kinase, activated by Ask-1 a MAPKKK that induces apoptosis	[105, 106]	16		54
STK_GCK	U07349	homologous to *S. cerevisiae* Ste20, activates JNK	[107]	16		324
STK_KIAA0619	AB014519	unknown kinase		16		239
STK_PHK-beta	X84906	phosphorylates Glycogen-Phosphorylase	[108]	15		246
OT_33a_Enx-1	AF070418	regulates expression of Homeobox-genes	[109, 110]	15		187
STK_Bub1	AF046078	controls segregation of chromatids; mutation in cancer causes increased mutation rate	[111, 112]	15		121
STK_NEK2	U11050	associates with centrosomes	[113, 114]	15		37
STK_PK428	U59305	related to family of myotonic dystrophy kinases	[115]	15		237
STK_KHS	U77129	homologous to *S. cerevisiae* Ste20, activates JNK	[116]	15		121
PP_PIR1	AF023917	dual specific, nuclear, dephosphorylates RNA, associated with speckles	[117, 118]	15		51

TABLE 5 (6/7)

STP_PP6	X92372	homologous to S. cerevisiae Sit4p and S. pombe ppe1, which regulate the cell cycle	[119]	15	30
STK_MNB	U52373	dual specific, homologous to DYRK kinase, located in region of chromosome 21 that is amplified in Down-Syndrom	[120, 121]	14	90
STK_VRK1	AB000449	homologous to Vaccinia Virus Kinase, nuclear	[122, 123]	14	286
STK_CHED	M80629	Homolog of cdc-2	[124]	14	75
STK_TTK	M86699	dual specific, expression correlates with cell cycle	[125, 126]	13	465
UK_PB-11	AF061944	unknown kinase		13	168
STP_PP2A-Cbeta	X12656	nuclear, dephosphorylates Bcl-2	[127, 128]	13	33
UK_UK20	NM_016507	unknown kinase		13	282
STK_GLK	AF000145	homologous to *S. cerevisiae* Ste20, activates JNK	[107]	13	140
STK_26b_CDC2_1_4	X05360	essential for G2/M progression	[96, 129, 130]	12	168
YP_Pri-1	U48297	may influence cell growth, nuclear but also associated with plasma membranes and endosomes	[131-133]	12	74
STK_cyclinK	AF060515	can regulate cdk-activity and transcription by RNA-polymerase II	[134]	12	31
STK_PHK-alphaL	X80497	subunit of phosphorylase kinase	[135]	12	24
STK_p70S6K	M60724	activated via PI3Kinase	[136-138]	11	824
YK_Yes	4885660	belongs to family of src-kinases	[139, 140]	11	661
STK_CaM-Kldelta	U73504	highly expressed in brain	[141]	11	216

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TABLE 5 (7/7)

YK_Ryk	X69970	receptor tyrosine kinase, doesn't belong to any known family of receptor tyrosine kinases, probably involved in Eph-signalling	[142, 143]	11	166
YP_PRL-3	AF041434	homologous to PRL-1	[131]	11	74
STP_PP1-Cgamma	X74008	catalytic subunit of PP1	[144]	11	59

Claims

1. A method for identifying nucleic acid molecules functionally associated with a desired phenotype comprising the steps:
   (a) providing a population of parental cells wherein said cell population substantially lacks the desired phenotype,
   (b) optionally subjecting said cell population to a procedure resulting in a rearrangement and/or mutation of the cell genome,
   (c) subjecting said cell population from (b) to a selection procedure for the desired phenotype,
   (d) identifying and optionally characterizing cells exhibiting said desired phenotype,
   (e) obtaining protein and/or mRNA from cells exhibiting said desired phenotype,
   (f) determining gene expression in cells exhibiting said desired phenotype and
   (g) comparing gene expression in cells exhibiting said desired phenotype with gene expression in cells substantially lacking the desired phenotype.

2. The method of claim 1 wherein the desired phenotype is selected from cancer cell properties.

3. The method of claim 2 wherein the cancer cell properties are selected from invasiveness, metastasis, loss of contact inhibition, loss of extracellular matrix requirement, growth factor independence, angiogenesis induction, immuno defense evasion, anti-apoptosis and/or increased levels of tumor markers.

4. The method of claim 2 wherein the desired phenotype is anti-apoptosis.

5. The method of claim 1 wherein the desired phenotpye is selected from production of secreted protein, susceptibility or resistance to pathogens, senescene, regulation of cell functions and modification of signal transduction pathways.

6. The method of any one of claims 1-5 wherein a parental cell is selected which is continuously in a process of genome rearrangement and mutagenesis.

7. The method of claim 6 wherein the parental cell is an immortalized or transformed cell.

8. The method of any one of claims 1-5 wherein a parental cell is selected which has a substantially stable genome.

9. The method of claim 8 wherein step (b) comprises a mutagenesis procedure.

10. The method of claim 9 wherein said mutagenesis procedure is selected from irradiation, chemical mutagenesis and combinations thereof.

11. The method of any one of claims 1-10 wherein step (d) comprises a cell sorting procedure.

12. The method of claim 11 wherein said cell sorting procedure is a Fluorescence Activated Cell Sorting Procedure (FACS).

13. The method of any one of claims 1-12 comprising obtaining mRNA in step (e) and hybridizing said mRNA or a nucleic acid made therefrom with a nucleic acid array.

14. The method of claim 13 wherein the nucleic acid made from mRNA is selected from the group consisting of cDNA and cRNA.

15. The method of any one of claims 13-14 wherein said nucleic acid array comprises a solid carrier having immobilized thereto a plurality of different nucleic acid molecules.

16. The method of any one of claims 13-15 wherein said nucleic acid array is selected from arrays of genomic DNA arrays, cDNA arrays and oligonucleotide arrays.

17. The method of any one of claims 13-16 wherein said nucleic acid array comprises nucleic acids encoding functional cellular polypeptides or portions thereof selected from kinases, phosphatases, enzymes and receptors.

18. The method of any one of claims 1-12 comprising obtaining protein in step (e) and analyzing the protein content in cells exhibiting the desired phenotype.

19. The method of claim 18 wherein said analyzing comprises 2D gel electrophoresis, mass spectrometry and/or binding to protein arrays.

20. The method of claim 18 or 19 wherein before analyzing a pretreatment step in order to reduce the complexity of the protein mixture is carried out.

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21. The method of any one of claims 1-20 further comprising the identification of a plurality of genes (gene cluster) which is associated with the desired phenotype.

22. The method of any one of claims 1-21 further comprising a validation step wherein the association of a defined gene or gene cluster with the desired phenotype is determined.

23. The method of claim 22 wherein the validation step comprises generating of dominant-negative mutants.

24. The method of any one of claims 1-23 further comprising a screening procedure wherein the activity of a test substance for a defined gene or gene cluster associated with the desired phenotype is determined.

25. Use of the method of any one of claims 1-24 for generating expression profiles of genes or gene clusters associated with a desired phenotype.

26. The use of claim 25 wherein the expression profile is compared with the expression profile in a biological sample.

27. The use of claim 26 wherein the sample is derived from a human patient.

28. Use of the nucleic acid shown in Table 1, Table 2 and Table 5 or fragments thereof or peptides or polypeptides encoded by said nucleic acids or fragments as targets.

29. The use of claim 28 for diagnostic applications.

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30. The use of claim 28 for therapeutic applications.

31. The use of claim 28 for a screening procedure to identify novel drugs.

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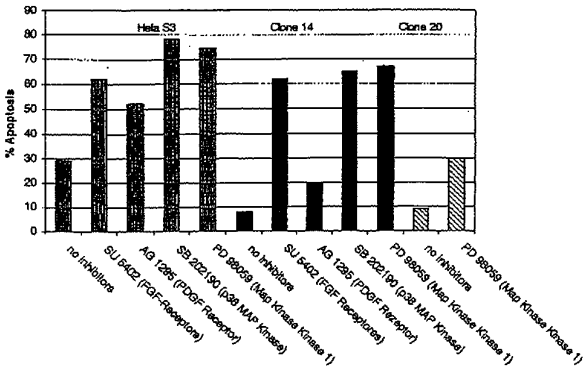
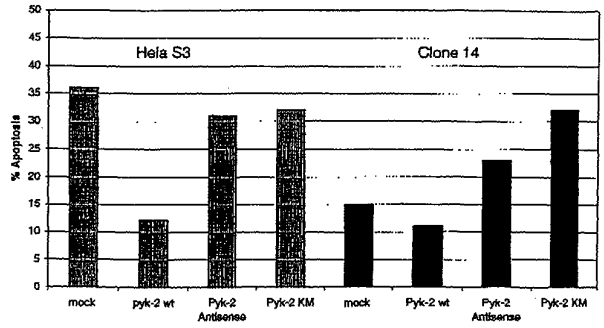
Figure 1


Hela S3
Clone 14
Clone 20
% Apoptosis
0
10
20
30
40
50
60
70
80
90
no Inhibitors
SU 5402 (FGF-Receptors)
AG 1295 (PDGF-Receptor)
SB 202190 (p38 MAP Kinase)
PD 98059 (Map Kinase Kinase 1)
no Inhibitors
SU 5402 (FGF Receptores)
AG 1295 (PDGF-Receptor)
SB 202190 (p38 MAP Kinase)
PD 98059 (Map Kinase Kinase 1)
no Inhibitors
PD 98059 (Map Kinase Kinase 1)

54

Figure 2


Hela S3
Clone 14
% Apoptosis
0
5
10
15
20
25
30
35
40
45
50
mock
pyk-2 wt
Pyk-2 Antisense
Pyk-2 KM
mock
Pyk-2 wt
Pyk-2 Antisense
Pyk-2 KM

55

FIGURE 3

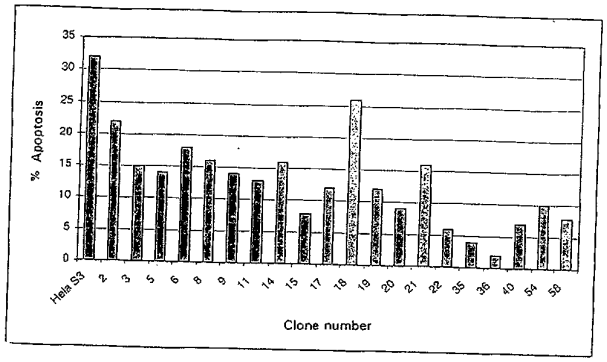
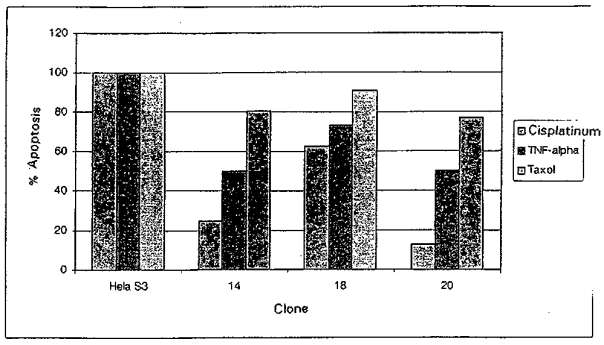

% Apoptosis
35
30
25
20
15
10
5
0
Hela S3
2
3
5
6
8
9
11
14
15
17
18
19
20
21
22
35
36
40
54
58
Clone number

FIGURE 4


120
100
80
60
40
20
0
% Apoptosis
Hela S3
14
18
20
Clone
Cisplatinum
TNF-alpha
Taxol

【国際公開パンフレット（コレクトバージョン）】

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(54) Title: METHOD FOR IDENTIFYING FUNCTIONAL NUCLEIC ACIDS

(57) Abstract: The present invention relates to a method for identifying nucleic acid molecules functionally associated with a desired phenotype.

【手続補正書】
【提出日】平成15年4月28日(2003.4.28)
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】特許請求の範囲
【補正方法】変更
【補正の内容】
【特許請求の範囲】
【請求項１】
以下の工程：
（ａ）親細胞のポピュレーションを準備し、その際、前記細胞ポピュレーションが実質的に所望の表現型を欠損し、かつ常にゲノム再配置及び突然変異誘発にある親細胞を選択し、
（ｂ）前記の細胞ポピュレーションに細胞ゲノムの再配置及び／又は突然変異をもたらす処置を施し、
（ｃ）前記の（ｂ）からの細胞ポピュレーションに所望の表現型のための選択処置を施し、
（ｄ）前記の所望の表現型を表す細胞を同定し、かつ場合により特徴付け、
（ｅ）前記の所望の表現型を表す細胞からｍＲＮＡを得て、
（ｆ）前記のｍＲＮＡ又はそれから得られたｃＤＮＡを核酸アレイにハイブリダイズさせ、かつ前記の所望の表現型を表す細胞において遺伝子発現を調査し、
（ｇ）前記の所望の表現型を表す細胞における遺伝子発現と所望の表現型を実質的に欠損している細胞における遺伝子発現とを比較する
を含む、所望の表現型と機能的に関連した核酸分子を同定するための方法。
【請求項２】
所望の表現型を癌細胞特性から選択する、請求項１記載の方法。
【請求項３】
癌細胞特性が浸潤性、転移、接触阻害の欠損、細胞外基質所要量の欠損、成長因子の独立性、血管形成誘導、免疫防御の回避及び／又は抗アポトーシスから選択される、請求項２記載の方法。
【請求項４】
所望の表現型が抗アポトーシスである、請求項２記載の方法。
【請求項５】
所望の表現型が分泌性タンパク質の産生、病原に対する感受性又は抵抗性、老化及び細胞機能の調節から選択される、請求項１記載の方法。
【請求項６】
親細胞が不死化細胞又は形質転換された細胞である、請求項１記載の方法。
【請求項７】
工程（ｄ）がセルソーティング処置を含む、請求項１から６までのいずれか１項記載の方法。
【請求項８】
前記のセルソーティング処置が蛍光活性化セルソーティング処置（ＦＡＣＳ）である、請求項７記載の方法。
【請求項９】
工程（ｅ）でｍＲＮＡを得ること及び該ｍＲＮＡ又はそれから生成される核酸を核酸アレイとハイブリダイズさせることを含む、請求項１から８までのいずれか１項記載の方法。
【請求項１０】
ｍＲＮＡから生成される核酸がｃＤＮＡ及びｃＲＮＡからなる群から選択される、請求項９記載の方法。
【請求項１１】

前記の核酸アレイが、複数の異なる核酸分子を固定されて有する固体担体を含む、請求項９又は１０記載の方法。
【請求項１２】
前記の核酸アレイがゲノムＤＮＡアレイ、ｃＤＮＡアレイ及びオリゴヌクレオチドアレイのアレイから選択される、請求項９から１１までのいずれか１項記載の方法。
【請求項１３】
前記の核酸アレイがキナーゼ、ホスファターゼ、酵素及び受容体から選択される機能的な細胞性ポリペプチド又はその部分をコードする核酸を含む、請求項９から１２までのいずれか１項記載の方法。
【請求項１４】
工程（ｅ）においてタンパク質を得ること及び所望の表現型を表す細胞におけるその細胞含量を分析することを含む、請求項１から１３までのいずれか１項記載の方法。
【請求項１５】
前記の分析が２Ｄゲル電気泳動、質量分析及び／又はタンパク質アレイへの結合を含む、請求項１４記載の方法。
【請求項１６】
分析の前にタンパク質混合物の複合性を低下させるために前処理工程を実施する、請求項１４又は１５記載の方法。
【請求項１７】
更に、所望の表現型と関連する複数の遺伝子（遺伝子クラスター）の同定を含む、請求項１から１６までのいずれか１項記載の方法。
【請求項１８】
更に、規定の遺伝子又は遺伝子クラスターと所望の表現型との関連を調査する確認工程を含む、請求項１から１７までのいずれか１項記載の方法。
【請求項１９】
確認工程が優性阻害型突然変異体の作成を含む、請求項１８記載の方法。
【請求項２０】
更に、所望の表現型と関連する規定の遺伝子又は遺伝子クラスターについて試験物質の活性を測定するスクリーニング処置を含む、請求項１から１９までのいずれか１項記載の方法。
【請求項２１】
所望の表現型と関連する遺伝子又は遺伝子クラスターの発現プロフィールの作成のための、請求項１から２０までのいずれか１項記載の方法の使用。
【請求項２２】
前記発現プロフィールを生物学的試料における発現プロフィールと比較する、請求項２１記載の使用。
【請求項２３】
試料をヒトの患者から得る、請求項２２記載の使用。
【請求項２４】
ターゲットとしての、第１表、第２表及び第５表に示される核酸又はその断片又は前記の核酸によってコードされるペプチド又はポリペプチド又は断片又は第３表及び第４表に示されるそれらの組み合わせの使用。
【請求項２５】
診断的用途のための請求項２４記載の使用。
【請求項２６】
治療的用途のための請求項２４記載の使用。
【請求項２７】
新規の医薬物質を同定するためのスクリーニング処置のための請求項２４記載の使用。

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International Application No
PCT/E 2/01073

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 059374 A (NEILSEN SOREN ;GOLDSMITH NEIL (DK); DEAKIN EDWARD (GB); STAFFORD A) 1 August 2002 (2002-08-01) page 2, line 21 -page 3, line 10 page 4, line 26-29 page 6, line 28 -page 7, line 8 claim 1; examples 1-12 ---	1,5,8, 11-19,25
X	WO 99 41371 A (KECK JAMES G ;WONG JUSTIN G P (US); STRATA BIOSCIENCES INC (US)) 19 August 1999 (1999-08-19)	1-5,11, 12,21,25
Y	page 5, line 2-17 page 7, line 7-32 page 10, line 12 -page 11, line 2 page 20, line 14 -page 21, line 23 page 22, line 4 -page 23, line 9 claims 1,10; examples 3,6,10,11 --- -/--	1-5,25

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2003	26. 03. 03
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Goetz, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/E 2/01073

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 01846 A (MORTIER KATHERINE ;DEVGEN NV (BE); BOGAERT THIERRY (BE); PLAETINCK) 13 January 2000 (2000-01-13) page 1, line 30 -page 2, line 9 page 3, line 2 -page 4, line 11 page 21, line 17 -page 23, line 2 page 27, line 1 -page 28, line 34 claim 1 ---	1
Y	WO 00 53813 A (MASSACHUSETTS INST TECHNOLOGY) 14 September 2000 (2000-09-14) page 2, line 9-11 page 3, line 1-33 page 7, line 15 -page 8, line 6 page 19, line 1-8 page 21, line 19-24 claims 10,11,19 ---	1-5,25
X	WO 98 32880 A (BARBER JACK ;IMMUSOL INC (US); TRITZ RICHARD (US); WELCH PETER (US) 30 July 1998 (1998-07-30)	1-5,8, 11,12, 21,25
Y	page 4, paragraph 2 page 21, paragraph 1 page 27, line 3 -page 28, line 1 page 35, paragraph 4 -page 42, paragraph 2 page 52, last paragraph -page 54, paragraph 1 page 76, paragraph 3 -page 77, paragraph 3 page 79, last paragraph -page 80, last paragraph claim 1 ---	1-5,25
X	US 6 019 966 A (COLEMAN ROGER ET AL) 1 February 2000 (2000-02-01) column 2, line 50 -column 3, line 49; claims 1-3 ---	28-31
X	WO 00 77195 A (BIOPH BIOTECH ENTW PHARM GMBH ;HANKE MICHAEL (DE); POHL JENS (DE);) 21 December 2000 (2000-12-21) page 1, line 10-20; claims 1,17-22 ---	28-31
X	US 5 811 098 A (CULOUSCOU JEAN-MICHEL ET AL) 22 September 1998 (1998-09-22) column 4, line 28 -column 5, line 33; claims 1-17 ---	28-31
X	WO 96 41523 A (YAYON AVNER ;YEDA RES & DEV (IL)) 27 December 1996 (1996-12-27) page 1, line 1.6; claims 1-20 ---	28-31
X	US 5 959 097 A (COWSERT LEX M ET AL) 28 September 1999 (1999-09-28) column 1, line 40-62 column 2, line 43-57; claims 1-20 --- -/--	28-31

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EI 2/01073

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 783 186 A (ARAKAWA TSUTOMU ET AL) 21 July 1998 (1998-07-21) column 2, line 58 -column 3, line 26; claims 1-13	28-31
X	US 5 990 109 A (IWANOWICZ EDWIN J ET AL) 23 November 1999 (1999-11-23) column 17, line 42-48 column 19, line 53 -column 20, line 16 claims 32,41	28-31
X	US 5 922 697 A (SHAHRIPOUR AURASH ET AL) 13 July 1999 (1999-07-13) column 1, line 10-44 column 12, line 34-37 claims 1,5,9	28-31
X	US 5 670 324 A (LITTMAN DAN ET AL) 23 September 1997 (1997-09-23) column 3, line 18-41; claims 1,2	28-31

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern[illegible]al application No.
[illegible]T/EP 02/01073

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

INTERNATIONAL SEARCH REPORT International Application No. PCT/EP 02/01073

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1 - 27

Method for the identification of nucleic acids functionally associated with a desired phenotype and use thereof.

2. Claims: 28 - 31 (partially)

Use of a panel of nucleic acids, depicted in Tables 1, 2 and 5, as target for diagnostic/therapeutic applications and for use in a screening procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/01073

Concerning the partial search for the second invention (claims: 28 - 31):

Claims 28 - 31 relate to the use of a panel of nucleic acids, depicted in Tables 1, 2 and 5, as target for diagnostic/therapeutic applications and for use in a screening procedure.

This use was found to bear no common technical relationship with the method and use of claims 1 - 27, which is generically directed to a method for identifying the genetic determinants of a desired phenotype. The applicant has therefore received the invitation pursuant to Rule 40.1 PCT and has paid additional search fees for the second invention.

The International Searching Authority considers that for this second invention recited in claims 28 - 31, a further lack of unity in view of the prior art as disclosed in

US5783186
US5959097
US6019966
US5811098
WO96/41523
WO00/77195

has been revealed during the search for those parts of the International Application which relate to the second invention in respect of which an additional fee has been paid.

The above prior art discloses the use of *HER2, MAPKK2, FGFR3, JAK2, EGFR* and *HER4* as target for diagnostic/therapeutic applications. The common technical concept involved in claims 28 - 31 (use of selected nucleic acids as targets) is therefore known. The use of each of the nucleic acids listed in Tables 1, 2 or 5 therefore constitutes a separate invention; the requirements of Rule 13.1 PCT are not therefore met.

Only subject-matter related to the first invention ("main invention") in those parts (i.e. **the first nucleic acid listed in Table 1)** thus identified has been the subject of a search.

This International Search Report has therefore been limited to:

Claims 28 - 31: use of *HCK* as target (for diagnostic/therapeutic applications, for a screening procedure to identify novel drugs)...)

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT).

The applicant is advised that the EPO policy when acting as International Preliminary Examining Authority is normally not to carry out a preliminary examination on non-unitary matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any International Preliminary Examination procedure.

Form PCT/ISA/210

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No PCT/E. .2/01073

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02059374	A	01-08-2002	WO 02059374	A1	01-08-2002
WO 9941371	A	19-08-1999	AU 2678999	A	30-08-1999
			CA 2319112	A1	19-08-1999
			EP 1053317	A1	22-11-2000
			WO 9941371	A1	19-08-1999
WO 0001846	A	13-01-2000	AU 4907999	A	24-01-2000
			BR 9911802	A	22-01-2002
			CA 2332619	A1	13-01-2000
			CN 1323354	T	21-11-2001
			DE 29924298	U1	12-09-2002
			DE 29924299	U1	12-09-2002
			DE 1093526	T1	11-10-2001
			WO 0001846	A2	13-01-2000
			EP 1197567	A2	17-04-2002
			EP 1093526	A2	25-04-2001
			GB 2349885	A ,B	15-11-2000
			GB 2362885	A ,B	05-12-2001
			GB 2370275	A ,B	26-06-2002
			HU 0103571	A2	28-01-2002
			JP 2002519072	T	02-07-2002
			NO 20010019	A	05-03-2001
			PL 347978	A1	06-05-2002
WO 0053813	A	14-09-2000	WO 0053813	A1	14-09-2000
WO 9832880	A	30-07-1998	AU 5927598	A	18-08-1998
			EP 0996741	A1	03-05-2000
			JP 2002514914	T	21-05-2002
			WO 9832880	A1	30-07-1998
US 6019966	A	01-02-2000	US 5914393	A	22-06-1999
WO 0077195	A	21-12-2000	AU 6262700	A	02-01-2001
			WO 0077195	A1	21-12-2000
US 5811098	A	22-09-1998	AU 3963295	A	06-05-1996
			CA 2202533	A1	25-04-1996
			EP 0787187	A1	06-08-1997
			FI 971532	A	11-06-1997
			JP 10507362	T	21-07-1998
			NO 971686	A	16-06-1997
			WO 9612019	A2	25-04-1996
			AU 676502	B2	13-03-1997
			AU 5180493	A	09-06-1994
			CA 2103323	A1	25-05-1994
			EP 0599274	A1	01-06-1994
			FI 935175	A	25-05-1994
			JP 7132084	A	23-05-1995
			MX 9307317	A1	30-06-1994
			NO 934163	A	25-05-1994
WO 9641523	A	27-12-1996	AT 213590	T	15-03-2002
			AU 715996	B2	17-02-2000
			AU 6014496	A	09-01-1997
			AU 721773	B2	13-07-2000
			AU 6014596	A	09-01-1997

Form PCT/ISA/210 (patent family annex) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/E 02/01073

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641523 A		CA 2223701 A1	27-12-1996
		CA 2224229 A1	27-12-1996
		CN 1195982 A	14-10-1998
		DE 69619526 D1	04-04-2002
		DE 69619526 T2	31-10-2002
		DK 836380 T3	22-04-2002
		EP 0833620 A1	08-04-1998
		EP 0836380 A1	22-04-1998
		ES 2173289 T3	16-10-2002
		WO 9641620 A1	27-12-1996
		WO 9641523 A1	27-12-1996
		JP 11508358 T	21-07-1999
		JP 11507828 T	13-07-1999
		US 2002193309 A1	19-12-2002
		US 6447783 B1	10-09-2002
		US 6517872 B1	11-02-2003
US 5959097 A	28-09-1999	AU 5122799 A	13-06-2000
		WO 0031103 A1	02-06-2000
US 5783186 A	21-07-1998	AU 723492 B2	31-08-2000
		AU 1278497 A	27-06-1997
		CA 2236913 A1	12-06-1997
		EP 0865448 A1	23-09-1998
		JP 2002502230 T	22-01-2002
		US 6458356 B1	01-10-2002
		WO 9720858 A1	12-06-1997
		US 2002164334 A1	07-11-2002
US 5990109 A	23-11-1999	AU 3313799 A	20-09-1999
		CA 2322311 A1	10-09-1999
		EP 1066286 A1	10-01-2001
		JP 2002505330 T	19-02-2002
		WO 9945009 A1	10-09-1999
		ZA 9901721 A	10-10-2000
US 5922697 A	13-07-1999	AU 7392696 A	28-04-1997
US 5670324 A	23-09-1997	US 5439819 A	08-08-1995
		US 5504000 A	02-04-1996

フロントページの続き

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4B063 QA01 QA18 QA19 QQ02 QQ08 QQ42 QQ53 QR08 QR32 QR42
QR50 QR55 QR59 QR66 QR72 QR77 QR82 QS25 QS28 QS34
QS36 QS39 QX01

专利名称(译)	鉴定功能性核酸的方法		
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[标]申请(专利权)人(译)	马普科技促进协会		
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IPC分类号	G01N33/50 C12N15/00 C12Q1/68 C12Q1/6837 C12Q1/6883 G01N27/447 G01N33/15 G01N33/53 G01N37/00		
CPC分类号	C12Q1/6883 C12Q1/6837 C12Q2600/158		
FI分类号	C12Q1/68.A G01N27/26.315.H C12N15/00 G01N33/15.Z G01N33/50.Z G01N33/53.D G01N33/53.M G01N37/00.102		
F-TERM分类号	2G045/AA35 2G045/BB09 2G045/BB14 2G045/BB20 2G045/BB50 2G045/BB51 2G045/CB01 2G045/DA12 2G045/DA13 2G045/DA14 2G045/DA36 2G045/FA11 2G045/FA14 2G045/FB02 2G045/FB07 2G045/FB12 2G045/GC11 2G045/GC15 4B063/QA01 4B063/QA18 4B063/QA19 4B063/QQ02 4B063/QQ08 4B063/QQ42 4B063/QQ53 4B063/QR08 4B063/QR32 4B063/QR42 4B063/QR50 4B063/QR55 4B063/QR59 4B063/QR66 4B063/QR72 4B063/QR77 4B063/QR82 4B063/QS25 4B063/QS28 4B063/QS34 4B063/QS36 4B063/QS39 4B063/QX01		
代理人(译)	矢野俊夫		
优先权	60/265631 2001-02-02 US		
外部链接	Espacenet		

摘要(译)

本发明涉及鉴定功能上与所需表型相关的核酸分子的方法。