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(54)【発明の名称】胃癌の悪性度診断用キット

(57)【要約】　（修正有）
【課題】胃癌の悪性度を決めるために使用するキットを提供する。
【解決手段】ヒトＬＡＴ１アミノ酸残基の、Ｎ末端から１～５２位を特異的に認識する抗ＬＡＴ１モノクローナル抗体を有する、免疫組織学染法によって胃癌の悪性度を決定するために使用されるキットを提供する。
【選択図】なし

【特許請求の範囲】
【請求項１】
抗ＬＡＴ１モノクローナル抗体を有する、免疫組織学染法によって胃癌の悪性度を決定するために使用されるキット。
【請求項２】
該モノクローナル抗体は、ヒトＬＡＴ１アミノ酸残基の、Ｎ末端から１～５２位を特異的に認識する、請求項１記載の胃癌の悪性度を決定するために使用されるキット。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は胃癌の悪性度を決めるために使用するキットである。詳しくは抗LAT1抗体を使用した免疫組織学的染色法によって、胃癌の悪性度を決めるためのキットである。
【背景技術】
【０００２】
細胞が増殖や成長のために細胞膜を介してアミノ酸を細胞内に取り込むためには正常細胞のみならず癌細胞でもアミノ酸トランスポーターが必須である。大型の中性アミノ酸の輸送にはL型アミノ酸トランスポーターが必要であり、多くはL型アミノ酸トランスポーター(LAT1，SLC7A5)に依存しており、このLAT1は金井らによって最初にクローニングされた[1]。続いて、２番目のタイプとして機能的にも分子的にも別個のLAT2 が分離された。このLAT2は大型のみならず小型のアミノ酸も輸送する[2]。
【０００３】
LAT1は主としてヒトの正常脳、脾臓、胸腺、精巣、胎盤組織や前立腺、食道、肺などの癌組織に発現している[3-5]。本発明者らは前立腺癌の予後の判定には従来のGleason の組織学的グレイドシステムとともにLAT1の発現が有効なマーカーであることを以前に示した。さらに幾つかの癌細胞株でもLAT1 mRNAの発現が高いことも認めている[6]。
【０００４】
しかしながら胃癌においてはこれまで不明であった。その為、胃癌におけるLAT1の免疫組織学的発現を本研究で検索した。すなわち我々が最近発明した抗LAT1モノクローナル抗体を使用して、非腫瘍性胃粘膜、腺腫、スキルス胃癌、非スキルス胃癌でLAT1発現を比較した。
【発明の概要】
【０００５】
アミノ酸トランスポーターは正常細胞、腫瘍細胞の維持・増殖にとって不可欠である。本研究では、我々が最近開発したモノクローナル抗体を使って、胃癌におけるL型アミノ酸トランスポーター1 (LAT1) の免疫組織化学的発現を胃腺腫及び非腫瘍性胃粘膜と比較して検索した。進行胃癌87症例を検索して、胃スキルス癌よりも非スキルス癌の方で癌細胞の細胞膜に有意に高いLAT1発現をみとめた。また、リンパ節転移のある胃癌症例では転移のない症例よりもLAT1発現が有意に高かった。LAT1発現と細胞増殖能のマーカーであるKi-67標識率（LI）とは正の相関がみとめられ、非スキルス胃癌では高LAT1発現症例が低発現症例に比較して、有意に予後が不良であった。Cox hazard test によって、非スキルス胃癌ではTNM stage (腫瘍の大きさ、リンパ節転移、血行性転移による進行度)とLAT1発現は互いに独立した予後因子になることを明らかにした。さらにスキルス胃癌を除いた未分化型胃癌では、高LAT1発現群で有意に予後が悪いことも示した。胃癌に比較して胃腺腫ではLAT1発現が有意に低かった。結論として、LAT1発現は胃癌の細胞増殖及び予後とリンクしており、LAT1を標的とした抑制剤が抗癌剤として将来役に立つ可能性があげられる。
【０００６】
このように本発明は、
[1]抗LAT1モノクローナル抗体を使用した免疫組織化学染色キットは胃癌の悪性度の判定に使用できる。
[2]本モノクローナル抗体はヒトLAT1のN末端1-52のペプチドを特異的に認識することから

、ヒト癌の悪性度を評価できる。

【図面の簡単な説明】

【０００７】

【図１】胃癌細胞膜での代表的なL型アミノ酸トランスポーター(LAT1) の免疫反応性発現：(a) 強さ 0, なし；1,弱陽性；2, 中等度陽性； 3, 強陽性。

【図２】正常胃粘膜、胃癌症例の背景胃粘膜(腸上皮化生, IMなし) 、胃癌症例の背景胃粘膜(腸上皮化生あり) 、胃腺腫、胃癌におけるLAT1 発現の(a) 強さと (b) スコアの比較。 胃癌分化型と未分化型とにおけるLAT1発現の強さとスコアの比較(c, d)。胃癌の非スキルス型とスキルス型とにおけるLAT1発現の強さとスコアの比較(e, f)。*p値<0.05.

【図３】胃正常粘膜 (a)、腸上皮化生(IM) (b), 胃腺腫 (c)、分化型腺癌 (d) の組織像(HE組織像)。それぞれのLAT1 発現。正常の胃粘膜（陰窩底部で強さ1の発現）(e)、腸上皮化生のある粘膜固有層の下1/2 で強陽性 (f)、腺腫（強さ2の発現）(g)、分化型腺癌(強さ3の強発現)(h)。

【図４】正常胃粘膜、胃癌症例の背景胃粘膜(腸上皮化生, IMなし) 、胃癌症例の背景胃粘膜(腸上皮化生あり)、胃腺腫、胃癌におけるKi-67(LI)の比較。*p値<0.05.

【図５】a) 全体の症例における生存曲線。LAT1発現低スコア(0-4) 群とLAT1発現高スコア(6-9) 群との間に有意な差なし(p =0.0997)。b) 非スキルス型胃癌症例での生存曲線。LAT1発現高スコア(6-9) 群はLAT1発現低スコア(0-4) 群と比較して予後が有意に不良(p =0.0270)。c) 非スキルス型胃癌症例中の進行度stage IB とII に限定した症例での解析。LAT1発現高スコア(6-9) 群はLAT1発現低スコア(0-4) 群と比較して、予後が有意に不良(p =0.0156)。

【図６】a) 進行癌Ｉ型（癌細胞の粘液が腸型）を除いた胃癌症例の生存曲線。LAT1発現高スコア(6-9) 群はLAT1発現低スコア(0-4) 群と比較して予後不良な傾向あり(p =0.057)。b) 未分化型胃癌症例の生存曲線。LAT1発現高スコア(6-9) 群はLAT1発現低スコア(0-4) 群と比較して予後不良な傾向あり(p =0.0558)。c) 非スキルス型胃未分化癌の生存曲線。LAT1発現高スコア(6-9) 群はLAT1発現低スコア(0-4) 群と比較して予後が有意に不良(p =0.0177)。

【具体的記載】

【０００８】

本発明の目的は、ヒト胃癌におけるLAT1発現が正常胃粘膜や非癌性病変とでは違うことを証明することである。本発明者は、既に報告されている脳グリオーマ(神経膠腫)、肺癌、食道癌と同様に、胃癌においてもLAT1高発現が悪性度を示すことを確認した[4,5,12]。しかし、スキルス型胃癌は非スキルス型胃癌に比較してLAT1発現スコアが低く、この型の癌細胞はアミノ酸輸送においてLAT1依存度が低いことが示唆される。この現象は、今後real time PCR等の他の方法で確認する必要がある。

【０００９】

胃腺腫はLAT1 発現スコアも強さも胃癌と正常腺腺窩上皮との中間の値を示した。本発明者は胃における良性及び悪性上皮性腫瘍のLAT1発現を確認した。他の臓器の良性腫瘍では、口腔の異形成病変、肺の異型腺腫様過形成病変でLAT1発現が報告されている。驚いたことに、胃癌症例の背景胃粘膜の腸上皮化生で胃癌と同様のLAT1高発現がみとめられた[13,14]。このことは、ヒト大腸の粘膜にLAT1が発現していることと関連しているかもしれない[15]。このように胃の腸上皮化生における高LAT1発現は粘膜の表現型として説明しうる。しかしながら、I型胃癌（癌細胞の粘液が腸型）とG型胃癌（癌細胞の粘液が胃型）の間にはLAT1発現において有意な差が認められなかった。すなわち、本発明者はG型胃癌でもLAT1高発現を確認した。腸型胃癌を除いた胃癌に限定すると、LAT1発現高スコア群が低スコア群に比較して予後が不良な傾向を認めた。

【００１０】

胃癌においてKi-67標識率とLAT1発現強さとの弱い相関が認められたことはLAT1が細胞増殖に寄与していることを意味しているかもしれない。この現象は肺の非小細胞癌でも報告されている[16]。しかしながら、他の幾つかの癌ではそのような相関が認められていない

ので、この食い違いについてはさらなる研究が必要である。本研究では、胃癌におけるLAT1とp53発現との間で弱い相関がみとめられたことは、LAT1発現がp53によって規制されている可能性がある。しかし、この点についてはやはりさらなる検索による確認が必要である。

【００１１】

既報では、肺癌において、LAT1発現が有意な予後規定因子であり、予後不良な結果を示している[3,16,17]。例えば、Kaira らによると、LAT1陽性肺癌は陰性肺癌に比べてリンパ節転移率が有意に高い[16]。この報告と同様に、本発明者はリンパ節転移のある胃癌症例ではリンパ節転移のない胃癌症例に比較して、LAT1発現スコアが有意に高いことを示した。しかしながら、我々の研究では、悪性度の高いスキルス癌を含む胃癌症例全体の検索ではLAT1発現と予後との有意な関連を見つけられなかった。一方、非スキルス癌に限定すると、LAT1発現高スコア群は有意に予後が不良であり、多変量解析でもLAT1発現が予後決定因子であることを確認できた。このことはLAT1発現とp53発現とに有意に相関があったことと関連しているかもしれない。また、スキルス胃癌症例の予後が不良であることは癌性腹膜炎を生じやすいなど、他の因子によっている可能性があげられる。

【００１２】

結論として、正常胃粘膜に比較して胃腺腫はLAT1発現が高く、胃癌細胞はさらに高い。LAT1発現の強さは胃癌の細胞増殖と予後に相関しているため、近い将来開発されるLAT1抑制物質が画期的な分子標的抗癌治療になりうる可能性を示している。LAT1発現が増強していることは、癌細胞が細胞増殖に必要な栄養物を強く要求していると考えられる。このように、LAT1機能を抑制することはヒトの多くの種類の癌治療法の開発につながる[18]。今後、癌細胞の増殖を抑制する低分子LAT1抑制物質が胃癌において有意に治療効果を示しうることは、大いに期待できる。

【実施例】

【００１３】

１．モノクローナル抗体の作成

LAT1のN末端の52 アミノ酸（アミノ酸1-52に相当）に対する抗ヒトLAT1モノクローナル抗体をハイスループットプロテオミクス法にて作成した[20,21]。この方法で、特異的な抗LAT1モノクローナル抗体を作るハイブリドーマを得た。このハイブリドーマ細胞をマウスBALB/c に腹腔内に注射した後に、腹水を回収してこれをアフィニティカラム(HiTrap protein G, GE Healthcare Bio-science AB) を通して精製抗体を得た。

【００１４】

２．患者と試料

1993年6月より2003年4月までに北里大学東病院で外科的に切除された進行胃癌（胃壁固有筋層内またはそれより深く浸潤）87症例を収集した。切除された胃は全て、10%フォルマリン固定し、腫瘍病変は5mmの厚さで全割して、パラフィンに包埋した。パラフィンブロックから、４μm厚さの切片を作り、HE染色を施した。全ての症例は日本胃癌取り扱い規約に則り[7]、組織学的に診断した。87症例中、28例がスキルス型胃癌であった。残りの59症例は非スキルス型胃癌であったが、その内訳は高分化型腺癌11例、中分化型腺癌15例、充実性低分化型腺癌20例、非充実性低分化型腺癌10例、印鑑細胞癌3例であった。

本発明者は、癌病変のLAT1発現に加えて、他に4群におけるLAT1発現を検索した。すなわち、正常胃粘膜20例（gastrointestinal stromal tumor 例や膵癌症例で胃粘膜に病変がないもの）、腸上皮化生のない背景胃粘膜32例(40才以下の胃癌症例の背景胃粘膜)、腸上皮化生のある背景胃粘膜37例(75才以上の胃癌症例で背景に腸上皮化生があるもの)、及び胃腺腫36症例である。

【００１５】

３．免疫組織化学

既に報告されている方法に従って[3]、外科的に切除されてフォルマリン固定・パラフィン包埋された組織の4μm厚さ切片を使って免疫組織学的染色を行った。使用した一次抗体、希釈、抗原賦活は表１にまとめた。

簡潔に記載すると、組織切片を脱パラフィンし、1%過酸化水素を含むメタノール中で３０分間処理して、内因性peroxidase をブロックした。その後、非特異的反応のブロックの為にProtein block Serum-Free (Dakocytomation, Kyoto, Japan)であらかじめ培養し、切片を一次抗体とともに37℃で１時間培養した。さらにperoxidaseでラベルしたpolymer (Envision, Dakocytomation, Kyoto, Japan), 続いて抗マウスIgG (Gout, Nichirei Bioscience, Tokyo, Japan)抗体で各30分間培養し、3,3’-diaminobenzidine で発色した。核はMeyerのヘマトキシリン溶液で対比染色した。

【００１６】

４．免疫組織染色の評価

LAT1の免疫反応性の評価はSinicropeの方法[3]の軽度変法を採用した。すなわち、癌細胞膜の免疫反応性をもとに、4カテゴリを次のように規定した。発現強さ0, 反応なし；1, 弱くないしは点状に陽性；2, 中等度に細胞膜全体が陽性；3, 細胞膜全体が強く陽性。各症例で癌組織中における最も高いLAT1発現の強さを採用した。胃癌細胞での代表的なLAT1発現の強さを図１に示した。さらにLAT1発現の領域を全体の胃癌組織を観察してその百分率であらわした。すなわち、0, 陽性なし；1, 局所的 1-10%陽性；2, 部分的 11-30% ; 3, びまん性 30%以上とした。LAT1発現スコアはLAT1発現強さX発現領域の数値であらわした。

p53 発現は核に強く発現している領域を癌組織全体の百分率として、0, 陽性なし；1, 局所的 1-10%陽性；2, 部分的 11-30% ; 3, びまん性 30%以上と評価した。

胃癌組織の粘液表現型の分類は、CD10 (刷子縁), MUC2 (腸杯細胞), MUC5AC (胃腺窩上皮細胞) 及びMUC6 (胃幽門腺)の免疫組織化学的反応性によって行い、4カテゴリ、胃型（G型）、腸型（I型）、胃腸型（GI型）及び分類不可型（U型）を規定した[9,10]。いずれのマーカーにも陰性な癌はU型とした。

Ki-67陽性細胞は1000以上の有核細胞から陽性細胞を数え、その%をKI-67標識率（LI）とした。

【００１７】

５．統計学的解析

各グループ間の比較はchi-squared, Mann-Whitney U 或いはKruskal-Wallis test を必要に応じて適用した。患者の生存曲線の比較の有意差はlog-rank testによった。LAT1, Ki-67 LI 及びp53の間での相関はSpearman’s rank correlation coefficient testによって解析した。全ての総計学的解析にはStatView software (Abacus Concepts, Inc. Berkeley, CA, USA)を採用し、p値が0.05以下を統計学的に有意とした。

【００１８】

６．結果

(1) 患者の特徴

検索した胃癌患者症例は男性54, 女性33からなっていた。年齢は33から85才にわたり、平均は60才であった。TNM分類[11]による病理学的進行度による症例の内訳は、pT IB 17, pT II 17, pT IIIA 15, pT IIIB 4, pT IV 34症例であった。本研究における胃癌症例の臨床病理学的因子は表２にまとめた。87症例中、56例が胃癌で死亡した。分化型胃癌は12, 未分化型胃癌は44例であった。12例の分化型胃癌の中, 3例に肝転移(25%)、1例に肺転移(8%)を認めた。一方、44例の未分化型胃癌では、2例に肝転移(4.5%)、11例に腹膜播種(25%),1例に癌性リンパ管症(2.3%)を認めた。

【００１９】

(2) LAT1 発現

正常胃粘膜における腺か上皮のLAT1発現はごくわずかで、LAT1発現の強さは低値（1.1±1.2, 平均 ± 標準偏差, 図2-a）であった。しかし腸上皮化生のある背景胃粘膜では、LAT1発現強さはより強かった (2.4 ± 0.9)。胃腺腫でもLAT発現は認められ (1.9 ± 1.2)、粘膜固有層の下1/2よりも上1/2の方が強かった。

胃癌では癌細胞膜に強く発現していた (2.6 ± 0.8)。分化型胃癌と未分化型胃癌の間でのLAT1発現に有意な差はなかった。しかし、非スキルス型胃癌はスキルス型胃癌に比べ

て、LAT1発現スコアが有意に高かった(図2-f)。またLAT1発現スコアは胃腺腫に比較して胃癌でより高かった。さらに胃腺腫や腸上皮化生のある背景胃粘膜におけるLAT1発現の強さは正常粘膜よりも有意に強かった。

正常粘膜、腸上皮化生のある背景胃粘膜、胃腺腫及び胃癌の代表的なLAT1発現を図3に示した。

【００２０】

(3) 胃癌の粘液表現型とLAT1発現

粘液の4マーカー発現に基づいて、87症例を4カテゴリへ分類した。26例はG型、22例はI型、38例はGI型、1例がU型であった。これら4カテゴリの間でLAT1発現を比較したが、有意な違いをみとめなかった。

【００２１】

(4) リンパ節転移とLAT1発現との関連

リンパ節転移のある症例は転移のない症例と比較して、癌細胞のLAT1発現の強さが高い傾向を認めた (p=0.0573)。さらにLAT1スコアはリンパ節転移例で転移のない症例と比較して有意に高かった(p=0.0077)。癌細胞のリンパ管や血管侵襲とLAT1発現とは有意な相関がみられなかった（データ省略）。

【００２２】

(5) p53 発現とLAT1発現との関連

Spearman’s rank correlation coefficient test によると、胃癌全症例でのp53スコアとLAT1発現の強さとの間には弱い相関を認めた(r=0.459, p<0.0001)。さらに胃癌全症例でのp53発現スコアとLAT1発現スコアとの間にも弱い相関を認めた(r=0.463, p<0.0001)。

【００２３】

(6) Ki-67 LI とLAT1発現との関連

図４に示したように、胃癌におけるKi-67 LIは他の胃病変グループより高かった。胃癌全症例でのKi-67 LI とLAT1発現強さとの間に弱い相関を認めた(r=0.428, p<0.001)。Ki-67 LI とLAT1発現スコアとの間には有意な相関を認めなかった。

【００２４】

(7) 患者の生存曲線

低及び高LAT1 発現スコア (低スコア0-4、高スコア6-9)で87症例を分けて生存曲線を比較した。全症例では、低LAT1スコア及び高LAT1 スコア群で有意な差がなかった(p=0.0997, 図5a)。しかし、非スキルス型胃癌では、高LAT1発現群は予後が有意に不良であった(p=0.0270, 図5b)。 特にstage IB とIIの非スキルス型胃癌に限定すると、高LAT1は予後が有意に不良であった(p=0.0156, 図5c)。

さらにI型(腸型)を除いた胃癌症例及び未分化癌では、高LAT1 スコア群が予後不良の傾向を示した(それぞれp=0.0570, 図6a；p=0.0558, 図6b)。

さらにスキルス型胃癌を除いた未分化癌（33例）では高LAT1 スコア群が有意に予後不良であった(p=0.0177, 図6c)。

【００２５】

(8) Cox hazard テスト

87症例全体では、癌の進行度stage のみが予後規定因子であった(表３)。非スキルス型胃癌に限定すると、LAT1 スコアとstage が単変量解析で、予後規定因子として認められた。続いて多変量解析でも両者は個々の独立した予後因子であった(表４)。

【表１】

表１．免疫組織化学染色に使用した一次抗体

Antibody	Clone	Source	Dilutation	Antigen retrieval
LAT1	Monoclonal	J-Pharma, Tokyo, Japan	Prediluted	Microwave treatment for 15 min
Ki-67	Monoclonal MIB-1	Dakocytomation, Glostrup, Denmark	1:100	Microwave treatment for 15 min
p53	Monoclonal DO7	Dakocytomation, Glostrup, Denmark	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (PH9))
CD10	Monoclonal 56C6	Novocastra, Newcastle-upon-tyne, UK	1:50	-
MUC2	Monoclonal Ccp58	Novocastra, Newcastle-upon-tyne, UK	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (PH9))
MUC5AC	Monoclonal CLH2	Novocastra, Newcastle-upon-tyne, UK	1:200	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (PH9))
MUC6	Monoclonal CLH5	Novocastra, Newcastle-upon-tyne, UK	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (PH6))

【表２】

表２．87 胃癌症例の臨床病理学的特徴

Age (years)	
Median (range)	60 (33-85)
Gender	
Male	54
Female	33
Tumor maximum diameter	
Mean (range) (cm)	8.8(2.0-24.0)
Stage	
ⅠB	17
Ⅱ	17
ⅢA	15
ⅢB	4
Ⅳ	34
Lymph node metastasis	
N(−)	23
N(+)	64
Histological grade	
differentiated type	26
undifferentiated type	61

【表３】

表３．外科的に切除された胃癌症例の予後に関する
単変量 Cox hazard 解析 (n=87)

variable	Univariate analysis OR (95% CI)	*P* value
Age (median, 60 years; range, 33-85 years	1.566(0.906-2.710)	0.1084
LAT1 intensity		
0 vs 1-3 (5:84)	1.451(0.353-5.962)	0.6059
0-1vs 2-3 (7:80)	1.033(0.411-2.596)	0.9451
0-2 vs 3 (17:70)	1.567(0.765-3.209)	0.2196
LAT1 score		
0 vs 1-9 (4:83)	1.451(0.353-5.962)	0.6059
0-1 vs 2-9 (6:81)	1.886(0.588-6.053)	0.2861
0-2 vs 3-9 (16:71)	1.561(0.736-3.312)	0.2459
0-3 vs 4-9 (50:37)	1.382(0.812-2.354)	0.2330
0-4 vs 6-9 (51:36)	1.557(0.915-2.651)	0.1027
0-6 vs 9(74:13)	1.116(0.526-2.365)	0.7751
Ki-67LI		
Cut off 40% (46:41)	0.907(0.532-1.547)	0.7202
Cut off 45% (55:32)	0.579(0.321-1.039)	0.0671
Stage (TNM)		
Ⅰb-Ⅲa vs Ⅲb-Ⅳ (49:38)	7.085(3.875-12.956)	<0.0001

CI, Confidence interval; LAT1 intensity, L-type amino-acid transporter 1 immunoreactivity (0, no staining; 1, weakly or patchily positive; 2, moderate complete cell membrane staining; 3, intense complete membrane staining. 0,); LAT-1 score, L-type amino-acid transporter 1 immunoreactivity score, multiplication of intensity and density (0, negative; 1, focal, 1-10% positive cells; 2, partial, 11-30% positive cells; 3 diffuse, >30%); LI, labeling index; OR, odds ratio.

【表４】

表４．外科的に切除された非スキルス胃癌症例の予後に関する
Cox hazard 多変量解析(n=59)

variable	Univariate analysis OR (95% CI)	*P* value	Multivariate analysis OR (95% CI)	*P* value
Age(median, 60years; range, 33-85 years)	1.500 (0.740-3.039)	0.2607		
LAT1 intensity				
0 vs 1-3 (2:57)	NA			
0-1vs 2-3 (3:56)	2.198(0.300-16.114)	0.4386		
0-2 vs 3 (9:50)	2.100(0.639-6.998)	0.2215		
LAT1 score				
0 vs 1-9 (2:57)	NA			
0-1 vs 2-9 (2:57)	NA			
0-2 vs 3-9 (7:50)	3.018(0.720-12.646)	0.1307		
0-3 vs 4-9 (29:30)	2.415(1.161-5.024)	0.0183		
0-4 vs 6-9 (30:29)	2.201(1.074-4.513)	0.0270	2.696(1.302-5.582)	0.0076
0-6 vs 9(47:12)	1.470(0.660-3.275)	0.3456		
Ki-67 LI				
Cut off 40% (22:37)	0.921(0.450-1.885)	0.8218		
Cut off 45%(32:27)	0.693(0.342-1.405)	0.3088		
TNM stage				
Ⅰb-Ⅲa vs Ⅲb-Ⅳ(36:23)	3.442(1.688-7.021)	<0.0001	7.153(3.295-15.529)	<0.0001

CI, Confidence interval; LAT1 intensity, L-type amino-acid transporter 1 immunoreactivity (0, no staining; 1, weakly or patchily positive; 2, moderate complete cell membrane staining; 3, intense complete membrane staining. 0,); LAT-1 score, L-type amino-acid transporter 1 immunoreactivity score, multiplication of intensity and density (0, negative; 1, focal, 1-10% positive cells; 2, partial, 11-30% positive cells; 3 diffuse, >30%); LI, labeling index; NA, not available; OR, odds ratio.

【００２６】

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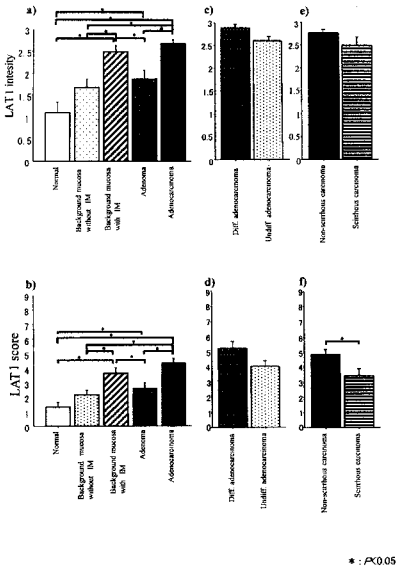
【図２】

図２

Figure2

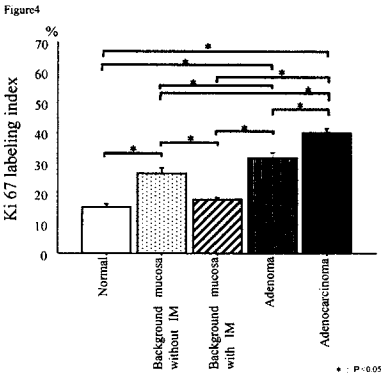


正常胃粘膜、胃癌症例の背景胃粘膜(腸上皮化生, IM なし) 、胃癌症例の背景胃粘膜(腸上皮化生あり) 、胃腺腫、胃癌における LAT1 発現の (a) 強さと (b) スコアの比較。 胃癌分化型と未分化型とにおける LAT1 発現の強さとスコアの比較(c, d)。胃癌の非スキルス型とスキルス型とにおける LAT1 発現の強さとスコアの比較(e, f)。*p 値<0.05.

【図４】

図４

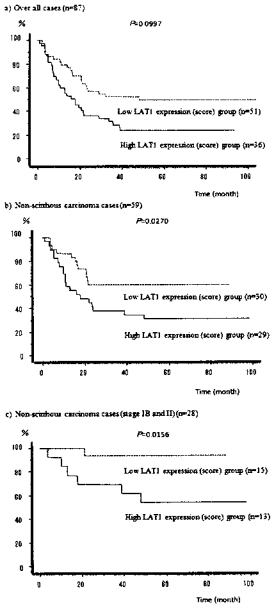
Figure4



正常胃粘膜、胃癌症例の背景胃粘膜(腸上皮化生, IM なし) 、胃癌症例の背景胃粘膜(腸上皮化生あり) 、胃腺腫、胃癌における Ki-67(LI)の比較。*p 値<0.05.

【図５】

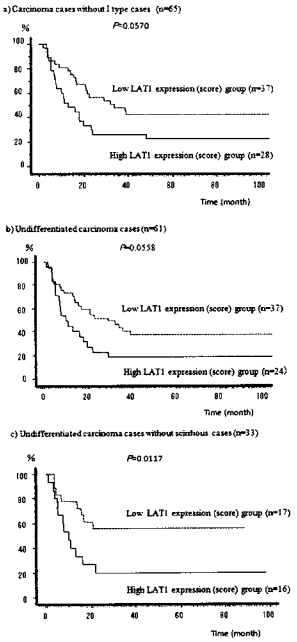
図５



a) 全体の症例における生存曲線。LAT1 発現低スコア(0-4) 群と LAT1 発現高スコア(6-9) 群との間に有意な差なし(p=0.0997)。
b) 非スキルス型胃癌症例での生存曲線。LAT1 発現高スコア(6-9) 群は LAT1 発現低スコア(0-4) 群と比較して予後が有意に不良(p=0.0270)。
c) 非スキルス型胃癌症例中の進行度 stage IB と II に限定した症例での解析。LAT1 発現高スコア(6-9) 群は LAT1 発現低スコア(0-4) 群と比較して、予後が不良(p=0.0156)

【図６】

図６

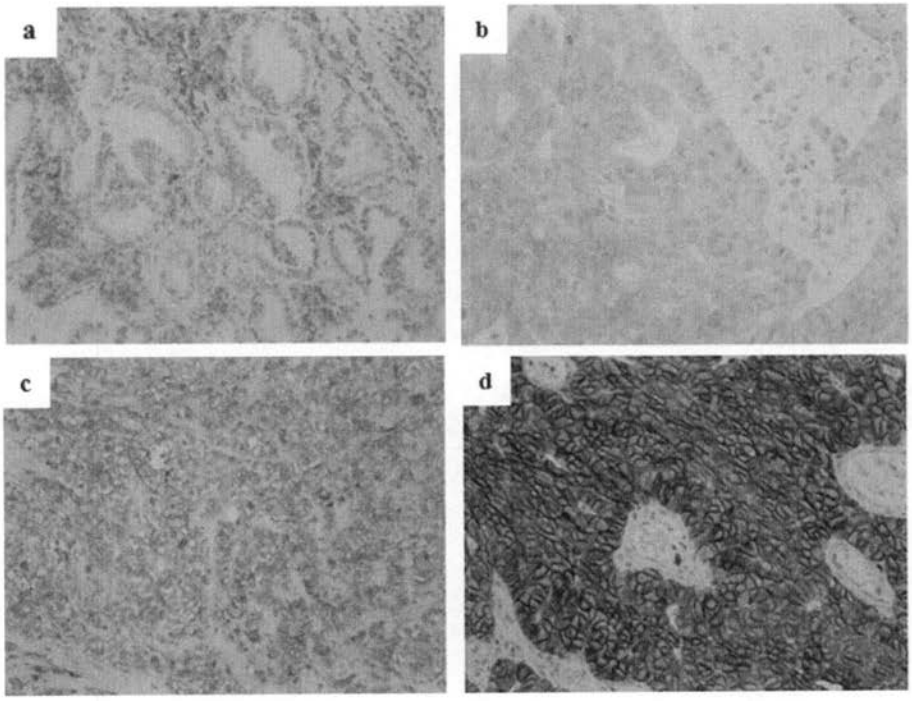


a) 胃癌 I 型 (癌細胞の粘液が腸型) をのぞいた進行胃癌症例の生存曲線。LAT1 発現高スコア(6-9) 群は LAT1 発現低スコア(0-4) 群と比較して予後が不良な傾向 (p=0.057)。
b) 未分化型胃癌症例の生存曲線。LAT1 発現高スコア(6-9) 群は LAT1 発現低スコア(0-4) 群と比較して予後が不良な傾向 (p=0.0558)。
c) 非スキルス型胃未分化癌の生存曲線。LAT1 発現高スコア(6-9) 群は LAT1 発現低スコア(0-4) 群と比較して予後が有意に不良 (p=0.0177)。

【図１】

図1

Figure 1

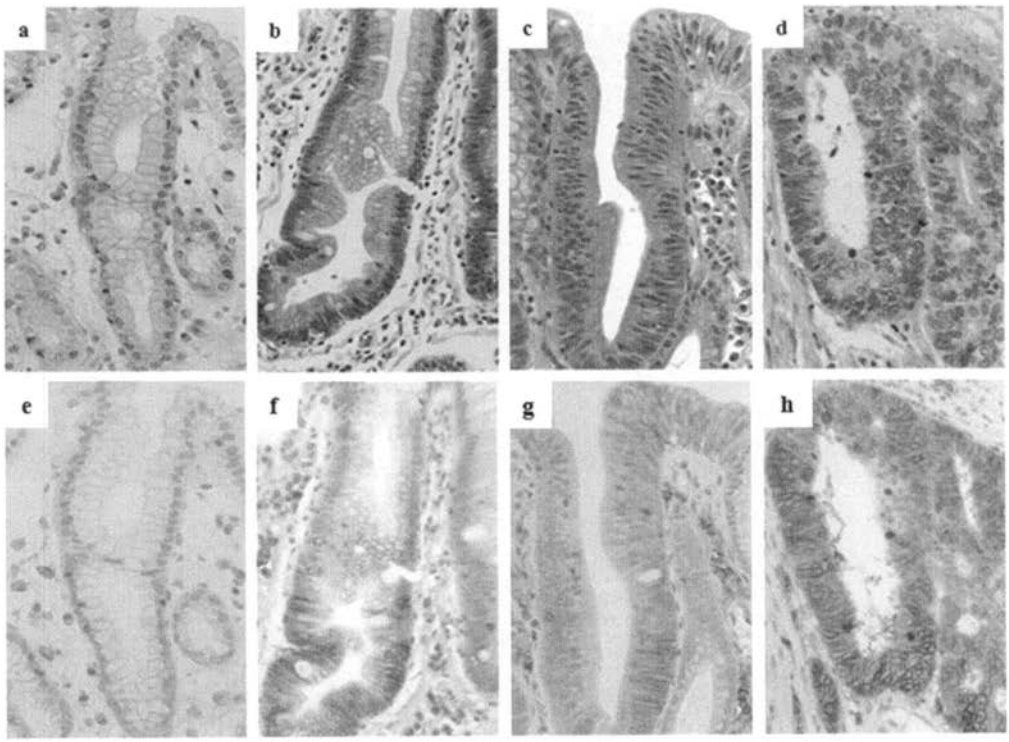


胃癌細胞膜での代表的な L 型アミノ酸トランスポーター(LAT1) の免疫反応性発現：(a) 強さ 0, なし；1, 弱陽性；2, 中等度陽性； 3, 強陽性。

【図３】

図３

Figure3



胃正常粘膜 (a)、腸上皮化生(IM) (b)， 胃腺腫 (c)、分化型腺癌 (d) の組織像。それぞれの LAT1 発現。正常の胃粘膜（粘膜底部で強さ１の発現）(e)、腸上皮化生の粘膜固有層の下 1/2 で強陽性 (f)、腺腫（強さ２の発現）(g)、分化型腺癌(強さ３の強発現)(h)。

フロントページの続き

Ｆターム(参考) 2G045 AA26 BA14 BB22 BB24 CB01 DA36 FA16 FB03
4H045 AA11 CA40 DA76 EA51 FA71

【外国語明細書】

TITLE OF INVENTION

A kit used to determine gastric cancer malignancy

DETAILED DESCRIPTION OF INVENTION

TECHNICAL FIELD

The present invention relates to a kit used to determine gastric cancer malignancy. More specifically, the present invention relates to a kit, comprising an anti-LAT1 monoclonal antibody, used to determine gastric cancer malignancy via immunohistochemical staining.

BACKGROUND ART

Amino acid transport across plasma membranes is mediated via amino acid transporters which are essential for the growth and proliferation of not only normal but also transformed cell. The L-amino acid transporter system responsible for the transport of large neutral amino acids largely depends on L-type amino acid transporter 1 (LAT1, SLC7A5), which was originally cloned by Kanai et al.[1] A second isoform, LAT2, has subsequently been isolated[2] with different functional properties and molecular identity. LAT2 transports not only large but also small neutral amino acids.

LAT1 is mainly expressed in human brain, spleen, thymus, testis, placenta, skeletal muscle and carcinoma cells such as prostatic, esophageal, and lung carcinoma.[3-5] Previously, the present inventors demonstrated that LAT1 expression can be a reliable prognostic marker in prostatic carcinoma, in agreement with the Gleason's histologic grading system. Furthermore, LAT1 mRNA expression is high in some tumor cell lines.[6] However, the situation in gastric carcinoma is unclear, and therefore the present study was conducted to assess immunoreactive LAT1 expression, comparing gastric carcinomas of scirrhous and non-scirrhous types with adenomas and non-neoplastic lesions, using our recently developed monoclonal antibody.

SUMMARY OF THE INVENTION

Amino acid transporters are essential for maintenance and proliferation of both

normal and transformed cells. In the present study, L-type amino acid transporter 1 (LAT1) immunoreactive expression was investigated in gastric carcinomas, in comparison with gastric adenomas and non-neoplastic lesions using our recently developed novel monoclonal antibody. In a total of 87 cases of advanced gastric cancer, high LAT1 expression was observed in carcinoma cells, predominantly at plasma membranes with greater intensity in non-scirrhous than scirrhous carcinomas. Gastric carcinoma cases with lymph node metastasis showed significantly higher LAT1 expression than cases without lymph node metastasis. A positive correlation with Ki-67 LIs was observed and the highly expressing non-scirrhous carcinomas showed a significantly poorer prognosis than the low LAT1 group. Cox hazard test revealed that TNM stage and LAT1 expression were independent prognostic factor in non-scirrhous carcinoma group. Further, a significant poor prognosis was confirmed in high LAT1 expression group, when limited to undifferentiated carcinoma cases excluding scirrhous carcinoma. Lower levels were found in adenomas. In conclusion, LAT1 expression may be linked with cell proliferation and prognosis of gastric carcinomas, and offers a potential target for future anticancer therapy by inhibitors.

Thus, the present invention relates to

[1] a kit, comprising an anti-LAT1 monoclonal antibody, used to determine gastric cancer malignancy via immunohistochemical staining and

[2] the kit used to determine gastric cancer malignancy according to above [1], wherein the monoclonal antibody recognizes human LAT1 amino acid residues specifically at positions 1 to 52 from the N-terminus.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1

Representative L type amino-acid (LAT1) expression in gastric carcinoma cell membranes: (a) Intensity 0, no immunoreactivity ; (b) Intensity 1, weakly positive s; (c) Intensity 2, moderately positive; and (d) Intensity 3, strong expression.

Figure 2

Comparison of LAT1 intensity (a) and scores (b) among normal mucosa, background mucosa without intestinal metaplasia (IM), background mucosa with IM, gastric adenomas, and carcinomas. Comparison of the LAT1 intensity and score between differentiated and undifferentiated types of gastric carcinoma (c, d), and between non-scirrhous and scirrhous types (e, f). *, P value<0.05.

Figure 3

Histologic photographs of (a) normal mucosa, (b) intestinal metaplasia (IM), (c) adenoma, and (d) differentiated type adenocarcinoma. (e) Normal mucosa with weak LAT1 expression, intensity 1 at the bottom of a crypt. (f) Strong LAT1 expression, intensity 3 in the lower half of an area of intestinal metaplasia. (g) Moderate expression of LAT1, intensity 2 in an adenoma. (h) Strong LAT1 expression in a differentiated adenocarcinoma, intensity 3.

Figure 4

Comparison of the Ki-67 labeling indice s among normal mucosa, background mucosa without intestinal metaplasia (IM), background mucosa with IM, adenoma, and adenocarcinomas *, P value<0.05.

Figure 5

a) Survival curves of overall cases. There was no significant difference between low (LAT1 score 0-4) and high expression (LAT1 score 6-9) groups (P=0.0997).

b) Survival curve of non-scirrhous carcinoma cases. There was significant difference between low (LAT1 score 0-4) and high expression (LAT1 score 6-9) groups (P=0.0270).

c) Analysis limited to stage IB and II cases of non-scirrhous carcinoma cases. High LAT1 expression (LAT1 score 6-9) was linked to a poorer prognosis, compared with

low LAT1 expression (LAT1 score 0-4) (Figure 5c, P =0.0156).

Figure 6

a) Survival curves of carcinoma cases without I type. There was tendency of difference between low (LAT1 score 0-4) and high expression (6-9) groups (P=0.057).

b) Survival curves of undifferentiated carcinoma cases. There was tendency of difference between low (LAT1 score 0-4) and high expression (6-9) groups (P=0.0558).

c) Survival curves of undifferentiated carcinoma cases without scirrhous carcinoma. There was significant difference between low LAT1expression (LAT1 score 0-4) and high expression (6-9) groups (P=0.0177).

DESCRIPTION OF THE EMBODIMENT

The aim of the present invention is to evaluate LAT1 expression in gastric carcinomas in comparison with normal tissue and non-cancerous lesions in the human stomach. The present inventors confirmed high expression in malignancies, in line with previously reports for other tumors such as gliomas, lung carcinomas, and esophageal carcinomas.[4, 5, 12] However, scirrhous carcinomas showed lower LAT1 scores than non-scirrhous carcinomas, suggesting reduced use of LAT1 as the main transporter of amino acids. This phenomenon now needs to be confirmed with different methods such as real time polymerase chain reaction.

Adenomas showed intermediate LAT1 scores and intensity between carcinomas and normal foveolar epithelium. The present inventors confirmed LAT1 expression both benign and malignant epithelial tumor in stomach. As for other benign tumor, LAT1 expression was reported in oral dysplastic lesion and atypical adenomatous hyperplasia in lung.[13-14] Surprisingly, background mucosa with IM showed as high LAT1 expression as carcinomas. This might be related to the previous report that LAT1 is expressed in human intestinal epithelial cells.[15] The high expression in intestinal metaplasia could thus be explained by the phenotype. However, there was no significant differences in LAT1 expression between I type carcinomas and G type carcinomas. So, the present inventors confirmed gastric carcinoma of G type had also high LAT1 expression. When limited to carcinoma without I type carcinoma, there was a tendency of poor prognosis in high LAT1 were group compared low LAT1 score.

The weak correlation observed between Ki67 LI and LAT1 intensity in gastric carcinomas might indicate that LAT1 contributes to cell growth, as reported in non

small cell lung cancers.[16] However, several carcinoma cases showed no correlation so that the discrepancies require further study. The weak correlation was also observed between LAT1 expression and p53 expression. LAT1 expression might be regulated by p53. However, this point needs further study clarification.

In previous reports, LAT1 expression was a significant prognostic factor predicting a poor outcome.[3, 16, 17] For example, Kaira reported LAT1 positive lung cancer showed higher lymph node metastasis than LAT1 negative cases.[16] In line with it, the present inventors have demonstrated that gastric carcinoma cases with lymph node metastasis showed higher LAT1 scores than cases without lymph node metastasis. However, our study showed no significant relation between LAT1 expression and prognosis in overall cases, including scirrhous carcinoma cases, which are considered to be highly malignant. When limited to only non-scirrhous carcinomas, the high LAT1 score group showed a significantly poor prognosis, and LAT1 expression was confirmed as a prognostic factor by multivariate analysis. This might be also related to significant correlation of LAT1 expression to p53 expression. Further, poor prognosis of scirrhous carcinoma cases might be caused by other factor including more prevalence of peritoneal dissemination.

In conclusion, gastric carcinoma cells showed higher LAT1 expression than adenomas, which in turn demonstrated increase over normal foveolar epithelium. Intensity was to some extent correlated with cell proliferation and prognosis in gastric carcinomas so that LAT1 inhibitors may have potential as novel drug therapuetics in the future. Increased expression of LAT1 is considered to be associated with high demand for nutrients caused by cell proliferation. Thus, inhibition of LAT1 function could be potential therapeutic strategy for many kinds of cancers. In fact, it has been reported that inhibition of LAT1/CD98hc reduces the growth of breast cancer cells.[18] Whether small molecule inhibitors of LAT1 inhibiting tumor cell growth[19] might also be active in the stomach clearly warrants attention.

EXAMPLES

1. MONOCLONAL ANTIBODY PRODUCTION

An anti-human LAT1 monoclonal antibody was prepared against the 52

amino acid N-terminal residue (corresponding to amino-acids 1-52) synthesized by high-throughput proteomics.[20, 21] A specific hybridoma cell producing the anti-LAT1 monoclonal antibody was obtained. The antibody was purified from the ascites of BALB/C mice, which were injected with the hybridoma cells, using an affinity column (HiTrap protein G, GE Healthcare Bio-science AB).

2. PATIENTS AND SAMPLES

A total of 87 cases of surgically resected advanced gastric carcinoma with invasion deeper than the muscularis propria between June 1993 and April 2003 at Kitasato University East hospital were collected. All of the resected stomachs were fixed in 10% buffered formalin and the tumor lesions were step-sliced at 5mm thickness and processed for embeddng in paraffin. Then 4 micrometer thick sections were cut and used for H&E staining. All cases were histologically diagnosed according to the histologic typing of the Japanese Research Society for Gastric Cancer.[7] Of the 87 cases, 28 were scirrhous carcinomas. Remaining 59 cases were treated as non-scirrhous carcinoma in this study, consisting of 11 cases of well differentiated adenocarcinoma, 15 cases of moderately differentiated adenocarcinoma, 20 cases of solid type poorly differentiated adenocarcinoma, 10 cases of non-solid type poorly differentiated adenocarcinoma, and 3 cases of signet ring cell carcinoma.

In addition to LAT1 expression in carcinoma lesions, the present inventors investigated LAT1 expression in 4 other groups as follows; 20 cases of normal gastric mucosa (little mucosal damage sampled from cases of gastrointestinal stromal tumors and pancreatic carcinomas), 32 cases of background mucosa without intestinal metaplasia (IM) (under 40 year old back ground mucosa of gastric carcinomas of the patients), 37 cases of background mucosa with intestinal metaplasia (back ground mucosa of gastric carcinoma cases with marked intestinal metaplasia in the patients over 75 years old), and 36 cases of gastric adenoma.

3. IMMUNOHISTOCHEMISTRY

Immunohistochemical staining was performed using 4-μm thick, formalin fixed paraffin embedded tissue sections from surgically removed samples according to the

methods previously described.[3] The primary monoclonal antibodies, dilutions and antigen retrieval were summalized in Table1.

Briefly, tissue sections were deparaffinized and endogenous peroxidase was blocked with 1% hydrogen peroxide in methanol for 30 minutes. After incubation with Protein block Serum –Free (Dakocytomation, Kyoto, Japan), slides were incubated with primary antibody for 1 hour at room temperature. After incubation with peroxidase-labeled polymer (Envision, Dakocytomation, Kyoto, Japan), anti-mouse IgG (Gout, Nichirei Bioscience Tokyo, Japan) for 30 minutes, 3,3'-diaminobenzidine was applied as the chromogen. Nuclei were counter-stained with Myer's hematoxylin to facilitate histological assessment.

4. EVALUATION OF THE IMMUNOHISTOCHEMICAL STAINING

Evaluation of immunoreactivity for LAT1 was performed according to the Sinicrope's method[8] with minor modification.[3] Briefly, based on the immunointensity of the carcinoma cell membranes, four categories were defined as follows: intensity 0, no staining; 1, weakly or patchily positive; 2, moderate complete cell membrane staining; 3, intense complete membrane staining. The highest LAT1 intensity observed was adopted in each case. Representative intensity of LAT1 expression in gastric carcinoma cells is shown in Figure 1. The stained area was also evaluated, expressed as the percentage of the whole carcinoma area, and classified as follows: 0, none; 1 (focal), 1-10%; 2 (partial), 11-30%; 3, >30%. Then, immunoreactive scores were calculated by multiplication of the values for the two parameters, intensity and area.

Evaluation of p53 expression was performed based on the percentage area with intense nuclear staining, classified as follows: 0, none; 1(focal), 1-10%; 2 (partial), 11-30%; 3 (diffuse),>30%.

Classification of phenotype was judged with the expression of CD10, MUC2, MUC5AC and MUC6. The phenotypes were classified into 4 categories, gastric type (G type), intestinal type (I type), gastrointestinal type (GI type), and unclassified type (U type). Based on the combination of the expression of CD10 (brush border), MUC 2 (intestinal goblet cells), MUC5AC (gastric foveolar epithelium), and MUC6 (gastric pyloric glands), cases with $MUC5AC^{+}$ or $MUC6^{+}$ and $CD10^{-}$, $MUC\ 2^{-}$ were judged as

G type; $MUC2^+$ or $CD10^+$, $MUC5AC^-$ and $MUC6^-$ were I type; cases with both $MUC\ 2^+$ or $CD10^+$ and $MUC5AC^+$ or $MUC\ 6^+$ were GI type; cases without all 4 marker's expression were U type. [9, 10]

Ki-67 positive cells were counted in > 1,000 cells, and Ki-67 labeling indices (LI) were calculated as percentage values.

5. STATISTICAL ANALYSIS

Comparisons between groups were conducted with the chi-squared, Mann-Whitney U or Kruskal-Wallis tests as appropriate. The statistical significance of differences between survival curves was tested by the log-rank test. Relations among LAT1, Ki-67 LI and p53 were analyzed using the Spearman's rank correlation coefficient test. StatView software (Abacus Concepts, Inc. Berkery, CA, USA) was employed for all statistical analyses and a *P* value less than 0.05 was considered to indicate statistical significance.

6. RESULTS

(1) Patient characteristics

The patients with gastric carcinoma comprised 54 men and 33 women. Their ages ranged from 33 to 85, with a mean of 60 years. Pathological stages by TNM classification[11] and case numbers were as follows; 17 cases of pT I B, 17 cases of pT II, 15 cases of pT III A, 4 cases of pT III B, 34 case of pT IV. Clinicopathological factors for the gastric carcinoma cases are shown in Table 2. Of the 87 cases, 56 died of the disease, 12 were differentiated type and 20 were undifferentiated type. Of the 12 cases of differentiated type, liver metastasis was found in 3 (25%), lung metastasis in one (8%), and peritoneal dissemination in one case (8%). On the other hand, of the 44 cases of undifferentiated type, liver metastasis was found in 2 (10%), peritoneal dissemination in 11 (4.5%), and lymphangitis carcinomatosa in one case (2.3%).

(2) LAT1 expression

In normal mucosa, LAT1 expression was faint in foveolar surface epithelium and LAT1 intensity was low (intensity: 1.1 ± 1.2, mean ± standard deviation, Figure 2-a). However, in background mucosa with IM, a higher LAT1 intensity was observed ($2.4 \pm$

0.9). In gastric adenomas, LAT1 expression was observed (intensity:1.9±1.2). Higher LAT1 expression in the upper part was usually found than in the lower part.

In gastric carcinomas, high LAT1 intensity was observed in carcinoma cells, predominantly on their plasma membranes (intensity: 2.6±0.8). There was no significant difference between differentiated and undifferentiated types. However, non-scirrhous carcinomas showed higher LAT1 scores than their scirrhous counterparts (Figure 2-f). LAT1 scores were also higher in gastric carcinomas than adenomas. Further, gastric adenoma and background mucosa with IM showed higher intensity than normal mucosa.

Representative LAT1 expression in normal mucosa, background mucosa with IM, adenomas, and carcinomas is illustrated in Figure 3.

(3) Phenotype of gastric carcinoma and LAT1 expression

When 87 cases were classified into 4 categories based on 4 markers expression. 26 cases were G type, 22 cases were I type, 38 cases were GI-type, and one case was U type. When LAT1 expression was compared among each category, there were no significant differences.

(4) Correlation between lymph node metastasis and LAT1 expression

There was a tendency that cases with lymph node metastasis showed higher LAT1 intensity than cases without lymph node metastasis (P=0.0573). LAT1 score also was significantly higher in cases with lymph node metastasis than in cases without lymph node metastasis (P=0.0077). There were no significant relations between lymphatic and vascular invasion to the LAT1 expression (data not shown).

(5) Correlation between p53 expression and LAT1 expression

With the Spearman's rank correlation coefficient test, a significant weak correlation was found between p53 score and LAT1 intensity in overall cases of gastric carcinoma (ρ=0.459, P<0.0001). Further, there was also significant correlation between p53 score and LAT1 score in overall cases of gastric carcinoma (ρ=0.463, P<0.0001).

(6) Correlation between Ki-67 LI and LAT1 expression

Ki-67 LI in gastric cancer were higher than in other groups, as shown in Figure 4. With the Spearman's rank correlation coefficient test, a weak correlation was found between Ki-67 LI and LAT1 intensity in gastric carcinoma cases overall (ρ=0.428, P<0.001). There was no significant correlation between Ki-67 LI and LAT1 intensity in adenomas.

(7) Survival curves of the patients

The overall survival was examined for 87 cases, divided into high and low expression groups according to the LAT1 score (0-4 as low and 6-9 as high). When all cases were tested, no significant difference between the low and high expression groups was found (Figure 5a, P value is 0.0997). However, for only non-scirrhous carcinomas, high LAT1 expression was related to a significantly poorer prognosis (Figure 5b, P value is 0.0270). When cases were limited to stage IB and II non -scirrhous carcinoma cases, high LAT1 expression was linked to a poorer prognosis (Figure 5c, P =0.0156).

Further, when cases were limited to carcinoma without I phenotype, and limited to undifferentiated carcinoma, there was a tendency of poor prognosis in high LAT1 expression group (Figure 6a, P =0.0570; Figure 6b, P =0.0558, respectively). Further, in undifferentiated carcinomas excluding scirrhous carcinoma (33 cases), significant poor prognosis was confirmed in high LAT1 expression group (Figure 6c, P =0.0177).

(8) Cox hazard analysis

For overall cases (n=87), only stage was judged as prognostic factor (Table 3). When cases were limited to non-scirrhous carcinomas, LAT1 score and stage were found as prognostic factor in univariate analysis. Subsequently, multivariate analysis revealed that both LAT1 score and stage were individual prognostic factors (Table 4).

Table 1

Table 1. Primary antibodies for the immunohistochemical study

Antibody	Clone	Source	Dilutation	Antigen retrieval
LAT1	Monoclonal	J-Pharma, Tokyo, Japan	Prediluted	Microwave treatment for 15 min
Ki-67	Monoclonal MIB-1	Dakocytomation, Glostrup, Denmark	1:100	Microwave treatment for 15 min
p53	Monoclonal DO7	Dakocytomation, Glostrup, Denmark	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (pH9))
CD10	Monoclonal 56C6	Novocastra, Newcastle-upon-tyne, UK	1:50	-
MUC2	Monoclonal Ccp58	Novocastra, Newcastle-upon-tyne, UK	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (pH9))
MUC5AC	Monoclonal CLH2	Novocastra, Newcastle-upon-tyne, UK	1:200	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (pH9))
MUC6	Monoclonal CLH5	Novocastra, Newcastle-upon-tyne, UK	1:100	Treatment in hot bath for 40 min (Dako Targeted Retrieval solution (pH6))

Table 2

Table 2. Clinicopathological features of 87 cases of gastric carcinoma

Age (years)		
	Median (range)	60(33-85)
Gender		
	Male	54
	Female	33
Tumor maximum diameter		
	Mean (range) (cm)	8.8 (2.0-24.0)
Stage		
	IB	17
	II	17
	IIIA	15
	IIIB	4
	IV	34
Lymph node metastasis		
	N(-)	23
	N(+)	64
Histological grade		
	differentiated type	26
	undifferentiated type	61

Table 3

Table 3. Cox hazard analysis of cause-specific survival in surgical treated gastric cancer (n=87)

variable		Univariate analysis OR (95% CI)	*P* value
Age (median, 60 years; range, 33-85 years)		1.566 (0.906-2.710)	0.1084
LAT1 intensity			
	0 vs 1-3 (5:84)	1.451 (0.353-5.962)	0.6059
	0-1vs 2-3 (7:80)	1.033 (0.411-2.596)	0.9451
	0-2 vs 3 (17:70)	1.567 (0.765-3.209)	0.2196
LAT1 score			
	0 vs 1-9 (4:83)	1.451 (0.353-5.962)	0.6059
	0-1 vs 2-9 (6:81)	1.886 (0.588-6.053)	0.2861
	0-2 vs 3-9 (16:71)	1.561 (0.736-3.312)	0.2459
	0-3 vs 4-9 (50-37)	1.382 (0.812-2.354)	0.233
	0-4 vs 6-9 (51:36)	1.557 (0.915-2.651)	0.1027
	0-6 vs 9 (74:13)	1.116 (0.526-2.365)	0.7751
Ki-67LI			
	Cut off 40% (46:41)	0.907 (0.532-1.547)	0.7202
	Cut off 45% (55:32)	0.579 (0.321-1.039)	0.0671
Stage (TNM)			
	Ib• IIIa vs IIIb• IV (49:38)	7.085 (3.875-12.956)	<0.0001

CI: Confidence interval;
LAT1 intensity: L-type amino-acid transporter 1 immunoreactivity (0: no staining; 1: weakly or patchily positive; 2: moderate complete cell membrane staining; 3: intense complete membrane staining);
LAT1 score: L-type amino-acid transporter 1 immunoreactivity score- given by multiplication of intensity and density (0: negative; 1: focal, 1-10% positive cells; 2: partial, 11-30% positive cells; 3: diffuse, >30%);
LI: labeling index; OR: odds ratio.

Table 4

Table 4. Cox hazard analysis of cause-specific survival in surgical treated non-scirrhous gastric cancer (n=59)

variable	Univariate analysis OR (95% CI)	*P* value	Multivariate analysis OR (95% CI)	*P* value
Age (median, 60 years; range, 33-85 years)	1.500 (0.740-3.039)	0.2607		
LAT1 intensity				
0 vs 1-3 (2:57)	NA			
0-1vs 2-3 (3:56)	2.198 (0.300-16.114)	0.4386		
0-2 vs 3 (9:50)	2.100 (0.639-6.998)	0.2215		
LAT1 score				
0 vs 1-9 (2:57)	NA			
0-1 vs 2-9 (2:57)	NA			
0-2 vs 3-9 (7:50)	3.018 (0.720-12.646)	0.1307		
0-3 vs 4-9 (29:30)	2.415 (1.161-5.024)	0.0183		
0-4 vs 6-9 (30:29)	2.201 (1.074-4.513)	0.0270	2.696 (1.302-5.582)	0.0076
0-6 vs 9 (47:12)	1.470 (0.660-3.275)	0.3456		
Ki-67LI		0.8218		
Cut off 40% (22:37)	0.921 (0.450-1.885)	0.3088		
Cut off 45% (32:27)	0.693 (0.342-1.405)	0.0671		
TNM Stage				
Ib+ IIIa vs IIIb+ IV (36:23)	3.442 (1.688-7.021)	<0.0001	7.153 (3.295-15.529)	<0.0001

CI: Confidence interval;
LAT1 intensity: L-type amino-acid transporter 1 immunoreactivity (0: no staining; 1: weakly or patchily positive; 2: moderate complete cell membrane staining; 3: intense complete membrane staining);
LAT1 score: L-type amino-acid transporter 1 immunoreactivity score- given by multiplication of intensity and density (0: negative; 1: focal, 1-10% positive cells; 2: partial, 11-30% positive cells; 3: diffuse, >30%);
LI: labeling index; OR: odds ratio.

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What is claimed is:

1. A kit, comprising an anti-LAT1 monoclonal antibody, used to determine gastric cancer malignancy via immunohistochemical staining.
2. The kit used to determine gastric cancer malignancy according to claim 1, wherein the monoclonal antibody recognizes human LAT1 amino acid residues specifically at positions 1 to 52 from the N-terminus.

1. Abstract

A kit, comprising an anti-LAT1 monoclonal antibody, used to determine gastric cancer malignancy via immunohistochemical staining.

2. Representative Drawing

None

Figure 1

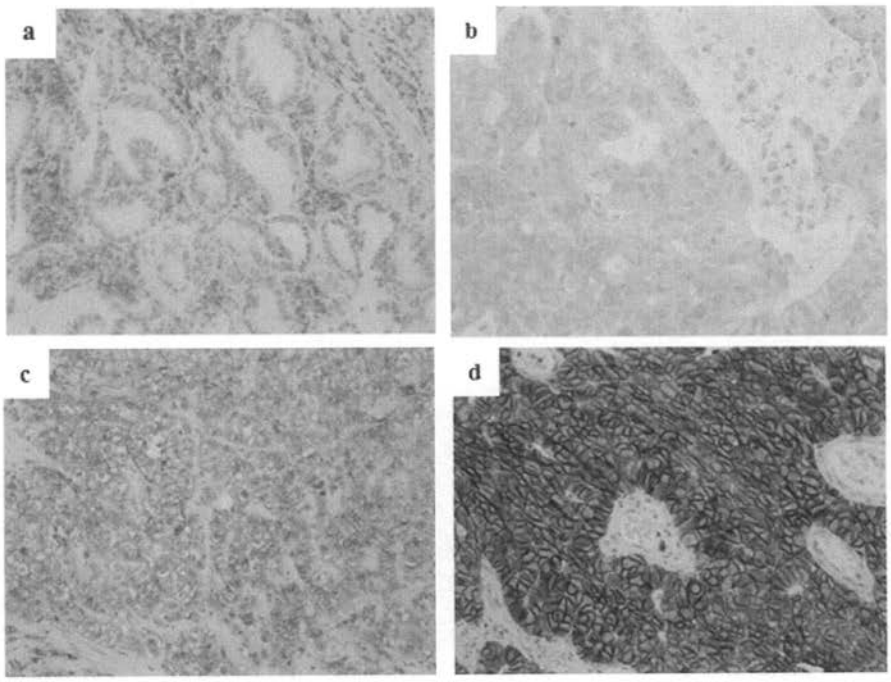
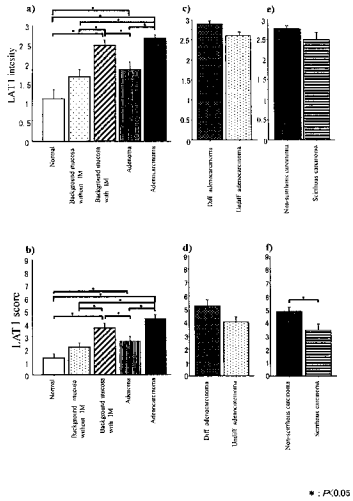


Figure 1
Representative L type amino-acid (LAT1) expression in gastric carcinoma cell membranes: (a) Intensity 0, no immunoreactivity ; (b) Intensity 1, weakly positive s; (c) Intensity 2, moderately positive; and (d) Intensity 3, strong expression.

114x120mm (400 x 400 DPI)

Figure2



Comparison of LAT1 intensity (a) and scores (b) among normal mucosa, background mucosa without intestinal metaplasia (IM), background mucosa with IM, gastric adenomas, and carcinomas. Comparison of the LAT1 intensity and score between differentiated and undifferentiated types of gastric carcinoma (c, d), and between non-scirrhous and scirrhous types (e, f). *, P value<0.05.
113x172mm (400 x 400 DPI)

Figure3

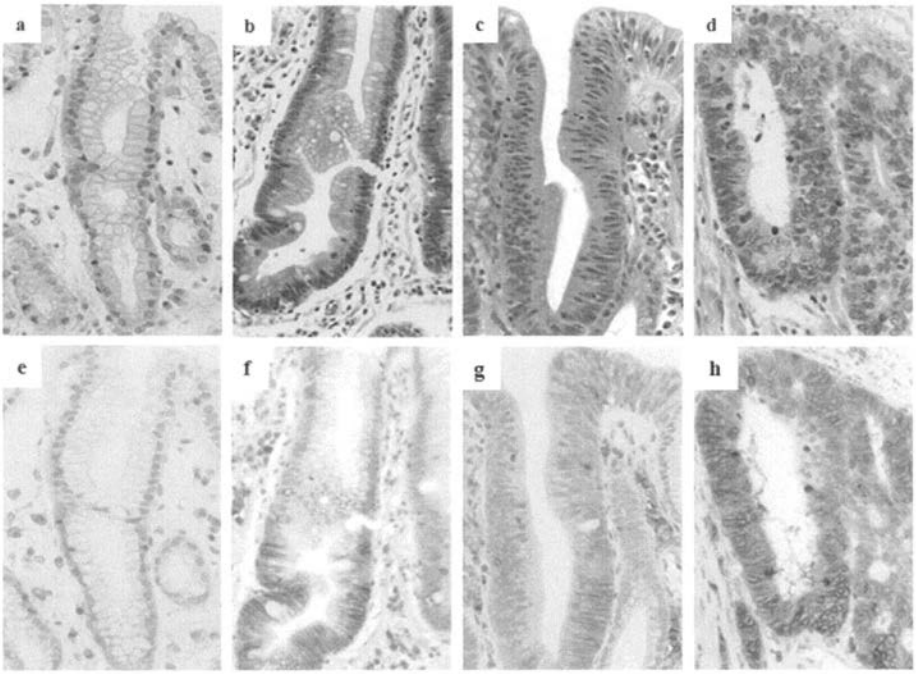


Figure 3
Histologic photographs of (a) normal mucosa, (b) intestinal metaplasia(IM), (c) adenoma, and (d) differentiated type adenocarcinoma. (e) Normal mucosa with weak LAT1 expression, intensity 1 at the bottom of a crypt. (f) Strong LAT1 expression, intensity 3 in the lower half cf an area of intestinal metaplasia. (g) Moderate expression of LAT1, intensity 2 in an adenoma. (h) Strong LAT 1 expression in a differentiated adenocarcinoma, intensity 3.

120x100mm (400 x 400 DPI)

Figure4

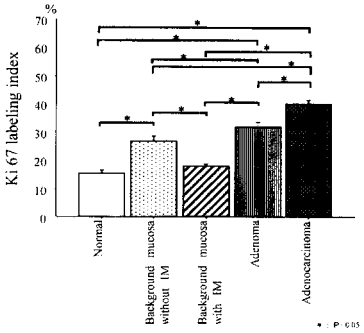


Figure 4
Comparison of the Ki-67 labeling indice s among normal mucosa, background mucosa without I intestinal metaplasia (IM), background mucosa with IM, adenoma, and adenocarcinomas *, P value<0.05.

120x100mm (400 x 400 DPI)

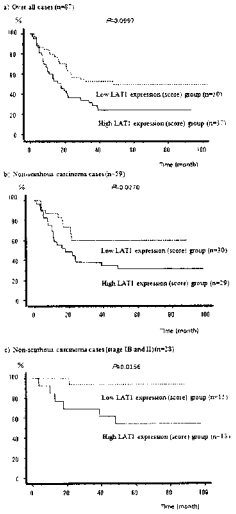


Figure 5
a) Survival curves of overall cases. There was no significant difference between low (LAT1 score 0-4) and high expression (LAT1 score 6-9) groups (P=0.0997).
b) Survival curve of non-scirrhous carcinoma cases. There was significant difference between low (LAT1 score 0-4) and high expression (LAT1 score 6-9) groups (P=0.0270).
c) Analysis limited to stage I B and II cases of non-scirrhous carcinoma cases. High LAT1 expression (LAT1 score 6-9) was linked to a poorer prognosis, compared with low LAT1 expression (LAT1 score0-4) (Figure 5c, P =0.0156).

209x296mm (400 x 400 DPI)

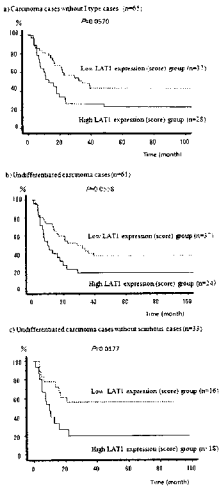


Figure 6
a) Survival curves of carcinoma cases without I type. There was tendency of difference between low (LAT1 score 0-4) and high expression (6-9) groups (P=0.057).
b) Survival curves of undifferentiated carcinoma cases. There was tendency of difference between low (LAT1 score 0-4) and high expression (6-9) groups (P=0.0558).
c) Survival curves of undifferentiated carcinoma cases without scirrhous carcinoma. There was significant difference between low LAT1expression (LAT1 score 0-4) and high expression (6-9) groups (P=0.0177).

209x296mm (400 x 400 DPI)

专利名称(译)	诊断胃癌恶性肿瘤的试剂盒		
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申请(专利权)人(译)	杰制药社		
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其他公开文献	JP5826495B2		
外部链接	Espacenet		

摘要(译)

要解决的问题：提供用于确定胃癌恶性程度的试剂盒。 用于通过免疫组化染色方法确定胃癌恶性程度的试剂盒，该试剂盒包含特异性识别N端人LAT1氨基酸残基1至52的抗LAT1单克隆抗体。 提供。 [选择图]无

表２．87 胃癌症例の臨床病理学的特徴

Age (years)	
Median (range)	60 (33-85)
Gender	
Male	54
Female	33
Tumor maximum diameter	
Mean (range) (cm)	8.8(2.0-24.0)
Stage	
I B	17
II	17
III A	15
III B	4
IV	34
Lymph node metastasis	
N (−)	23
N (+)	64
Histological grade	
differentiated type	26
undifferentiated type	61