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(54) **IDENTIFICATION OF JAK/STAT PATHWAY MODULATING GENES BY GENOME WIDE RNAI SCREENING**

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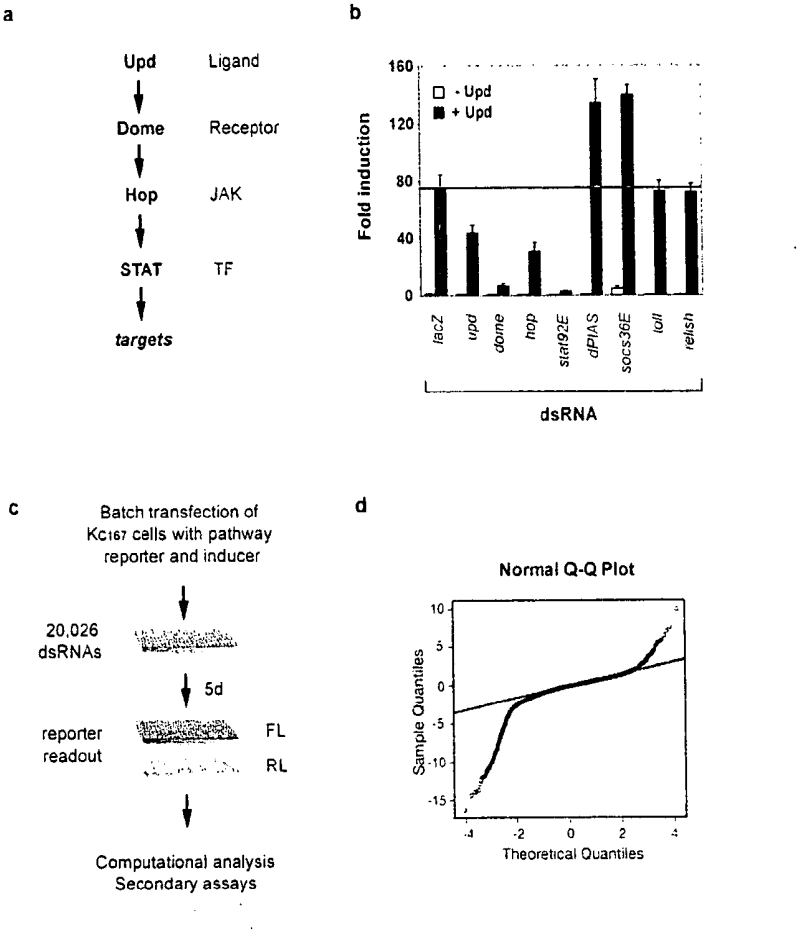
(57) **ABSTRACT**

The present invention relates to a method for identifying a compound capable of modulating the activity of the JAK/STAT pathway and to the use of different JAK/STAT pathway components as a target for the modulation of the activity of the JAK/STAT pathway. Moreover, the present invention is concerned with a method for modulating the activity of the JAK/STAT pathway. Furthermore, the present invention pertains to a pharmaceutical composition and to the use of different JAK/STAT pathway components and/or effector molecules thereof for the manufacture of such composition for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder.



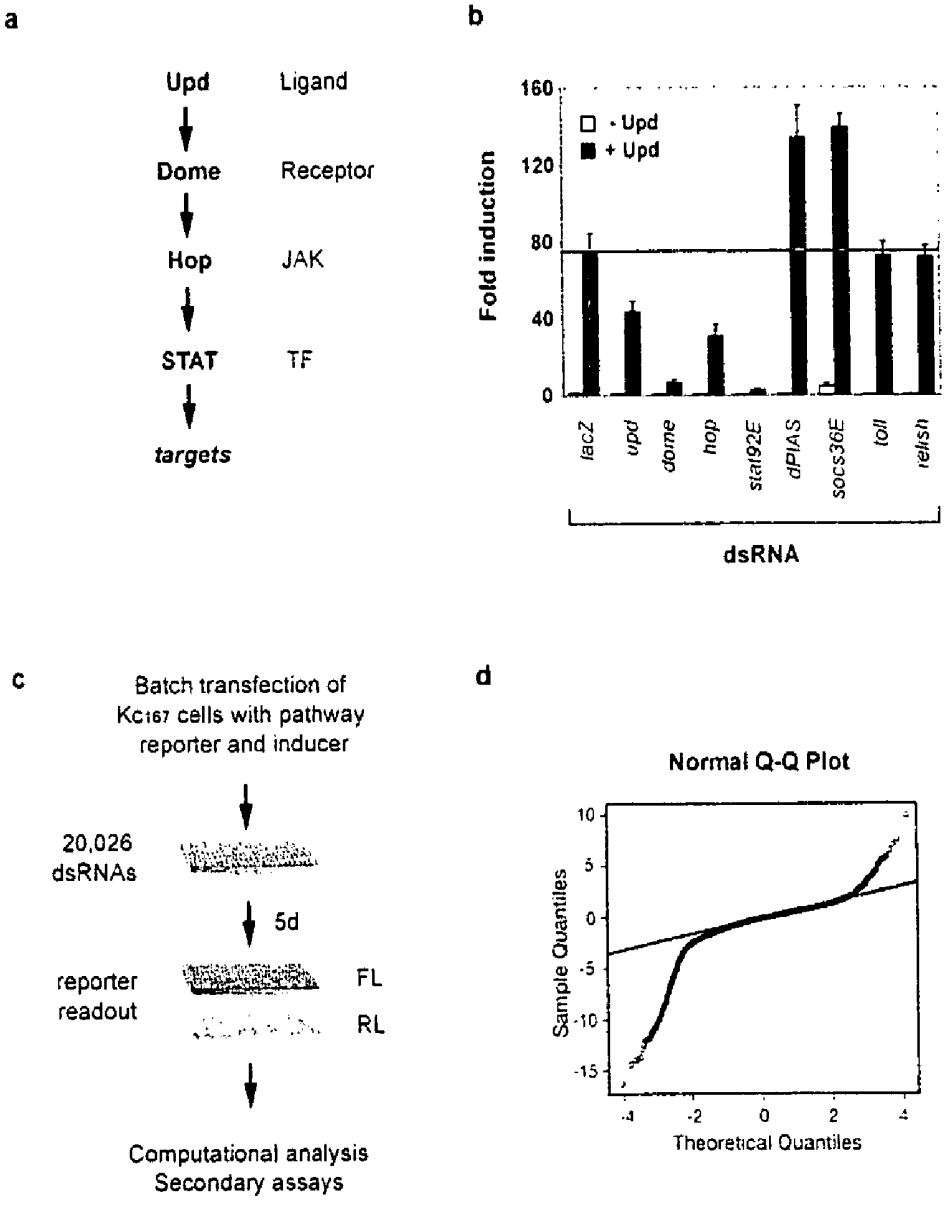
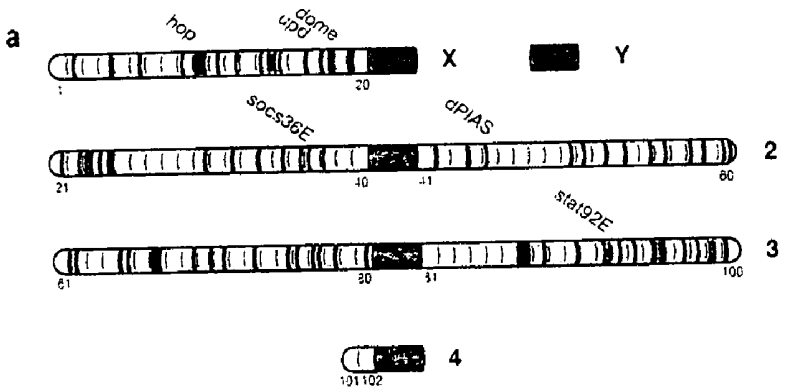
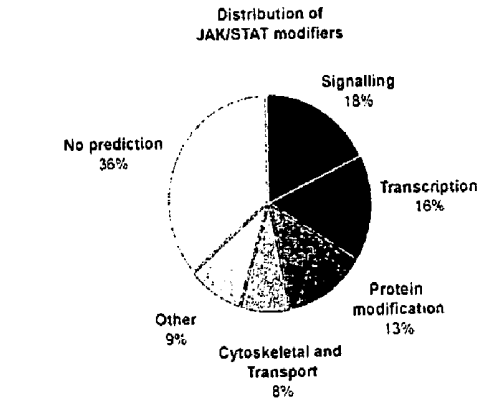

a
Upd
Ligand
Dome
Receptor
Hop
JAK
STAT
TF
targets
b
Fold induction
160
120
80
40
0
- Upd
+ Upd
lacZ
upd
dome
hop
stat92E
dPIAS
socs36E
toll
relish
dsRNA
c
Batch transfection of
Kc167 cells with pathway
reporter and inducer
20,026
dsRNAs
5d
reporter
readout
FL
RL
Computational analysis
Secondary assays
d
Normal Q-Q Plot
Sample Quantiles
10
5
0
-5
-10
-15
-4
-2
0
2
4
Theoretical Quantiles

Figure 1


a
hop
dome
upd
X
Y
socs36E
dPIAS
2
stat92E
3
4


b
Distribution of
JAK/STAT modifiers
Signalling
18%
Transcription
16%
Protein
modification
13%
Cytoskeletal and
Transport
8%
Other
9%
No prediction
36%

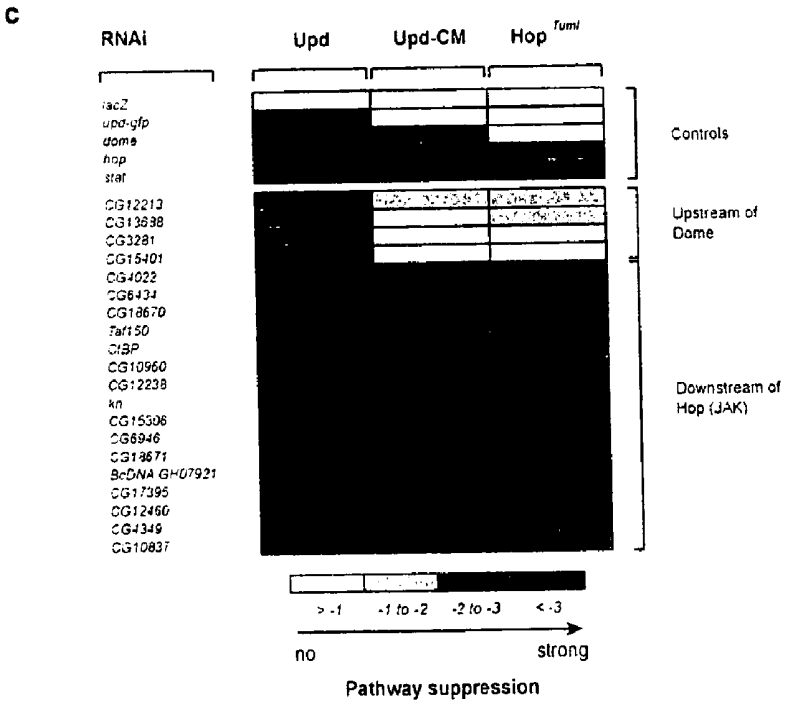

c
RNAi
Upd
Upd-CM
Hop Tumi
lacZ
upd-gfp
dome
hop
stat
CG12213
CG13638
CG3281
CG15401
CG4022
CG6434
CG18670
Taf150
CtBP
CG10960
CG12238
kn
CG15306
CG6946
CG18671
BcDNA GH07921
CG17395
CG12460
CG4349
CG10837
Controls
Upstream of
Dome
Downstream of
Hop (JAK)
> -1
-1 to -2
-2 to -3
< -3
no
strong
Pathway suppression

Figure 2

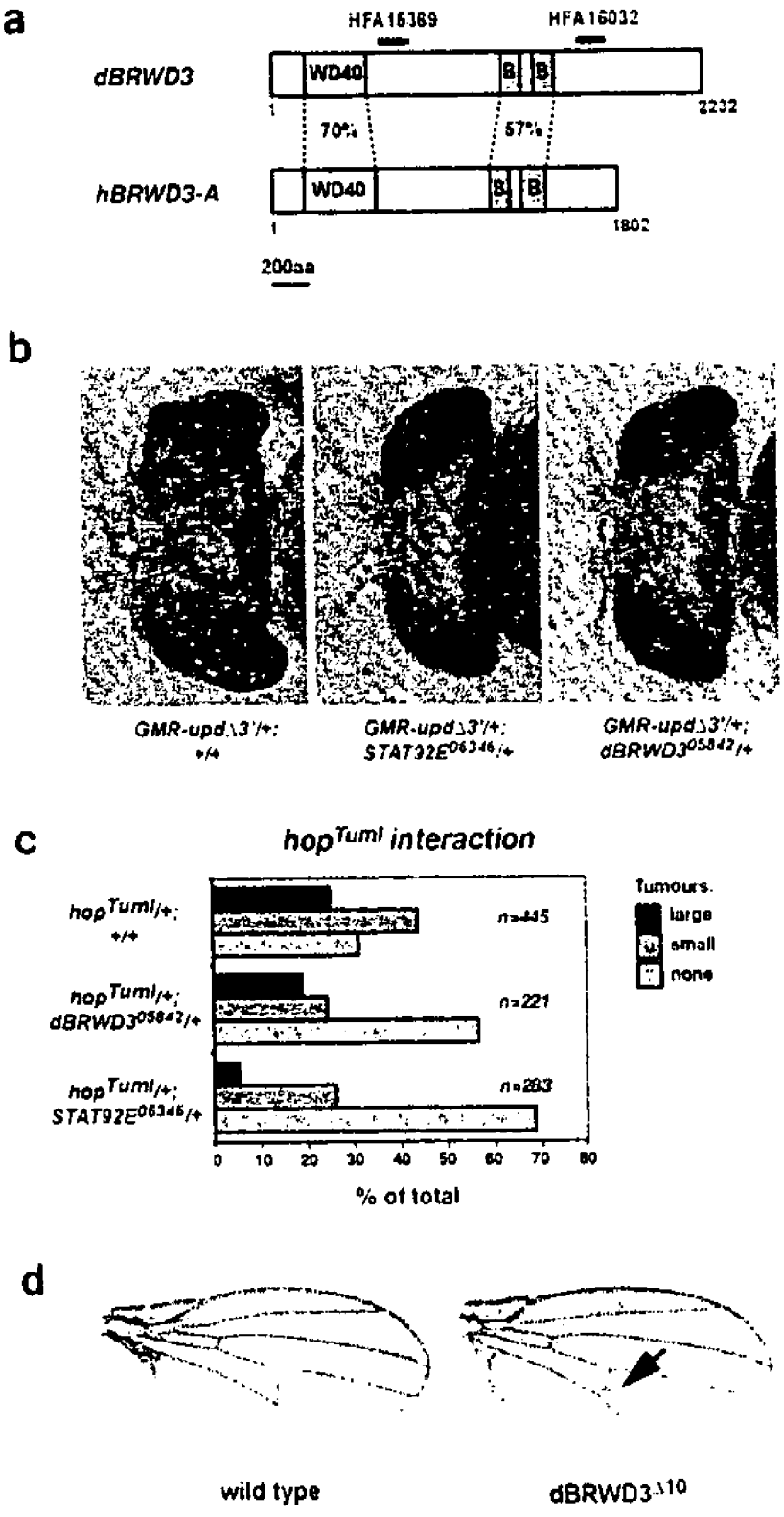

a
HFA16369
HFA16032
dBRWD3
WD40
B
B
2232
70%
57%
hBRWD3-A
WD40
B
B
1802
200aa
b
GMR-upd3'/+;
+/+
GMR-upd3'/+;
STAT92E06346/+
GMR-upd3'/+;
dBRWD305842/+
c
hopTuml interaction
hopTuml/+;
+/+
n=445
hopTuml/+;
dBRWD305842/+
n=221
hopTuml/+;
STAT92E06346/+
n=283
Tumours:
large
small
none
0 10 20 30 40 50 60 70 80
% of total
d
wild type
dBRWD3Δ10

Figure 3

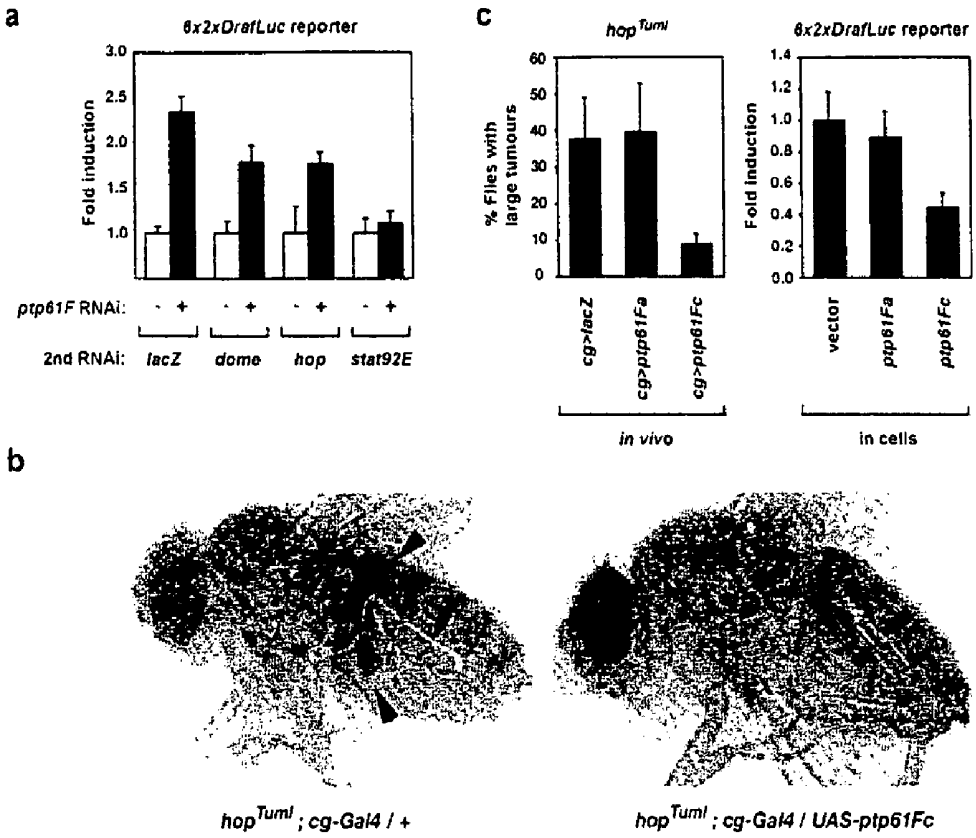

a
8x2xDrafLuc reporter
Fold induction
3.0
2.5
2.0
1.5
1.0
ptp61F RNAi: - + - + - + - +
2nd RNAi: lacZ dome hop stat92E
c
hop Tuml
% Files with large tumours
60
50
40
30
20
10
0
cg>lacZ
cg>ptp61Fa
cg>ptp61Fc
in vivo
8x2xDrafLuc reporter
Fold induction
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
vector
ptp61Fa
ptp61Fc
in cells
b
hop Tuml ; cg-Gal4 / +
hop Tuml ; cg-Gal4 / UAS-ptp61Fc

Figure 4

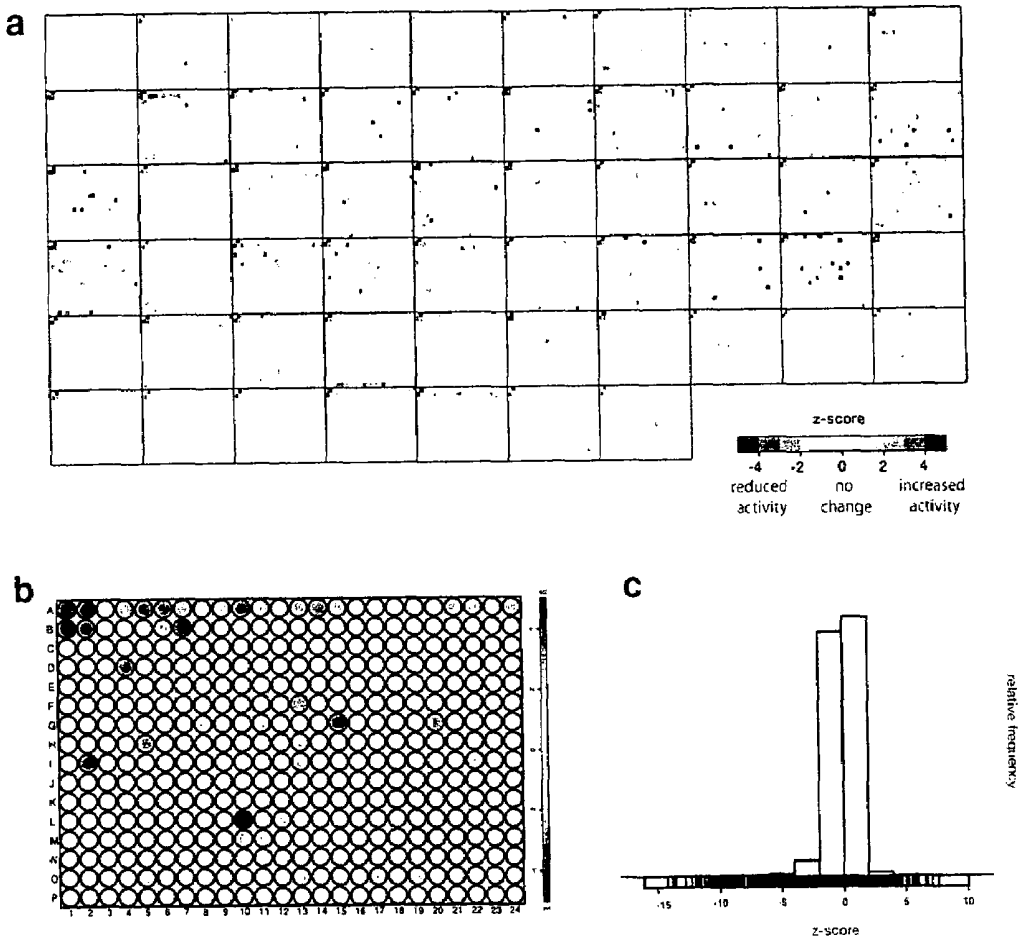
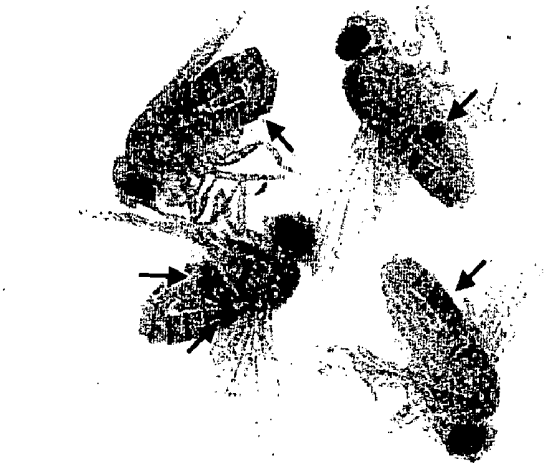

a
z-score
-4 -2 0 2 4
reduced activity
no change
increased activity
b
c
relative frequency
-15 -10 -5 0 5 10
z-score

Figure 5

Figure 6

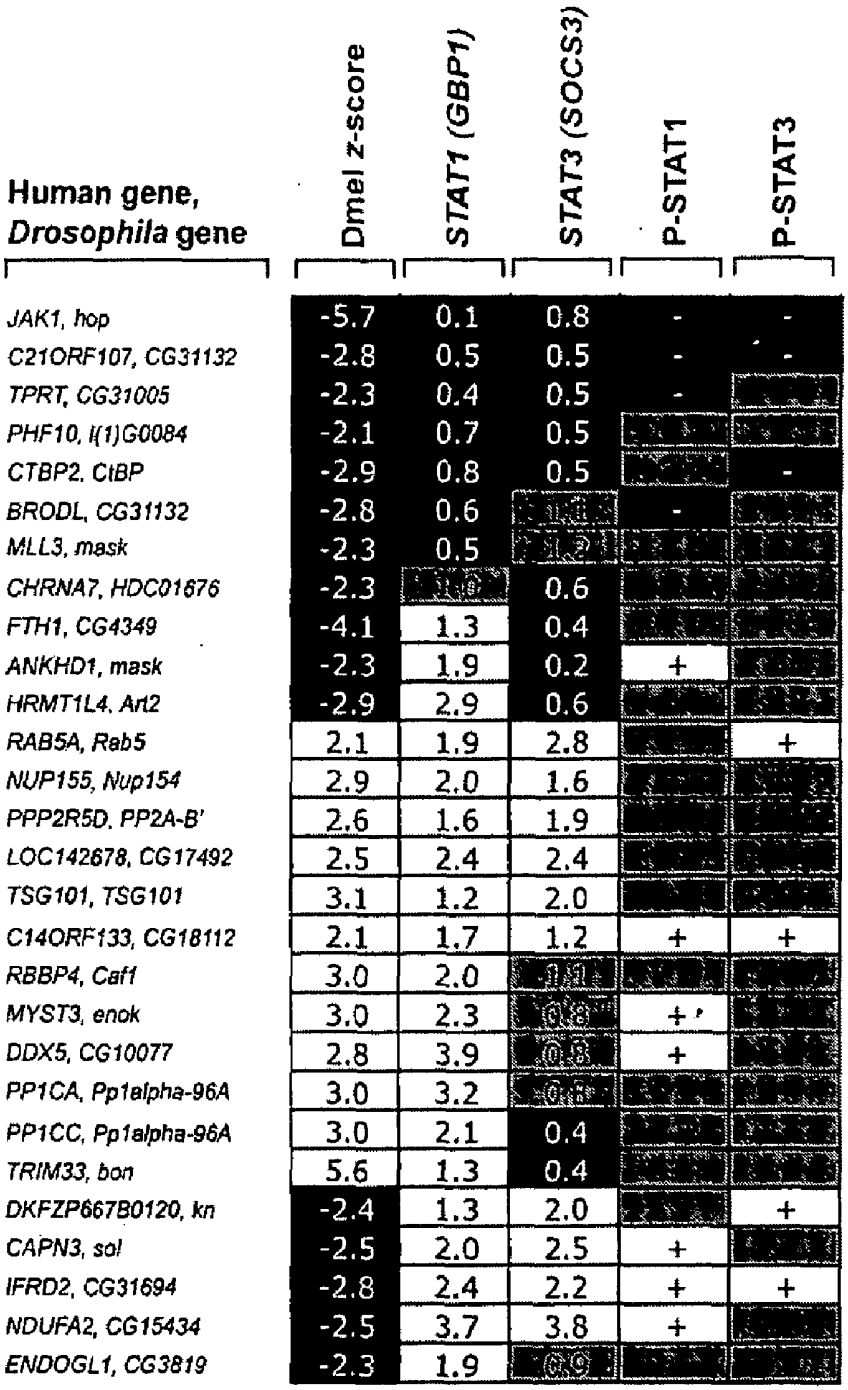


hop^{Tum-l} / + ; +/+

hop^{Tum-l} / + ; $stat92E^{06342}$/+

hop^{Tum-l} / + ; $dBRWD3^{05842}$/+

Figure 7


Human gene, Drosophila gene
Dmel z-score
STAT1 (GBP1)
STAT3 (SOCS3)
P-STAT1
P-STAT3
JAK1, hop -5.7 0.1 0.8 - -
C21ORF107, CG31132 -2.8 0.5 0.5 - -
TPRT, CG31005 -2.3 0.4 0.5 -
PHF10, l(1)G0084 -2.1 0.7 0.5
CTBP2, CtBP -2.9 0.8 0.5 -
BRODL, CG31132 -2.8 0.6 -
MLL3, mask -2.3 0.5
CHRNA7, HDC01676 -2.3 0.6
FTH1, CG4349 -4.1 1.3 0.4
ANKHD1, mask -2.3 1.9 0.2 +
HRMT1L4, Art2 -2.9 2.9 0.6
RAB5A, Rab5 2.1 1.9 2.8 +
NUP155, Nup154 2.9 2.0 1.6
PPP2R5D, PP2A-B' 2.6 1.6 1.9
LOC142678, CG17492 2.5 2.4 2.4
TSG101, TSG101 3.1 1.2 2.0
C14ORF133, CG18112 2.1 1.7 1.2 + +
RBBP4, Caf1 3.0 2.0
MYST3, enok 3.0 2.3 +
DDX5, CG10077 2.8 3.9 +
PP1CA, Pp1alpha-96A 3.0 3.2
PP1CC, Pp1alpha-96A 3.0 2.1 0.4
TRIM33, bon 5.6 1.3 0.4
DKFZP667B0120, kn -2.4 1.3 2.0 +
CAPN3, sol -2.5 2.0 2.5 +
IFRD2, CG31694 -2.8 2.4 2.2 + +
NDUFA2, CG15434 -2.5 3.7 3.8 +
ENDOGL1, CG3819 -2.3 1.9

IDENTIFICATION OF JAK/STAT PATHWAY MODULATING GENES BY GENOME WIDE RNAI SCREENING

[0001] The present invention relates to a method for identifying a compound capable of modulating the activity of the JAK/STAT pathway and to the use of different JAK/STAT pathway components as a target for the modulation of the activity of the JAK/STAT pathway. Moreover, the present invention is concerned with a method for modulating the activity of the JAK/STAT pathway. Furthermore, the present invention pertains to a pharmaceutical composition and to the use of different JAK/STAT pathway components and/or effector molecules thereof for the manufacture of such composition for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder.

[0002] Signalling pathways mediating the transduction of information between cells are essential for development, cellular differentiation and homeostasis (Brivanlou, A. H. & Darnell, J. E., Jr., Science 295, 813-8. (2002)). Their dysregulation is also frequently associated with human malignancies. The JAK/STAT pathway represents one such signalling cascade whose evolutionarily conserved roles include cell proliferation and haematopoiesis (Hombria, J. C. & Brown, S., Curr Biol 12, R569-75 (2002)).

[0003] Developmental genetic screens in *Drosophila* have identified multiple JAK/STAT pathway components on the basis of their segmentation phenotype (Binari, R. & Perrimon, N., Genes Dev 8, 300-12. (1994); Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. & Perrimon, N., Genes Dev 12, 3252-63. (1998); Hou, X. S., Melnick, M. B. & Perrimon, N., Cell 84, 411-9 (1996)) and subsequent analysis of the pathway has characterised evolutionarily conserved roles during immune responses, haematopoiesis and cellular proliferation (Lagueux, M., Perrodou, E., Levashina, E. A., Capovilla, M. & Hoffmann, J. A., Proc Natl Acad Sci USA 97, 11427-32. (2000); Boutros, M., Agaisse, H. & Perrimon, N., Dev Cell 3, 711-22. (2002); Meister, M. & Lagueux, M., Cell Microbiol 5, 573-580 (2003); Mukherjee, T., Castelli-Gair Hombria, J. & Zeidler, M. P., Oncogene in press (2005)). The JAK/STAT signalling cascade in *Drosophila* is comprised of the extracellular ligand Unpaired (Upd) (Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. & Perrimon, N., Genes Dev 12, 3252-63. (1998)), a trans-membrane receptor with homology to the IL6 receptor family termed Domeless (Dome) (Brown, S., Hu, N. & Castelli-Gair Hombria, J., Curr Biol 11, 1700-5. (2001)), a single Janus tyrosine kinase (JAK) called Hopscotch (Hop) (Binari, R. & Perrimon, N., Genes Dev 8, 300-12. (1994)) and the STAT92E transcription factor (Hou, X. S., Melnick, M. B. & Perrimon, N., Cell 84, 411-9 (1996); Yan, R., Small, S., Desplan, C., Dearolf, C. R. & Darnell, J. E., Jr., Cell 84, 421-30 (1996)) (FIG. **1***a*). Known regulators of JAK/STAT signalling including a family of SOCS-like genes (Callus, B. A. & Mathey-Prevot, B.; Oncogene 21, 4812-4821 (2002); Karsten, P., Hader, S. & Zeidler, M. P., Mech Dev 117, 343-6 (2002)), dPIAS/Su(var)2-10 (Betz, A., Lampen, N., Martinek, S., Young, M. W. & Darnell, J. E., Jr., Proc Natl Acad Sci USA 98, 9563-8 (2001)) and STAM (Mesilaty-Gross, S., Reich, A., Motro, B. & Wides, R., Gene 231, 173-86 (1999)) are functionally conserved and were identified based on their homology to components originally characterised in mammalian cell culture studies (Hombria, J. C. & Brown, S., Curr Biol 12, R569-75 (2002)). Although successful in identifying the pathway members Upd, Dome, Hop and STAT92E, it is probable that forward genetic approaches have missed components possibly due to non-saturating mutagenesis, genetic redundancy or phenotypic pleiotropy (Nagy, A., Perrimon, N., Sandmeyer, S. & Plasterk, R., Nat Genet 33 Suppl, 276-84 (2003)).

[0004] In order to identify novel pathway components and circumvent limitations of classical genetic screens, the inventors of the present invention have undertaken a genome-wide RNA interference (RNAi) screen, a powerful technique for the identification of new components of diverse cellular pathways (Kamath, R. S. et al., Nature 421, 231-7 (2003); Kittler, R. et al., Nature 432, 1036-40 (2004); Berns, K. et al., Nature 428, 431-7 (2004); Paddison, P. J. et al., Nature 428, 427-31 (2004); Boutros, M. et al., Science 303, 832-5 (2004)). Using this screen, a systematic genome-wide survey for genes required for JAK/STAT pathway activity could be performed. Analysis of 20,026 RNAi-induced phenotypes in cultured *Drosophila* melanogaster haemocyte-like cells identified interacting genes encoding 4 known and 84 previously uncharacterised proteins. Subsequently, cell based epistasis experiments have been used to classify these based on their interaction with known components of the signalling cascade. In addition to multiple human disease gene homologues, the inventors of the present invention have identified the tyrosine phosphatase Ptp61F and the *Drosophila* homologue of BRWD3, a bromo-domain containing protein disrupted in leukaemia. Moreover, in vivo analysis demonstrates that disrupted dBRWD3 and overexpressed Ptp61F function as suppressors of leukaemia-like blood cell tumours. This screen represents a comprehensive identification of novel loci required for JAK/STAT signalling and provides molecular insights into an important pathway relevant for human diseases.

[0005] A first aspect of the present invention, therefore, relates to a method for identifying a compound capable of modulating the activity of the JAK/STAT pathway, comprising

(a) contacting a compound with at least one target molecule selected from

[0006] (i) nucleic acid molecules, comprising

[0007] (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

[0008] (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

[0009] (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

[0010] (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3); and

[0011] (ii) polypeptide molecules

[0012] (ii.1) encoded by the nucleic acid molecules of (i) and/or

[0013] (ii.2) having the sequences as shown in SEQ ID NOs. 1-87, and

(b) determining the degree of modulation of the at least one target molecule by the compound.

[0014] In accordance with the present invention, it is to be understood, that the term "modulating the activity of the JAK/STAT pathway", when used herein, means activating or inhibiting the activity of the JAK/STAT signalling pathway. An activation or inhibition of the activity of the JAK/STAT signalling pathway may e.g. be mediated by an activation or

inhibition of at least one component of the JAK/STAT pathway, either directly or indirectly.

[0015] According to the present invention, step (a) of the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprises contacting a compound with at least one target molecule selected from the nucleic acid molecules of (i) and the polypeptide molecules of (ii).

[0016] The nucleic acid molecules of (i) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprise in one embodiment of the present invention a nucleotide sequence of (i.1) as show in SEQ ID NOs. 88 to 265. Preferably, the nucleic acid molecules of (i) comprise a nucleic acid sequence of (i.1) as shown in SEQ ID NOs. 88 to 174. More preferably, the nucleic acid molecules of (i) comprise a nucleic acid sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174.

[0017] It is to be understood that the *Drosophila* gene sequences of SEQ ID Nos. 175-265 encompasse respective splice variants.

[0018] Moreover, nucleic acid molecules of (i) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprise in another embodiment of the present invention a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1). Preferably, the nucleic acid molecules of (i) comprise a nucleic acid sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174. More preferably, the nucleic acid molecules of (i) comprise a nucleic acid sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174.

[0019] In a further embodiment of the present invention, the nucleic acid molecules of (i) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprise a nucleotide sequence of (i.3) which has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80% to a nucleotide sequence of (i.1) or (i.2). Within the context of the present application, the term "has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80%", as used herein, means that the sequence identity is at least 65, 66, 67, 6, 69, preferably at least 70, 71, 72, 73, 74, more preferably at least 75, 76, 77, 78, 79 and most preferably at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%. Preferably, the nucleic acid molecules of (i) comprise a nucleotide sequence of (i.3) which has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80% to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174 or a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174. More preferable, the nucleic acid molecules of (i) comprise a nucleotide sequence of (i.3) which has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80% to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174 or a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174.

[0020] Finally, the nucleic acid molecules of (i) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprise in a further embodiment of the present invention a nucleotide sequence of (i.4) which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3). The term "hybridizes under stringent conditions" according to the present application is used as described in Sambrook et al. Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, Laboratory Press (1989), 1.101-1.104. Consequently, hybridization under stringent conditions occurs when a positive hybridization signal is still detected after washing for 1 h with 1×SSC and 0.1% SDS at 55° C., preferably at 62° C. and most preferably at 68° C., in particular for 1 h in 0.2×SSC and 0.1% SDS at 55° C., preferably at 62° C. and most preferably at 68° C. It is preferred that the nucleic acid molecules of (i) comprise a nucleotide sequence of (i.4) which hybridizes under stringent conditions to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174, a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174 or a nucleotide sequence of (i.3) which has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80% to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174 or a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 88 to 174. More preferably, the nucleic acid molecules of (i) comprise a nucleotide sequence of (i.4) which hybridizes under stringent conditions to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174, a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174 or a nucleotide sequence of (i.3) which has an identity of at least 65, preferably at least 70, more preferably at least 75 and most preferably at least 80% to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174 or a nucleotide sequence of (i.2) which is complementary to a nucleotide sequence of (i.1) as shown in SEQ ID NOs. 91, 116, 124, 133, 136, 152, 154, 155 to 174.

[0021] The nucleic acid molecules of (i) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway may be present in single-stranded or double-stranded form and may be selected from RNA, DNA or nucleic acid analog molecules, such as sugar- and backbone-modified ribonucleic acids or deoxyribonucleic acids. It should be noted, however, that other nucleic acid analogs, such as peptide nucleic acids (PNA) or locked nucleic acids (LNA), are also suitable.

[0022] Moreover, according to the present invention, the nucleic acid molecules of (i) used according to the present invention may be non-recombinant nucleic acid molecules, recombinant nucleic acid molecules generated by recombinant methods, e.g. by known amplification procedures such as PCR, or chemically synthesized nucleic acid molecules. The nucleic acid molecules of (i) may be present in isolated, i.e. purified, form or in non-isolated form, i.e. in a cellular environment.

[0023] In a preferred embodiment of the present invention, the nucleic acid molecules of (i) used according to the present invention are present in a vector, which may be any prokaryotic or eukaryotic vector, on which the nucleic acid sequence is present preferably under control of a suitable expression signal, e.g. promoter, operator, enhancer etc. Examples for

prokaryotic vectors are chromosomal vectors, such as bacteriophages, and extrachromosomal vectors, such as plasmids, wherein circular plasmid vectors are preferred. Examples for eukaryotic vectors are yeast vectors or vectors suitable for higher cells, e.g. insect cells or mammalian cells, plasmids or viruses.

[0024] The polypeptide molecules of (ii) used according to the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway are encoded by the nucleic acid molecules of (i) described above and or have a sequence as shown in SED ID Nos. 1-87. According to a preferred embodiment of the present invention, the polypeptide molecules of (ii) have an amino acid sequence as shown in SEQ ID NO. 4, 29, 37, 46, 49, 65, 67 to 87.

[0025] The compound used in step (a) of the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway may be selected from compounds capable of directly and/or indirectly inhibiting or activating the transcription or translation of a nucleic acid molecule of (i). Preferably, the compounds capable of directly and/or indirectly inhibiting or activating the transcription or translation of a nucleic acid molecule of (i) comprise polypeptides such as proteins, enzymes, antibodies, polypeptide inhibitors, polypeptide activators, agonist, antagonists, mimetics, low molecular weight substances, antisense molecules, RNAi molecules and ribozymes. More preferably, the compounds capable of directly and/or indirectly inhibiting or activating the transcription or translation of a nucleic acid molecule of (i) are antisense molecules directed against a nucleic acid molecule of (i) or RNAi molecules. The antisense molecules and RNAi molecules may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesising oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, said molecules may be generated by in vitro and in vivo transcription of DNA sequences.

[0026] Moreover, the compound used in step (a) of the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway may also be selected from compounds capable of directly and/or indirectly inhibiting or activating a polypeptide molecule of (ii). Preferably, the compounds capable of directly and/or indirectly inhibiting or activating a polypeptide molecule of (ii) comprise polypeptides such as proteins, enzymes, antibodies, polypeptide inhibitors, polypeptide activators, agonist, antagonists, mimetics, oligopeptides, low molecular weight substances and polypeptide cofactors. More preferably, the compounds capable of directly and/or indirectly inhibiting or activating a polypeptide molecule of (ii) are antibodies or fragments thereof directed against a polypeptide molecule of (ii). Within the context of the present invention, the term "antibody", as used herein, encompasses polyclonal antibodies, monoclonal antibodies, e.g. chimeric antibodies, humanized antibodies, human antibodies or recombinant antibodies, e.g. single-chain antibodies. Further, the term "antibody fragment" encompasses common antibody fragments, e.g. proteolytic fragments such as Fab, $F(ab)_2$, Fab' or recombinant fragments such as scFv. The antibodies or fragments thereof may be obtained using hybridoma cell lines or recombinant DNA methods using techniques well known in the art. However, the antibodies or fragments thereof may also be isolated from phage antibody libraries using techniques described in the art.

[0027] According to the present invention, step (b) of the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway comprises determining the degree of modulation of the at least one target molecule by the compound. Preferably, the degree of modulation of the at least one target molecule by the compound may be determined either by measuring the amount and/or expression rate of the nucleic acid molecules of (i) or by measuring the amount and/or activity of the polypeptide molecules of (ii). A variety of protocols including, for example, ELISA, RIA, and FACS, for measuring nucleic acid molecules and/or proteins are known in the art and provide a basis for measuring the amount and/or expression rate of a nucleic acid molecule or the amount and/or activity of a polypeptide molecule. Particularly, the capability of a substance to modulate the activity of the JAK/STAT pathway is determined as described in the Example.

[0028] According to the present invention, the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway may be a molecular based assay or a cellular assay. Therefore, the at least one target molecule may be provided either in vivo in a cellular system, preferably a cellular system overexpressing the at least one target molecule, or in vitro in cell fractions containing the at least one target molecule or with the at least one target molecule in a substantially isolated and purified form. Methods for providing the at least one target molecule are well known in the art and may be used in performing the present invention. According to the present invention, it is preferred that the method for identifying a compound capable of modulating the activity of the JAK/STAT pathway is performed in a high-throughput format.

[0029] A second aspect of the present invention pertains to the use of at least one molecule selected from

(i) nucleic acid molecules, comprising

[0030] (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

[0031] (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

[0032] (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

[0033] (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3); and

(ii) polypeptide molecules

[0034] (ii.1) encoded by the nucleic acid molecules of (i) and/or

[0035] (ii.2) having the sequences as shown in SEQ ID NOs. 1-87,

as a target for the modulation of the activity of the JAK/STAT pathway.

[0036] Within the context of the present invention, the nucleic acid molecules of (i), comprising (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265, (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1), (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2), and/or (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3), and the polypeptide molecules of (ii) encoded by the nucleic acid molecules of (i) used as afore-mentioned are as described above.

[0037] A third aspect of the present invention relates to a method for modulating the activity of the JAK/STAT pathway comprising contacting a cell with at least one molecule selected from

(i) nucleic acid molecules, comprising

[0038] (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

[0039] (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

[0040] (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

[0041] (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

(ii) polypeptide molecules

[0042] (ii.1) encoded by the nucleic acid molecules of (i) and/or

[0043] (ii.2) having the sequences as shown in SEQ ID NOs. 1-87; and

(iii) effector molecules of (i) and/or (ii).

[0044] The method for modulating the activity of the JAK/STAT pathway may suitably be performed as molecular based assay or cellular assay. Preferably, the cell used in the method for modulating the activity of the JAK/STAT pathway is a cell showing the JAK/STAT pathway, e.g. an animal cell.

[0045] According to the present invention, the nucleic acid molecules of (i), comprising (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265, (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1), (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2), and/or (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3), and the polypeptide molecules of (ii) encoded by the nucleic acid molecules of (i) used according to the method for modulating the activity of the JAK/STAT pathway are as described above.

[0046] Moreover, the effector molecules of (i) and/or (ii) used according to the method for modulating the activity of the JAK/STAT pathway are selected from polypeptides such as proteins, enzymes, antibodies, polypeptide inhibitors, polypeptide activators, agonist, antagonists, mimetics, oligopeptides, cofactors, low molecular weight substances, antisense molecules, RNAi molecules and ribozymes. Preferably, the effector molecules of (i) and/or (ii) are compounds identified by the method for identifying compounds of modulating the activity of the JAK/STAT pathway described above. More preferably, the effector molecules of (i) and/or (ii) are antibodies or fragments thereof directed against a polypeptide molecule of (ii), antisense molecules directed against a nucleic acid molecule of (i) and/or RNAi molecules.

[0047] Further, the present invention is concerned in a fourth aspect with a pharmaceutical composition comprising as an active agent at least one molecule selected from

(i) nucleic acid molecules, comprising

[0048] (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

[0049] (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

[0050] (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

[0051] (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

(ii) polypeptide molecules

[0052] (ii.1) encoded by the nucleic acid molecules of (i) and/or

[0053] (ii.2) having the sequences as shown in SEQ ID NOs. 1-87; and

(iii) effector molecules of (i) and/or (ii).

[0054] According to the present invention, the nucleic acid molecules of (i), comprising (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265, (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1), (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2), and/or (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3), the polypeptide molecules of (ii) encoded by the nucleic acid molecules of (i) and the effector molecules (of (iii)) of (i) and/or (ii) comprised in the pharmaceutical composition of the invention are as described above.

[0055] In addition to the at least one active ingredient, the pharmaceutical composition of the invention may contain suitable pharmaceutically acceptable carriers, diluents and/or adjuvants, which facilitate processing of the active ingredient into preparations, which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

[0056] The pharmaceutical composition of the present invention is particularly suitable for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder. Preferably, the JAK/STAT pathway associated disorder is selected from the group consisting of papillary thyroid carcinoma, Refsum disease, blood-brain barrier glucose transport defect, X-linked nonsyndromic mental retardation, long QT syndrome 4, subcortical laminar heterotopia, leukemia, steroid-resistant nephrotic syndrome, invasive pituitary tumor, sporadic Sotos syndrome, autosomal dominant iron overload, hereditary pancreatitis, stomatocytosis I, atypical Rett syndrome, phosphoglycerate dehydrogenase deficiency, Wolman disease, neurophysiologic defect in schizophrenia, autosomal recessive SCID (T-negative/B-positive type), atelostogenesis (type I), Larson syndrome, spondylocarpotarsal synostosis syndrome, frontometaphyseal dysplasia, diabetes mellitus (type II), susceptibility to insulin resistance, Griscelli Syndrome, limb-girdle muscular dystrophy (type 2A), growth hormone insensitivity with immunodeficiency and breast cancer.

[0057] In one embodiment of the present invention the pharmaceutical composition is used for the prevention or treatment of a JAK/STAT pathway associated disorder. Pharmaceutical compositions suitable for the prevention or treatment of a JAK/STAT pathway associated disorder include compositions wherein the at least one active ingredient is contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compounds, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

[0058] The actual amount of the pharmaceutical composition administered, will of course, be dependent on the subject being treated, on the subject's weight, the severity of the JAK/STAT pathway associated disorder, the manner of administration and the judgement of the prescribing physician. For the pharmaceutical composition of the invention, a daily dosage of 1 to 200 mg of the at least one active ingredient per kg and day, particularly 10 to 100 mg of the at least one active ingredient per kg and day, is suitable. Suitable routes of administration may, for example, include oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal administrations. Preferably, the subject being treated is an animal, in particular a human being.

[0059] In another embodiment of the present invention the pharmaceutical composition is used for the diagnosis of a JAK/STAT pathway associated disorder, e.g. a disorder characterized by or associated with the over- or underexpression of a nucleic acid molecule of (i) or a polypeptide molecule of (ii). Diagnostic assays include methods which utilize the pharmaceutical composition and a label to detect the nucleic acid molecule of (i) or polypeptide molecule of (ii) in human body fluids or extracts of cells or tissues.

[0060] Finally, a further aspect of the present invention relates to the use of at least one molecule selected from

(i) nucleic acid molecules, comprising

[0061] (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

[0062] (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

[0063] (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

[0064] (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

(ii) polypeptide molecules

[0065] (ii.1) encoded by the nucleic acid molecules of (i) and/or

[0066] (ii.2) having the sequences as shown in SEQ ID NOs. 1-87; and

(iii) effector molecules of (i) and/or (ii);

for the manufacture of a pharmaceutical composition for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder.

[0067] According to the present invention, the nucleic acid molecules of (i), comprising (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265, (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1), (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2), and/or (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3), the polypeptide molecules of (ii) encoded by the nucleic acid molecules of (i) and the effector molecules (of (iii)) of (i) and/or (ii) used according to the present invention for the manufacture of a pharmaceutical composition for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder are as described above.

[0068] Moreover, according to the present invention, the pharmaceutical composition and the JAK/STAT pathway associated disorder are as described above.

[0069] Methods for the manufacture of a pharmaceutical composition, comprising the step of admixing at least one molecule selected from nucleic acid molecules of (i), comprising (i.1) a nucleotide sequence as shown in SEQ ID NOs. 88 to 265, (i.2) a nucleotide sequence which is complementary to a nucleotide sequence of (i.1), (i.3) a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2), and/or (i.4) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3), polypeptide molecules of (ii) encoded by the nucleic acid molecules of (i) and effector molecules (of (iii)) of (i) and/or (ii) with a pharmaceutically acceptable excipient, vehicle or carrier and optionally other ingredients are well known to those skilled in the art and may be used in performing the present invention.

[0070] Further, the present invention shall be explained by the following Tables, Figures and Example.

Tables

[0071] Table 1 shows the RNAi JAK/STAT phenotypes.

[0072] Table 2 shows the functional groups classified by InterPro prediction and GO.

[0073] Table 3 shows the genetic interactions with hop^{Tuml}.

[0074] Table 4 shows sequence and cytological information.

[0075] Table 5 shows human homologues of *Drosophila* genes with JAK/STAT phenotypes.

[0076] Table 6 shows human disease homologues of *Drosophila* genes with JAK/STAT phenotypes.

[0077] Supplementary Table 7 shows the expected and observed phenotype frequency.

[0078] Table 7 shows preferred human JAK/STAT homologues ranked according to their involvement in a human disease.

[0079] Table 8 shows evolutionary and functional conservation of JAK/STAT pathway components.

FIGURES

[0080] FIG. **1** shows the genome-wide RNAi screen for JAK/STAT signalling factors. a: Schematic representation of the *Drosophila* JAK/STAT signalling pathway. b: Knock-down of known JAK/STAT components leads to loss of pathway induction by Upd whereas knock-down of lacZ, toll and relish show no effect. The red line indicates a 70-fold reporter induction relative to negative control dsRNA. Error bars represent standard deviations of six experiments. c: Screening approach. 20,026 dsRNA were screened in duplicate in 384-well plates prior to computational analysis and retesting. FL: firefly luciferase (indicated in red); RL: *Renilla* luciferase (indicated in yellow) d: Q-Q plot of normally distributed quantiles against actual screening result quantiles in the pathway reporter channel. A perfect fit to a normal distribution is represented by the red line. Tails of positively and negatively interacting dsRNAs at each extreme with a z-score threshold of >2 and <−2 represent RNAi experiments with significant phenotypes ($p<0.05$).

[0081] FIG. **2** shows the analysis of JAK/STAT activity modulators. a: Schematic representation of positive (red) and negative (green) regulator loci distributed within the *Drosophila* genome. An interactive version of this panel is available at http://www.dkfz.de/signaling/jak-pathway/cytomap.php. b: Distribution of predicted gene functions. c: Epistasis analysis of the indicated positive pathway regulators showing inter-

actions graded from none (yellow) to strong (red). Results shown have been obtained in two independent octuplicate experiments. Upd: Upd ectopic expression; Upd-CM: Upd conditioned medium; hop^{Tuml}: expression of a constitutively active JAK-allele. Colour coding of z-scores is shown in the key.

[0082] FIG. **3** shows that dBRWD3 functions as a JAK/STAT pathway component. a: Domain structure and sequence similarity of *Drosophila* and human BRWD3 proteins. Percentages show the similarity in the amino acid sequence and regions targeted by two independent dsRNAs independently recovered in the screen are shown. b: Adult *Drosophila* heads heterozygous for the GMR-updA3' transgene crossed to wild type (left), stat92E (middle) and dBRWD3 mutants (right). Note the strong reduction in eye size following removal of pathway components. c: hop^{Tuml} induced tumour formation is significantly decreased in both size and frequency of tumours in stat92E and dBRWD3 heterozygous backgrounds. d: By comparison to adult wild type wings (left), ectopic wing vein material (arrow) is present in homozygous $dBRWD3^{\Delta 10}$ mutant (a putative hypomorphic allele, right), a phenotype reminiscent of the $stat92EH^{HJ}$ mutant.

[0083] FIG. **4** shows that Ptp61F is a tumour suppressor in vivo. a: Epistasis analysis of ptp61F dsRNA in cell culture revealed that it acts downstream of Hop and upstream or parallel to STAT92E. b: Haemocyte specific misexpression of ptp61F can protect hop^{Tuml} mutants from melanotic tumour formation. Compare large black tumours in controls (arrow heads, left) with small tumours present in ptp61F expressing individual (right). c: Quantitative analysis of large tumour formation in hop^{Tuml} mutants expressing cytoplasmic Ptp61Fa and nuclear Ptp61Fc shows specificity of rescue for the nuclear isoform (left), an effect that is mirrored by overexpression of the same isoforms in tissue culture based reporter assays (right). Error bars represent standard deviations of 3 or 4 independently tested transgenic lines or eight parallel cell culture experiments.

[0084] FIG. **5** shows an overview of primary RNAi screen data. a: False colour representation of the genome-wide screen showing averaged z-scores for each well present in the fifty seven 384-well duplicate plates. Key indicates the colours associated with the z-scores: −4 (red) represents a strong decrease in reporter activity, +4 (blue) represents an increase in activity. Four controls were included in the top left corner of each plate and are visible in all plates except 1 and 9 for which these dsRNA controls failed. b: False colour representation of average z-scores for a representative example from the genome-wide screen (plate 34). Controls present in the lop left corner of each plate were hop (A1), dome (A2), stat92E (B1) and socs36E (B2). dsRNAs from the library were present in all other wells including position B07 which targets hopscotch. L10 which targets CG2033 was excluded from the final list because of a cell viability phenotype previously identified in both Kc and S2R+ cells (Boutros, M. et al., Science 303, 832-5 (2004)). Similarly, 102 and G20 (which both target sbr/CG17335) were excluded due to variability in retesting and a previously described bi-nucleate phenotype (Kiger, A. A. et al., J Biol 2, 27 (2003)). Colour coding for z-scores is shown in the key and uses the same scheme shown in a. c: Histogram of z-scores for the genome-wide screen indicates that the majority of dsRNA experiments do not modify JAK/STAT signaling activity.

[0085] FIG. **6** shows the loss of JAK/STAT pathway components and hop^{Tuml} induced tumour formation. hop^{Tuml}/+; +/+ females (top) frequently contain large black melanotic tumours (arrows). hop^{Tuml}/+;$stat92E^{06342}$/+ heterozygotes which lack one copy of stat92E (middle) contain fewer and smaller tumours. hop^{Tuml}/+;$dBRWD3^{05842}$/+ (bottom) also contain fewer and smaller tumours. Flies were grown in parallel independent experiments at 25° C. and are representative examples of the individuals recovered (see Table 3 for further information).

[0086] FIG. **7** shows a heat-map showing human JAK/STAT pathway regulating genes identified. Data shown are: the original *Drosophila* interactions (col 1) expressed as z-scores, fold change in the expression levels of STAT1 and STAT3 target genes (col 2 & 3, respectively) and the levels of phosphorylated STAT1 and STAT3 (col 4 & 5, respectively). In all columns black represents a decrease, white an increase and grey no change in activity.

EXAMPLE

[0087] Signalling pathways mediating the transduction of information between cells are essential for development, cellular differentiation and homeostasis (Brivanlou, A. H. & Darnell, J. E., Jr., Science 295, 813-8. (2002)). Their dysregulation is also frequently associated with human malignancies. The JAK/STAT pathway represents one such signalling cascade whose evolutionarily conserved roles include cell proliferation and haematopoiesis (Hombria, J. C. & Brown, S., Curr Biol 12, R569-75 (2002)). Here, the inventors of the present invention describe a systematic genome-wide survey for genes required for JAK/STAT pathway activity. Analysis of 20,026 RNAi-induced phenotypes in cultured *Drosophila* melanogaster haemocyte-like cells identified interacting genes encoding 4 known and 84 previously uncharacterised proteins. Subsequently, cell based epistasis experiments have been used to classify these based on their interaction with known components of the signalling cascade. In addition to multiple human disease gene homologues, the inventors of the present invention have identified the tyrosine phosphatase Ptp61F and the *Drosophila* homologue of BRWD3, a bromodomain containing protein disrupted in leukaemia (Kalla, C. et al., Genes Chromosomes Cancer 42, 128-43 (2005)). Moreover, in vivo analysis demonstrates that disrupted dBRWD3 and overexpressed Ptp61F function as suppressors of leukaemia-like blood cell tumours. This screen represents a comprehensive identification of novel loci required for JAK/STAT signalling and provides molecular insights into an important pathway relevant for human diseases.

1. Experimental Procedures

1.1. Constructs and Pathway Reporter

[0088] The JAK/STAT firefly luciferase reporter 6x2xDrafLuc was constructed by multimerisation of a molecularly characterised STAT92E binding site present in the promoter of the endogenous target genes Draf (Kwon, E. J. et al., J Biol Chem 275, 19824-19830 (2000)) while the 4xsocsLuc reporter is based on a single region containing four potential STAT92E binding sites present within the first intron of socs36E (Karsten, P., Hader, S. & Zeidler, M. P., Mech Dev 117, 343-6 (2002)). A *Renilla* luciferase reporter gene under the control of the constitutively active Actin5C promoter was co-transfected and used to monitor cell number.

[0089] Strictly speaking, the JAK/STAT reporter 6x2xDrafLuc was constructed by multimerisation of STAT92E binding sites. Specifically, a 165 bp blunted

BamHI/Xbal fragment from the original p2xDrafSTAT(wt) (Kwon, E. J. et al., J Biol Chem 275, 19824-19830 (2000)) (a kind gift of M. Yamaguchi and M.A. Yoo) was inserted into the Smal cut p2xDrafSTAT(wt). The same fragment was amplified by PCR with NotI sites on both ends and inserted into compatible sites to yield the 3x2xDrafLuc reporter containing six STAT92E binding sites. These fragments were amplified again and the resulting 540 bp fragment was inserted into the SacI cut 3x2xDrafLuc vector to generate the 6x2xDrafLuc reporter with an enhancer of approximately 1000 bp containing a total of 12 STAT92E binding sites. A second independent JAK/STAT pathway reporter, 4xsocs-Luc, was generated by amplifying a 745 bp product from genomic DNA using the primers 5'-GTTAGGTAC-CGGGTCGCAGTATCGTTGGCG-3' and 5'-CGMG-GATCC CTGTCACTTCTCAGAAATCGGTC-3'. This was then cut with EcoRI/BamHI to give a 285 bp fragment, subcloned into pBS(KS+) (Stratagene) and re-excised with Asp718/BamHI. This 340 bp fragment, containing four predicted STAT92E binding sites (Karsten, P., Hader, S. & Zeidler, M., Mech Dev 117, 343. (2002)), was cloned into Asp718/BgIII sites of pGL3 vector (Promega).

[0090] The pAct-RL vector expressing *Renilla* luciferase from a constitutive reporter was generated by cloning a 974 bp fragment coding for *Renilla* luciferase from pRLSV40 (Invitrogen) into the BamHI/Xbal cut pP_{Ac5c}-PL vector (a kind gift from Dan Curtis). To generate the pAct-UpdGFP vector, a cDNA coding for Upd (Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. & Perrimon, N., Embo J 14, 2857-65 (1995)) was fused in frame to EGFP via a BamHI site and inserted into the BamHI/Xbal cut pP_{Ac5c}-PL vector. A vector expressing the dominant gain-of-function allele Hop^{TumL} was cloned by inserting the open reading frame obtained from pUAS-hop^{TumL} (Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. & Perrimon, N., Embo J 14, 2857-65 (1995)) into the NotI/Xbal cut pAc5.1A vector (Invitrogen). A pAc5.1-Sid-1 expression construct which was used to facilitate uptake of dsRNA was a gift of Craig Hunter (Feinberg, E. H. & Hunter, C. P., Science 301, 1545-7 (2003)).

[0091] To generate Ptp61F. expression constructs, cDNAs encoding Ptp61Fc (LP01280) and Ptp61Fa (RE01370) were obtained from the *Drosophila*Genomics Resource Center (University of Indiana). cDNA clones were analysed by restriction analysis and end sequencing to confirm their integrity before subcloning into pAc5.1A and pUAST (Brand, A. H. & Perrimon, N., Development 118, 401-15 (1993)). For Ptp61Fc, the coding region of LP01280 was excised as an EcoRI/XhoI (partial digest) fragment of 1.8 kb and cloned into pUAST. Subsequently, the insert was re-excised with EcoRI/Xbal and cloned into pAc5.1A (Invitrogen). For Ptp61Fa, the coding region of RE01370 was cut out with EcoRI/Asp718(filled) and cloned into pAc5.1A cut EcoRI/Xbal(filled). The generate a pUAST construct, an EcoRI/Asp718 fragment was used.

[0092] To clone p[w^+,UAS-dPIAS-GFP], the EST clone LD09022 was used as a template in conjunction with the oligos 5'-CATCGGATCCTGCAAAAAGGGG TCCAACG-TACC GGAT-3' and 5'-GGGGTACCAAAAATGGTG-CATATGCTT CGA-3' to amplify a region coding for 522 amino acids. The resulting product was sequenced, cut with Asp718/BamHI and subcloned into pBS-EGFP-B to generate an in frame C-terminal EGFP fusion protein. This gene was then subcloned as an Asp718/Xbal fragment into pUAST (Brand, A. H. & Perrimon, N., Development 118, 401-15 (1993)).

[0093] Multiple independent transgenic *Drosophila* stocks of each transformation vector construct were generated by microinjection of embryos using standard techniques (Spradling, A. C. & Rubin, G. M., Science 218, 341-347 (1982)).

1.2. Genome-Wide RNAi Screening

[0094] A genome-wide RNAi library based on PCR templates with an average length of 408 bp flanked by T7-promotor binding sites was generated by in vitro transcription (Boutros, M. et al., Science 303, 832-5 (2004)). Therefore, PCR fragments containing T7 promoter sequences on each end (Hild, M. et al., Genome Biol 5, R3 (2003)) were used as templates to generate 20,026 dsRNAs by in vitro transcription (Boutros, M. et al., Science 303, 832-5 (2004)). After DNAse I treatment, dsRNAs were purified by ethanol precipitation and individually quality controlled by gel electrophoresis. RNAs were diluted to a working stock concentration and aliquoted in ready-to-screen 384-well tissue culture plates (Greiner). Computational mapping predict that the 20,026 RNA fragments target >91% of all predicted genes in the *Drosophila* genome (Annotation 4.0) (Misra, S. et al., Genome Biol 3, RESEARCH0083-3 (2002)). Protocols and supplemental material can be found at http://www.dkfz-heidelberg.de/signaling/jak-pathway/. Complete primer and amplicon sequence information for double-stranded RNAs including calculation of predicted efficiency and off-target effects for the RNAi library is publicly accessible at http://rnai.dkfz.de.

[0095] For screening experiments, *Drosophila* Kc_{167} cells were maintained in Schneider's medium (Invitrogen) supplemented with 10% foetal bovine serum (PAA) and 100 μg/ml penicillin-streptomycin (Invitrogen). Cells were grown at 25° C. at subconfluent densities. The RNAi screening experiments were performed in white, polystyrene 384-well tissue culture plates (Greiner 781 073). A total of fiftyseven 384-well screening plates were loaded with an average of 75 nM (500 ng) dsRNA in 5 μl of 1 mM Tris pH 7. Kc_{167} cells were transfected in batch in 6-well plates with 0.25 μg of the 6x2xDrafLuc JAK/STAT signalling reporter, 0.6 μg of pAct-UpdGFP expression vector, 0.25 μg pAc5.1-Sid-1 (to facilitate RNA uptake (Feinberg, E. H. & Hunter, C. P., Science 301, 1545-7 (2003))) and 0.025 μg of pAct-RL vector as a co-reporter. The total plasmid amount was normalised to 2 μg with a pAc5.1 plasmid (Invitrogen) and 5×10^6 cells were transfected with Effectene (Qiagen). After 7 hours incubation at 25° C., batch transfected cells were resuspended in serum-free medium. Subsequently 15,000 cells in 20 μl were dispensed per dsRNA containing well using an automated liquid dispenser (MultiDrop, Thermo Labsystems). Cells were incubated for 45 min and 30 μl of serum-containing medium was added to each well. Cells were grown for 5 days to allow for protein depletion. Pathway activity was measured for using a luminescence assay for firefly and *Renilla* luciferase on a Mithras LB940 plate reader (Berthold Technologies). Luminescence of the *Renilla* luciferase was measured using a 490 nm filter set. Screens were performed in duplicate. Each plate contained dsRNA targeting stat92E, dome, hop and socs36E in A1, A2, B1, B2 which were used as positive controls (see also FIG. **5***b*). For retests, an independent JAK/STAT pathway reporter (4xsocsLuc) was used which con-

tained a STAT-binding site from the endogenous JAK/STAT-pathway target socs36E (Karsten, P., Hader, S. & Zeidler, M., Mech Dev 117, 343. (2002)).

[0096] To identify candidate genes that significantly increase or decrease JAK/STAT signalling pathway activity, the raw luciferase results were normalised by median centering of each 384-well plate (separately by channel). Z-scores were calculated as the number standard deviation that a particular well differed from the median of the 384-well plate. To minimise false negatives, the inventors of the present invention applied a set of low-stringency criteria to generate a list of candidate genes to be used in specific retests. First, the inventors filtered dsRNA treatments with z-scores >2 for negative regulators or <−2 for positive regulators, respectively. Treatments that showed a high variability between duplicates were excluded. Further, RNAi experiments that showed z-scores of >2 or <−2 in the control channel were not selected for retesting. The inventors also filtered against previously identified cell viability modifiers that show a phenotype in cultured *Drosophila* cells (Boutros, M. et al., Science 303, 832-5 (2004)). The inventors also excluded genes that showed phenotypes in other screens. These filtering steps led to a final list of approximately 107 candidates that were selected for retesting. New dsRNA was re-synthesized as described above and repeat assays were performed in quadruplicate. 89 of the candidates were confirmed using a second JAK/STAT reporter assay (4xsocsLuc) employed to exclude reporter-specific artefacts. Data analysis and representation were performed using R and Bioconductor (Gentleman, R. C. et al., Genome Biol 5, R80 (2004)).

[0097] The predicted genes targeted by 91 dsRNAs were classified according to InterPro (Mulder, N. J. et al., Nucleic Acids Res 33 Database Issue, D201-5 (2005)) and GO (Harris, M. A. et al., Nucleic Acids Res 32, D258-61 (2004); Drysdale, R. A. et al., Nucleic Acids Res 33 Database Issue, D390-5 (2005)) and manual inspection was used to order genes into functional groups. Predicted proteins without InterPro domain or GO annotation were classified as "Unknown" although these sequences might encode structurally conserved proteins. To determine whether *Drosophila* proteins have homologues in other species, the inventors used BLASTP searches against the protein predictions from H. sapiens (NCBI build 35) with a cut-off of $E<10^{-10}$. Databases were obtained from Ensembl (http://www.ensembl.org) (Clamp, M. et al., Nucleic Acids Res 31, 38-42 (2003)) and Flybase (hftp://www.flybase.org) (Drysdale, R. A. et al., Nucleic Acids Res 33 Database Issue, D390-5 (2005)). Reciprocal best BLASTP analysis was used to identify the human homologue of CG31132. CG31132 and human BRWD3 are classified as orthologous pairs by InParanoid (http://inparanoid.cgb.ki.se/).

1.3. Cell-Based Epistasis Experiments

[0098] To undertake epistasis experiments, cells were transfected with vectors to stimulate pathway activity (see below) for 7 hours and 30,000 cells in 50 μl of serum-free medium were seeded into wells of clear bottom 96-well plates (Greiner), which contained 1.5 μg of the dsRNAs to be tested (listed in FIG. **2***c*). Following 1 hour incubation, 75 μl medium supplemented with 10% foetal bovine serum was added to the cells, plates were sealed and cells lysed after 5 days to measure luciferase activities.

[0099] Each dsRNA was tested for its ability to suppress pathway activity under three conditions: (1) in Upd-expressing cells (screening conditions), (2) in cells treated with Upd-conditioned medium (Upd-CM), and (3) in cells expressing the activated form of JAK, Hop^{Tuml} (Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. & Perrimon, N., Embo J 14, 2857-65 (1995); Luo, H., Hanratty, W. P. & Dearolf, C. R., Embo J 14, 1412-20 (1995)). Specifically, for Upd overexpression 5×10^6 Kcl_{67} cells were transfected with 600 ng pAct-UpdGFP, 500 ng 6x2xDrafLuc reporter, 250 ng pAc5.1-Sid-1, 25 ng pAct-RL and pAc5.1 to a total of 2 μg DNA. For Hop^{TumL} overexpression, 5×10^6 Kc_{167} cells were transfected with 200 ng pAct-hop^{TumL}, 500 ng 6x2xDrafLuc reporter, 250 ng pAc5.1-Sid-1, 25 ng pAct-RL and pAc5.1 to a total of 2 μg DNA. To analyse processes upstream of Upd, two batches of cells were transfected separately to generate 'responder' and 'Upd-producer' cells. The 'responder' cells were batch transfected with 500 ng 6x2xDrafLuc reporter, 250 ng pAc5.1-Sid-1, 25 ng pAct-RL and pAc5.1 to a total of 2 μg plasmid DNA and subsequently seeded into 96-well plates containing the respective dsRNAs as described above. The 'Upd-producing' cells were transfected with 2 μg pAct-UpdGFP and cultured in 10 cm dishes (Falcon). Three days after transfection, cells were treated with 50 μg/ml Heparin (Sigma). After 24 hours, the supernatant was harvested, cleared by centrifugation and passed through a 0.2 μm filter (Millipore). 50 μl of this Upd-conditioned medium were then used to stimulate pathway activity in the 'responder' cells for 24 hours. Control medium from untransfected Heparin treated cells did not elicit pathway activity (not shown).

[0100] Experiments were performed in eight replicates and repeated at least twice. Reporter activity in the firefly luciferase channel was divided by the *Renilla* luciferase channel to normalise for cell number. Z-scores were calculated as the multiples of the standard deviation that a specific RNAi treatment differed from cells treated with lacZ dsRNA as negative controls. Z-scores were subsequently transformed into a false-colour representations as depicted in FIG. **2***c*.

[0101] RNA controls as shown in FIG. **2***c* were in vitro transcribed from PCR templates generated using the following gene-specific primer sequences: 5T7lacZ: GAATAATACGACTCACTATAGGGAGA-CAGTGGCGTCTGGCGGAAAA (SEQ ID NO. 448), 37lacZ: GMTTMTACGACTCACTATAGGGAGATC-CGAGCC AGTTTACCCGCT (SEQ ID NO. 449), 5T7gfp: TMTACGACTCACTATAGGACGGC CGCCATTMCMG-CAAAAG (SEQ ID NO. 450) and 3T7gfp: TAATACGACT-CACT ATAGGCTGGGCGGAGCGGATGATG (SEQ ID NO. 451). Note that the gfp dsRNA was used to target the Upd-GFP transgene and leads to a loss-of pathway activity. lacZ dsRNA was used as a negative control.

[0102] For epistasis analysis of the putative negative regulator ptp61F, cells were batch transfected with reporter and Upd inducer as described above. Subsequently, these cells were treated with 1.5 μg of dsRNA targeting the ptp61F transcript and 1.5 μg of dsRNA against lacZ, dome, hop or stat92E. In parallel, cells from the same transfection batch were treated with lacZ, dome, hop or stat92E dsRNAs alone. After normalisation, the values of experiments with control dsRNA alone were set to one. To examine the JAK/STAT phenotype of ptp61F in cells, 5×10^6 Kc_{167} cells were transfected with 0.6 μg pAct-UpdGFP, 0.5 μg 6x2xDrafLuc reporter, 0.25 μg pAc5.1-Sid-1, 0.025 μg pAct-RL and pAc5.1 to a total of 2 μg DNA. To assess the effects of the different Ptp61F splice forms, cells were transfected as described before with additional 0.5 μg of pAct-Ptp61Fa,

pAct-Ptp61Fc or vector control, respectively. JAK/STAT pathway activation was expressed in relation to control cells.

1.4. Genetics

[0103] A P-element insertion termed I(3)05842 (Spradling, A. C. et al., Genetics 153, 135-77 (1999)) was identified in the fourth intron of dBRWD3/CG31132 as part of a Flybase search (Drysdale, R. A. et al., Nucleic Acids Res 33 Database Issue, D390-5 (2005)). A I(3)05842 stock was obtained from the Bloomington stock centre (University of Indiana). The P-element insertion I(3)05842 is homozygous lethal and fails to complement the Df(3R)crb874 and Df(3R)crb87-5 deficiencies. Twenty three independent stocks in which the ry^+ marker present in the P[ry^+,PZ] insertion had been lost following a cross to a transposase source were established. Of these, seven were viable revertants (30%) and include two stocks with the wing vein phenotype (FIG. **3***d*), two are semi-lethal with occasional escapers and the remainder were lethal.

[0104] For genetic interaction assays, females of the stock y,w,hop^{Tuml}/FM7; P [w^+,cg-Gal4.A]2 (Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. & Perrimon, N., Embo J 14, 2857-65 (1995)) were crossed to wild type controls (OreR and w^{1118}) and mutations in stat92E and I(3)05842. The haemocyte specific Gal4 driver line P[w^+,cg-Gal4.A]2 allowed specific UAS insertions to be tested for their potential influence on tumour formation. Transgenic animals expressing UAS-EGFP or UAS-β-galactosidase were used as negative controls while UAS-dPIAS-EGFP served as a positive control (Betz, A., Lampen, N., Martinek, S., Young, M. W. & Darnell, J. E., Jr., Proc Natl Acad Sci USA 98, 9563-8 (2001)) (see Table 3).

[0105] Crosses were incubated at 25° C. and adult females heterozygous for the hop^{Tuml} chromosome were scored within 24 hours of eclosion for the presence of tumours classified as small (one or two small melanotic spots as shown in FIG. **4***b* [right]) or large (one or more large melanised growths or more than three small spots; FIG. **4***b* [left]). Survival rates for hop^{Tuml} females appear to be independent of tumour frequency at the time point counted (not shown). Assays were repeated at least twice for each genotype and a representative example from one experiment is shown (FIG. **4***b*).

[0106] Genetic interaction with P[w^+,GMR-updΔ3']'19 was undertaken as described in Genetics 165, 1149-66 ((2003), Bach, E. A., Vincent, S., Zeidler, M. P. & Perrimon, N.) using OreR and $STAT92E^{06346}$ as negative and positive controls, respectively. Suppression of P[w^+,GMR-updΔ3']'19 induced eye overgrowth by $dBRWD3^{05842}$ was observed in multiple independent experiments in a majority of individuals of the appropriate genotype. *Drosophila* heads were photographed using a Zeiss STEMI 2000-C binocular microscope and Axiocam camera.

2. Results and Discussion

[0107] Developmental genetic screens in *Drosophila* have identified multiple JAK/STAT pathway components on the basis of their segmentation phenotype (Binari, R. & Perrimon, N., Genes Dev 8, 300-12. (1994); Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. & Perrimon, N., Genes Dev 12, 3252-63. (1998); Hou, X. S., Melnick, M. B. & Perrimon, N., Cell 84, 411-9 (1996)) and subsequent analysis of the pathway has characterised evolutionarily conserved roles during immune responses, haematopoiesis and cellular proliferation (Lagueux, M., Perrodou, E., Levashina, E. A., Capovilla, M. & Hoffmann, J. A., Proc Natl Acad Sci USA 97, 11427-32. (2000); Boutros, M., Agaisse, H. & Perrimon, N., Dev Cell 3, 711-22. (2002); Meister, M. & Lagueux, M., Cell Microbiol 5, 573-580 (2003); Mukherjee, T., Castelli-Gair Hombria, J. & Zeidler, M. P., Oncogene in press (2005)). The JAK/STAT signalling cascade in *Drosophila* is comprised of the extracellular ligand Unpaired (Upd) (Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. & Perrimon, N., Genes Dev 12, 3252-63. (1998)), a trans-membrane receptor with homology to the IL6 receptor family termed Domeless (Dome) (Brown, S., Hu, N. & Castelli-Gair Hombria, J., Curr Biol 11, 1700-5. (2001)), a single Janus tyrosine kinase (JAK) called Hopscotch (Hop) (Binari, R. & Perrimon, N., Genes Dev 8, 300-12. (1994)) and the STAT92E transcription factor (Hou, X. S., Melnick, M. B. & Perrimon, N., Cell 84, 411-9 (1996); Yan, R., Small, S., Desplan, C., Dearolf, C. R. & Darnell, J. E., Jr., Cell 84, 421-30 (1996)) (FIG. **1***a*). Known regulators of JAK/STAT signalling including a family of SOCS-like genes (Callus, B. A. & Mathey-Prevot, B.; Oncogene 21, 4812-4821 (2002); Karsten, P., Hader, S. & Zeidler, M. P., Mech Dev 117, 343-6 (2002)), dPIAS/Su(var)2-10 (Betz, A., Lampen, N., Martinek, S., Young, M. W. & Darnell, J. E., Jr., Proc Natl Acad Sci USA 98, 9563-8 (2001)) and STAM (Mesilaty-Gross, S., Reich, A., Motro, B. & Wides, R., Gene 231, 173-86 (1999)) are functionally conserved and were identified based on their homology to components originally characterised in mammalian cell culture studies (Hombria, J. C. & Brown, S., Curr Biol 12, R569-75 (2002)). Although successful in identifying the pathway members Upd, Dome, Hop and STAT92E, it is probable that forward genetic approaches have missed components possibly due to non-saturating mutagenesis, genetic redundancy or phenotypic pleiotropy (Nagy, A., Perrimon, N., Sandmeyer, S. & Plasterk, R., Nat Genet 33 Suppl, 276-84 (2003)).

[0108] In order to identify novel pathway components and circumvent limitations of classical genetic screens, the inventors of the present invention have undertaken a genome-wide RNA interference (RNAi) screen, a powerful technique for the identification of new components of diverse cellular pathways (Kamath, R. S. et al., Nature 421, 231-7 (2003); Kittler, R. et al., Nature 432, 1036-40 (2004); Berns, K. et al., Nature 428, 431-7 (2004); Paddison, P. J. et al., Nature 428, 427-31 (2004); Boutros, M. et al., Science 303, 832-5 (2004)). To this end, the inventors devised a quantitative assay for JAK/STAT signalling activity in cultured *Drosophila* cells by multimerising a STAT92E-binding site from the Draf promotor (Kwon, E. J. et al., J Biol Chem 275, 19824-19830 (2000)) to generate the 6x2xDrafLuc firefly luciferase reporter. Given the role for JAK/STAT signalling in haematopoiesis (Meister, M. & Lagueux, M., Cell Microbiol 5, 573-580 (2003)), the inventors used *Drosophila* hemocyte-like Kc_{167} cells due to their endogenous ability to respond to pathway activation (FIG. **1***b*). On transfection of the 6x2xDrafLuc reporter and a plasmid to constitutively express the ligand Upd, a robust induction of the reporter gene activity was observed (FIG. **1***b*). The inventors first examined whether depletion of known pathway components by RNAi (Clemens, J. C. et al., Proc Natl Acad Sci U S A 97, 6499-6503 (2000)) modifies JAK/STAT signalling activity in Kc_{167} cells. The inventors assessed the effect of double-stranded (ds) RNAs targeting the mRNA of the genes dome, stat92E and hop, as well as dsRNAs directed against the negative regulators socs36E and dPIAS. As shown in FIG. **1***b*, knock down of JAK/STAT

components results in significant changes in reporter activity while reporter activity in uninduced cells remains at low levels (FIG. **1***b*).

[0109] The inventors then set out to systematically identify genes required for JAK/STAT signalling by generating a library of 20,026 dsRNAs targeting 91% of the predicted transcripts in the *Drosophila* genome. Using this library the inventors performed duplicate genome-wide screens as outlined in FIGS. **1***c* and **5**. After computational analysis (FIG. **1***d*), dsRNAs targeting candidates were resynthesised and assayed with an independent reporter, derived from the promoter of the pathway target gene socs36E (Karsten, P., Hader, S. & Zeidler, M. P., Mech Dev 117, 343-6 (2002)) to exclude reporter specific artefacts. These approaches confirmed the identification of 71 dsRNAs which decrease pathway activity (targeting putative positive regulators) and 19 dsRNAs which increase pathway activity (putative negative regulators) (see Table 1). While most modifiers are distributed throughout the genome (FIG. **2***a*), the X chromosome is devoid of negative regulators, a finding which may be linked to the role of the pathway in *Drosophila* sex determination (Sefton, L., Timmer, J. R., Zhang, Y., Beranger, F. & Cline, T. W., Nature 405, 970-3 (2000)).

[0110] Based on InterPro and GO annotations, pathway modifiers were classified according to their predicted functions. Signalling factors, enzymes mediating post-translational protein modifications and transcription factors cumulatively represent 47% of the genes identified (FIG. **2***b*). Furthermore, 74% of the identified loci possess human homologues (E-value $<10^{-10}$), 33% of which have been implicated in human disease (Tables 5 and 6). Examples of genes identified in the screen include CG11501 encoding a putatively secreted negative regulator of JAK/STAT signalling previously demonstrated to be a pathway target gene (Boutros, M., Agaisse, H. & Perrimon, N., Dev Cell 3, 711-22. (2002)), enok/CG11290 encoding an acetyl-transferase and the tumor suppressor protein 101/CG9712 gene which encodes a ubiquitin conjugating enzyme. The molecular role of these genes in the regulation of JAK/STAT signalling remains to be determined.

[0111] A genetic technique to characterise signalling molecules is the determination of their epistatic relationship with respect to defined pathway components. The inventors therefore performed cell-based epistatic assays to determine the pathway response to Upd expression, Upd conditioned medium or expression of the constitutively active JAK allele hop^{Tuml} (Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. & Perrimon, N., Genes Dev 12, 3252-63. (1998); Sefton, L., Timmer, J. R., Zhang, Y., Beranger, F. & Cline, T. W., Nature 405, 970-3 (2000)) while simultaneously targeting a subset of positive regulators. In this way, dsRNA-inactivated genes required upstream in the pathway can be characterised on the basis of their rescue by pathway activation further downstream (FIG. **2***c*). For example, while depletion of the interferon-related protein encoded by CG15401 results in down-regulation of signalling stimulated by Upd expression, stimulation by Upd conditioned medium or hop^{Tuml} is unaffected (FIG. **2***c*). This suggests that CG15401 is required for the production and/or activity of the Upd ligand. Conversely, loss of pathway activity resulting from the knock down of CG18670 and CG6400 (now annotated as one gene termed CG31132) cannot be rescued by any form of pathway stimulus implying a function downstream of JAK (FIG. **2***c*). Although this analysis suggests a role for multiple genes upstream of Dome, this classification is based on the lack of interaction observed under the differing experimental conditions and the molecular basis of these results remains to be confirmed.

[0112] In order to confirm the function of candidate genes in vivo, the inventors tested examples of both positive and negative regulators of the JAK/STAT signalling pathway. One positive regulator mentioned above is CG31132 which encodes a 2232 amino acid WD40 and bromo-domain containing protein homologous to human BRDW3 (FIG. **3***a*). BRDW3 is a functionally uncharacterised locus recently identified at the break point of t(X;11) (q13;q23) translocations derived from multiple B-cell chronic lymphocytic leukaemia (B-CLL) patients (Kalla, C. et al., Genes Chromosomes Cancer 42, 128-43 (2005)). In the screen, reduction of pathway activity was observed for two independent dsRNAs present in the library that target different regions of the transcript (FIG. **3***a*).

[0113] A previously uncharacterised mutagenic P-element inserted in the fourth intron of CG31132 (henceforth termed $dBRDW3^{05842}$) has been deposited in public stock collections as part of the *Drosophila* genome project and remobilisation of this transposon indicates that the insertion is responsible for late embryonic lethality. The inventors therefore tested for genetic interactions between dBRDW3 and JAK/STAT signalling by crossing the $dBRDW3^{05842}$ allele to GMR-updΔ3' (Bach, E. A., Vincent, S., Zeidler, M. P. & Perrimon, N., Genetics 165, 1149-66 (2003)). The GMR-upd□3' transgene ectopically misexpress Upd during eye development resulting in cellular overproliferation and an enlarged adult eye (FIG. **3***b* (left)). Furthermore, removal of one copy of stat92E significantly suppresses eye overgrowth (FIG. **3***b* (middle)) due to a reduction in the potency of JAK/STAT signalling (Bach, E. A., Vincent, S., Zeidler, M. P. & Perrimon, N., Genetics 165, 1149-66 (2003)). Removal of a single copy of dBRDW3 was also able to suppress the GMR-updΔ3' phenotype (FIG. **3***b* (right)) as expected for a positive regulator of JAK/STAT signalling. In addition, a chromosomal deficiency removing the region has also been independently identified as a suppressor of GMR-updΔ3' (Bach, E. A., Vincent, S., Zeidler, M. P. & Perrimon, N., Genetics 165, 1149-66 (2003)).

[0114] One phenotypic consequence of constitutive JAK/STAT activation caused by the gain-of-function JAK allele hop^{Tuml} is the overproliferation of haemocytes and the frequent formation of melanotic tumours, a phenotype previously described as a *Drosophila* model for leukaemia (Luo, H., Hanratty, W. P. & Dearolf, C. R., Embo J 14, 1412-20 (1995); Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. & Perrimon, N., Embo J 14, 2857-65 (1995)). The inventors found that the removal of one copy of dBRWD3 is sufficient to reduce the size and the frequency of hop^{Tuml} induced melanotic tumours (FIG. **3***c* and Table 3). Moreover, homozygous escapers of a putative hypomorphic allele of dBRWD3, generated by excision of the original P-element, frequently develop ectopic wing vein material (FIG. **3***d*) reminiscent of the weak loss-of-function $stat92E^{HJ}$ allele (Yan, R., Luo, H., Darnell, J. E., Jr. & Dearolf, C. R., Proc Natl Acad Sci USA 93, 5842-7 (1996)). Taken together, these experiments suggest a role for dBRWD3 in JAK/STAT signalling.

[0115] As a second example the inventors analysed the ptp61F gene which encodes a protein tyrosine phosphatase. dsRNA knocking down all mRNA splice forms transcribed from this locus leads to an increase in JAK/STAT signalling

activity. The inventors performed epistasis analysis in which the inventors removed known pathway components and tested for the effect of simultaneously targeting ptp61F. Double RNAi against ptp61F together with lacZ, dome or hop results in pathway stimulation (FIG. **4***a*). However, simultaneous removal of ptp61F and stat92E is sufficient to prevent signalling (FIG. **4***a*). Loss of this phosphatase therefore results in the stimulation of STAT92E activity even in the absence of upstream components indicating that Ptp61F negatively regulates the pathway downstream of JAK. The inventors next asked whether Ptp61F also interferes with JAK/STAT signalling in vivo by using the cg-Gal4 driver to misexpress ptp61F in blood cells of hop^{Tuml} mutants. Misexpression of Ptp61Fc in a hop^{Tuml} mutant background resulted in a suppression of melanotic tumour formation with the average frequency of large tumours reduced by approximately 4 fold, an effect also observed following the misexpression of the known negative regulator dPIAS (Betz, A., Lampen, N., Martinek, S., Young, M. W. & Darnell, J. E., Jr., Proc Natl Acad Sci USA 98, 9563-8 (2001)) (FIG. **4***b* and Table 3). Alternative splicing of ptp61F leads to nuclear and cytoplasmic protein forms which both contain the same phosphatase domain (McLaughlin, S. & Dixon, J. E., J Biol Chem 268, 6839-42 (1993)). However, the tumour suppressor phenotype is only observed with nuclear Ptp61Fc (FIG. **4***c*), an effect that is reproduced by over-expression of the nuclear localised protein in cell culture (FIG. **4***c*). These results are consistent with our identification of ptp61F as a negative regulator of pathway activity and suggest that it may function by targeting phosphorylated, nuclear localised STAT92E for deactivation.

[0116] Aberrant JAK/STAT signalling has been implicated in multiple human malignancies and its components have been proposed as molecular targets for the development of therapeutic compounds (O'Shea, J. J., Pesu, M., Borie, D.C. & Changelian, P. S., Nat Rev Drug Discov 3, 555-64 (2004)). The genome-wide screen presented here identified known and previously unknown genes and the inventors have characterised their likely level of interaction with defined pathway components using cell-based epistasis analysis. Of the 89 JAK/STAT modifiers identified, many have human homologues that remain to be characterised. The inventors have here performed an analysis of two examples in vivo and demonstrate their roles in regulating the pathway during development and tumour genesis in *Drosophila*. One of these is a homologue of human BRWD3, a gene recently identified at the break-point of a translocation isolated from multiple B-CLL patients (Kalla, C. et al., Genes Chromosomes Cancer 42, 128-43 (2005)). Given our functional analysis of dBRWD3 and the known roles for JAK/STAT signalling during normal haematopoiesis, it is possible that a breakdown in BRWD3 mediated STAT regulation may represent a key molecular mechanism leading to the development of B-CLL. Thus, comprehensive reverse genetic surveys for signalling pathway components using *Drosophila*as a model organism represent a potentially powerful approach with which insights relevant to human disease can be obtained.

Example 2

[0117] Novel components regulating the JAK/STAT pathway in *Drosophila*melanogaster have been previously been identified using a robust STAT92E responsive reporter assay in combination with genome-wide RNAi (Müller, P., Kuttenkeuler, D., Gesellchen, V. Zeidler, M.P. and Boutros M. (2005) "Identification of JAK/STAT signalling components by genome-wide RNAi" Nature 436 871-875). Having identified the essential components in *Drosophila*, a second crucial step is the identification of human functional orthologs. Given that many of the potential human orthologs have been implicated in human disease, these proteins, and the mRNAs that encode them, may represent targets for therapeutic interventions by small molecules or RNAi based approaches. Using a HeLa cell model we have assayed the activity of endogenous STAT1 and STAT3 following treatment with siRNA targeting potential pathway modulating genes. Assays of hSTAT phosphorylation state and the expression levels of their targets, have identified 27 human genes, which function as modulators of human JAK/STAT signal transduction. These have been ranked on the basis of potential significance and are listed in Table 7 together with the human diseases they have previously been associated with.

Results

[0118] Compared to *Drosophila*, the JAK/STAT pathway in mammalians is much more complex in that multiple paralogs exist for the pathway ligand, receptor, JAK and STAT. As an initial approach towards identifying regulators of human JAK/STAT signaling, we have analyzed phenotypes caused by siRNA-mediated knockdown of candidate pathway modifiers in human cells. Human genes for this analysis were selected based on their homology to *Drosophila* JAK/STAT pathway regulators previously identified (Müller et al. 2005). Homology prediction by a variety of methods yielded 73 candidates homologous to 56 *Drosophila* genes. Pools of 4 siRNAs per candidate (Dharmacon SMARTpools) were used to ensure the efficiency and specificity of knockdown. As an easily tractable model, we have used human cancer-derived HeLa cells which express multiple STATs and which respond to stimulation by a variety of cytokine ligands (Ehret G.B., Reichenbach P., Schindler U., Horvath C.M., Fritz S., Nabholz M., Bucher P. (2001) "DNA binding specificity of different STAT proteins. Comparison of in vitro specificity with natural target sites" *J Biol Chem* 276 6675-6688).

[0119] Two approaches have been used to determine the activity of STAT1 and STAT3 in the HeLa cell system tested. Firstly, the levels of tyrosine-701-phosphorylated STAT1 (pSTAT1) and tyrosine-705-phosphorylated STAT3 (pSTAT3) were determined in HeLa cell lysates that had been stimulated with human Interferon gamma (INFγ) or Oncostatin M (OSM) for 15 min, respectively. These cells had previously been treated with siRNA targeting either controls or the putative pathway interactors for 72hs. After determination of the overall level of STAT1/3, the western blots were stripped and re-probed with pSTAT1 and pSTAT3 antibodies and with antibodies to determine β-ACTIN levels as a normalization control. The relative levels of pSTAT1/3 versus STAT1/3 were assessed with regard to the overall level of β-ACTIN detected and a call made representing either an increase in PSTAT levels (+), a decrease in PSTAT (−) or no change (FIG. **7** column 4 & 5).

[0120] As a second independent approach to determine the level of STAT1 and STAT3 activity, the expression levels of the previously characterized pathway target genes GBPI (a STAT1 target) and SOCS3 (a STAT3 target) were determined 6 hrs after stimulation of HeLa cells with INFy and OSM, respectively. As before, cells had previously been treated with siRNA targeting either controls or putative pathway regulators for 72 hrs. Target gene levels were determined using

branched DNA technology (QuantiGene, Panomics) and normalized to the level of β-actin mRNA. Results from duplicate assays are expressed as fold changes in target gene expression levels relative to cells treated with control siRNA. Statistically significant changes in response ($p<0.05$) are shown in black (decrease in expression level) or white (increase in expression) (FIG. 7 column 2 & 3, Table 8 column 5 & 6). In this table the scores relating to hSTAT1 and hSTAT3 target genes are expressed such that 1 is the expression level induced by pathway ligands following treatment with a control siRNA. Numbers below 1 therefore indicate a reduction in expression while scored above 1 represent an increase. Scores for *Drosophila* STAT92E are expressed as z-scores—a measure of statistical significance in which significant suppression of activity is represented by numbers <−2.0 while significant enhancement is represented by values >+2.0. Statistically significant changes are indicated by the change in colour of the boxes shown in FIG. **7**. Note that only genes which interact via at least one assay are shown and other human homologues of interacting *Drosophila* genes not listed did not show any interaction with the STAT1 or STAT3 assays used.

[0121] Analysis of these two independent data sets, in conjunction with the scores originally obtained for the *Drosophila* orthologs (FIG. **7** column 1 and Table 8, column 4) has identified positively acting factors that are required for both STAT1 & 3 as well as factors that are required specifically by only STAT1 or STAT3. In addition negatively acting factors acting on either or both STATs have been found. Finally, some factors act positively for one STAT and negatively for another (this may be a result of redundancy within the pathway) while others act as positive regulators in *Drosophila* but as negative regulators in human cells. This analysis has lead to the compilation of a list of human genes playing a role in the regulation of human JAK/STAT signaling (Table 7). These genes have been ranked by order of interest (highest at the top; Table 7) as judged by criteria such as involvement in human disease, predicted sub-cellular localization and strength of interaction.

TABLE 1

JAK/STAT phenotypes by RNAi

Gene name	dsRNA ID	z-score [6 × 2 × Draf-luc]	z-score [4 × SOCS-luc]	Functional group assignment (based on GO and Interpro evidence)	Interpro 8.0 evidence	GO Evidence	SEQ ID NO.
				Positive Regulators			
Art2	HFA00627	−2.9	−3.2	Protein modifying enzymes/Metabolism	IPR000051	GO: 0016274; protein-arginine N-methyltransferase activity	SEQ ID NO. 175
asf1	HFA11324	−2.3	−2.5	Others	IPR008967	GO: 0003682; chromatin binding	SEQ ID NO. 176
bin3	HFA04919	−3.1	−3.3	Unknown	IPR000051	na; na	SEQ ID NO. 177
CG10007	HFA14173	−3.2	−2.9	Unknown	noIPR	na; na	SEQ ID NO. 178
CG10730	HFA02102	−2.1	−2.3	Unknown	IPR004245	na; na	SEQ ID NO. 179
CG10960	HFA09807	−2.0	−2.1	Protein modifying enzymes/Metabolism	IPR005829	GO: 0005355; glucose transporter activity	SEQ ID NO. 180
CG11307	HFA11648	−2.3	−2.4	Unknown	noIPR	GO: 0016757; transferase activity	SEQ ID NO. 181
CG11696	HFA19417	−2.0	−2.3	Transcription regulators	IPR007087	GO: 0003677; DNA binding	SEQ ID NO. 182
CG12213	HFA14478	−3.3	−3.2	Unknown	IPR009053	na; na	SEQ ID NO. 183
CG12460	HFA20970	−3.3	−3.4	Transcription regulators	IPR000504	GO: 0030528; transcription regulator activity	SEQ ID NO. 184
CG12479	HFA19459	−2.3	−2.4	Unknown	IPR007512	na; na	SEQ ID NO. 185
CG13243	HFA01920	−2.7	−2.6	Unknown	IPR003117	na; na	SEQ ID NO. 186
CG13473	HFA10017	−2.4	−2.1	Cytoskeleton and Transport	IPR006662	GO: 0005489; electron transporter activity	SEQ ID NO. 187
CG14434	HFA17927	−2.0	−2.3	Unknown	IPR008173	na; na	SEQ ID NO. 188
CG15306	HFA17993	−3.3	−3.1	Signal transduction	IPR001715	GO: 0005102; receptor binding	SEQ ID NO. 189
CG15418	HFA00432	−2.1	−2.1	Protein modifying enzymes/Metabolism	IPR002223	GO: 0004866; endopeptidase inhibitor activity	SEQ ID NO. 190
CG15434	HFA00449	−2.5	−2.9	Protein modifying enzymes/Metabolism	IPR007741	GO: 0003954; NADH dehydrogenase activity	SEQ ID NO. 191
CG15555	HFA15093	−2.3	−2.6	Others	IPR001873	GO: 0015268; alpha-type channel activity	SEQ ID NO. 192
CG15784	HFA18090	−2.4	−2.6	Unknown	IPR009072	na; na	SEQ ID NO. 193
CG16903	HFA18561	−2.8	−2.8	Transcription regulators	IPR011028	GO: 0016251; general RNA polymerase II transcription factor activity	SEQ ID NO. 194
CG17179	HFA10258	−2.1	−2.8	Unknown	IPR001680	na; na	SEQ ID NO. 195
CG18160	HFA21006	−3.1	−2.4	Unknown	noIPR	na; na	SEQ ID NO. 196
CG30069	HFA06272	−2.9	−2.2	Protein modifying enzymes/Metabolism	noIPR	GO: 0016491; oxidoreductase activity	SEQ ID NO. 197
CG3058	HFA00563	−3.4	−3.5	Cytoskeleton and Transport	IPR006663	GO: 0005489; electron transporter activity	SEQ ID NO. 198
CG31005	HFA15507	−2.3	−3.0	Protein modifying enzymes/Metabolism	IPR000092	GO: 0000010; trans-hexaprenyltranstransferase activity	SEQ ID NO. 199
CG31132	HFA16032	−2.8	−3.5	Unknown	IPR001487	na; na	SEQ ID NO. 200
CG31132	HFA15369	−2.3	−3.6	Unknown	IPR001487	na; na	SEQ ID NO. 201
CG31358	HFA15235	−2.0	−2.2	Cytoskeleton and Transport	IPR001972	GO: 0005200; structural constituent of cytoskeleton	SEQ ID NO. 202
CG31694	HFA00415	−2.8	−2.7	Signal transduction	IPR006921	GO: 0005102; receptor binding	SEQ ID NO. 203
CG32406	HFA09966	−2.1	−2.2	Signal transduction	IPR000980	na; na	SEQ ID NO. 204
CG32573	HFA19906	−3.1	−2.9	Unknown	IPR000719	na; na	SEQ ID NO. 205
CG3281	HFA15470	−3.1	−3.0	Transcription regulators	IPR007087	GO: 0030528; transcription regulator activity	SEQ ID NO. 206
CG3819	HFA10378	−2.3	−2.3	Unknown	IPR001604	na; na	SEQ ID NO. 207
CG4022	HFA10395	−3.4	−3.7	Unknown	noIPR	na; na	SEQ ID NO. 208
CG40351	HFA20930	−2.6	−2.7	Transcription regulators	IPR001214	GO: 0030528; transcription regulator activity	SEQ ID NO. 209
CG4349	HFA19892	−4.1	−2.1	Others	IPR009040	GO: 0008199; ferric iron binding	SEQ ID NO. 210
CG4446	HFA10420	−2.7	−2.7	Protein modifying enzymes/Metabolism	IPR004625	GO: 0008478; pyridoxal kinase activity	SEQ ID NO. 211
CG4653	HFA19909	−3.2	−3.0	Protein modifying enzymes/Metabolism	IPR001254	GO: 0004263; chymotrypsin activity	SEQ ID NO. 212
CG4781	HFA04488	−2.5	−2.5	Unknown	IPR003591	na; na	SEQ ID NO. 213
CG6422	HFA16036	−3.3	−3.2	Unknown	IPR007275	na; na	SEQ ID NO. 214
CG6434	HFA10635	−2.8	−2.8	Unknown	IPR001680	na; na	SEQ ID NO. 215
CG6946	HFA16145	−2.3	−2.9	RNA processing and Translation	IPR000504	GO: 0003723; RNA binding	SEQ ID NO. 216
CG7635	HFA20054	−2.9	−2.8	Cytoskeleton and Transport	IPR001972	GO: 0005200; structural constituent of cytoskeleton	SEQ ID NO. 217

TABLE 1-continued

JAK/STAT phenotypes by RNAi

Gene name	dsRNA ID	z-score [6 × 2 × Draf-luc]	z-score [4 × SOCS-luc]	Functional group assignment (based on GO and Interpro evidence)	Interpro 8.0 evidence	GO Evidence	SEQ ID NO.
CG8108	HFA09675	−2.7	−2.7	Transcription regulators	IPR007087	GO: 0003676; nucleic acid binding	SEQ ID NO. 218
CG9086	HFA20148	−2.8	−2.9	Signal transduction	IPR009030	GO: 0005057; receptor signaling protein activity	SEQ ID NO. 219
CkIIalpha	HFA11946	−2.1	−2.5	Signal transduction	IPR000719	GO: 0004702; receptor signaling protein serine/threonine kinase activity	SEQ ID NO. 220
CkIIbeta	HFA20230	−2.7	−2.6	Signal transduction	IPR000704	GO: 0004702; receptor signaling protein serine/threonine kinase activity	SEQ ID NO. 221
comm3	HFA09995	−2.2	−2.2	Unknown	noIPR	na; na	SEQ ID NO. 222
CtBP	HFA16617	−2.9	−2.8	Transcription regulators	IPR006139	GO: 0003714; transcription corepressor activity	SEQ ID NO. 223
dome	HFA19583	−6.2	−4.9	Signal transduction	IPR000194	GO: 0004907; interleukin receptor activity	SEQ ID NO. 224
eIF-4B	HFA20983	−3.2	−3.0	RNA processing and Translation	IPR000504	GO: 0003723; RNA binding	SEQ ID NO. 225
HDC01676	HFA01091	−2.3	−2.6	Unknown	IPR006202	na; na	SEQ ID NO. 226
HDC11198	HFA11427	−2.3	−2.2	Unknown	noIPR	na; na	SEQ ID NO. 227
hop	HFA20340	−5.7	−4.1	Signal transduction	IPR001245	GO: 0004718; Janus kinase activity	SEQ ID NO. 228
Ipk2	HFA00357	−2.6	−4.0	Signal transduction	IPR005522	GO: 0050516; inositol-polyphosphate multikinase activity	SEQ ID NO. 229
jbug	HFA04167	−2.7	−3.2	Cytoskeleton and Transport	IPR001298	GO: 0005200; structural constituent of cytoskeleton	SEQ ID NO. 230
kn	HFA07637	−2.4	−2.4	Transcription regulators	IPR003523	GO: 0030528; transcription regulator activity	SEQ ID NO. 231
l(1)G0084	HFA19450	−2.1	−2.1	Transcription regulators	IPR001965	GO: 0003677; DNA binding	SEQ ID NO. 232
larp	HFA16984	−2.5	−2.4	Unknown	IPR006630	na; na	SEQ ID NO. 233
mask	HFA15370	−2.3	−2.7	Signal transduction	IPR002110	GO: 0005102; receptor binding	SEQ ID NO. 234
mst	HFA20582	−2.2	−2.6	Unknown	noIPR	na; na	SEQ ID NO. 235
nonA	HFA20357	−3.0	−3.3	RNA processing and Translation	IPR000504	GO: 0030528; transcription regulator activity	SEQ ID NO. 236
Obp93a	HFA15220	−2.4	−2.9	Cytoskeleton and Transport	IPR006170	GO: 0005549; odorant binding	SEQ ID NO. 237
Rrp1	HFA00784	−4.3	−4.3	Others	IPR000097	GO: 0004520; endodeoxyribonuclease activity	SEQ ID NO. 238
sol	HFA20587	−2.5	−3.0	Others	IPR001876	GO: 0005516; calmodulin binding	SEQ ID NO. 239
Stat92E	HFA16870	−5.0	−5.2	Signal transduction	IPR001217	GO: 0004871; signal transducer activity	SEQ ID NO. 240
Taf2	HFA11298	−2.7	−2.9	Transcription regulators	IPR002052	GO: 0016251; general RNA polymerase II transcription factor activity	SEQ ID NO. 241
				Negative regulators			
bon	HFA16914	5.6	4.8	Protein modifying enzymes/Metabolism	IPR001841	GO: 0004842; ubiquitin-protein ligase activity	SEQ ID NO. 242
Caf1	HFA16596	3.0	2.6	Protein modifying enzymes/Metabolism	IPR001680	GO: 0035035; histone acetyltransferase binding	SEQ ID NO. 243
CG10077	HFA09691	2.8	4.0	RNA processing and Translation	IPR001410	GO: 0003724; RNA helicase activity	SEQ ID NO. 244

TABLE 1-continued

JAK/STAT phenotypes by RNAi

Gene name	dsRNA ID	z-score [6 × 2 × Draf-luc]	z-score [4 × SOCS-luc]	Functional group assignment (based on GO and Interpro evidence)	Interpro 8.0 evidence	GO Evidence	SEQ ID NO.
CG11400	HFA06070	2.6	2.2	Unknown	noIPR	na; na	SEQ ID NO. 245
CG11501	HFA14317	3.7	3.1	Unknown	noIPR	na; na	SEQ ID NO. 246
CG13499	HFA04144	2.5	3.1	Unknown	noIPR	na; na	SEQ ID NO. 247
CG14247	HFA14742	3.2	3.4	Unknown	IPR002557	na; na	SEQ ID NO. 248
CG15706	HFA06577	2.2	2.1	Unknown	IPR011701	na; na	SEQ ID NO. 249
CG16975	HFA02552	2.7	2.7	Transcription regulators	IPR001660	GO: 0030528; transcription regulator activity	SEQ ID NO. 250
CG17492	HFA02623	2.5	2.1	Protein modifying enzymes/Metabolism	IPR001841	GO: 0004842; ubiquitin-protein ligase activity	SEQ ID NO. 251
CG18112	HFA15304	2.1	2.1	Unknown	IPR001829	na; na	SEQ ID NO. 252
CG30122	HFA06935	3.3	2.8	Transcription regulators	IPR003034	GO: 0003677; DNA binding	SEQ ID NO. 253
CG4907	HFA15673	3.3	3.5	Unknown	IPR007070	na; na	SEQ ID NO. 254
dre4	HFA08714	2.6	2.5	Transcription regulators	IPR000994	GO: 0003712; transcription cofactor activity	SEQ ID NO. 255
enok	HFA04096	3.0	3.0	Transcription regulators	IPR001965	GO: 0030528; transcription regulator activity	SEQ ID NO. 256
lig	HFA07247	2.2	2.1	Unknown	IPR009060	na; na	SEQ ID NO. 257
Nup154	HFA03384	2.9	2.9	Cytoskeleton and Transport	IPR011045	GO: 0005487; nucleocytoplasmic transporter activity	SEQ ID NO. 258
par-1	HFA07660	4.4	4.2	Signal transduction	IPR000719	GO: 0004674; protein serine/threonine kinase activity	SEQ ID NO. 259
Pp1alpha-96A	HFA16795	3.0	3.8	Signal transduction	IPR006186	GO: 0004722; protein serine/threonine phosphatase activity	SEQ ID NO. 260
PP2A-B'	HFA16344	2.6	2.5	Signal transduction	IPR002554	GO: 0008601; protein phosphatase type 2A regulator activity	SEQ ID NO. 261
Ptp61F	HFA08683	5.9	8.1	Signal transduction	IPR000863	GO: 0004725; protein tyrosine phosphatase activity	SEQ ID NO. 262
Rab5	HFA00777	2.1	2.1	Signal transduction	IPR001806	GO: 0005525; GTP binding	SEQ ID NO. 263
Socs36E	HFA02455	3.2	2.3	Signal transduction	IPR000980	GO: 0007259; JAK-STAT cascade	SEQ ID NO. 264
TSG101	HFA11098	3.1	3.4	Protein modifying enzymes/Metabolism	IPR001440	GO: 0004842; ubiquitin-protein ligase activity	SEQ ID NO. 265

InterPro Evidence was obtained from: Mulder et al. (2005). InterPro, progress and status in 2005.
GO Evidence was obtained from: R. A. Drysdale, M. A. Crosby and The FlyBase Consortium (2005). FlyBase: genes and gene models. Nucleic Acids Research 33: D390-D395. http://flybase.org/
All 384-well screening plates contained dsRNAs against known JAK/STAT pathway components.
Controls for the 57 screening plates were stat92E RNAI (identified 55 times), hop RNAi (identified 37 times), dome RNAi (identified 55 times) and socs36E RNAi (identified 45 times)
An Interactive table with links to the Interpro records is available at http://www.dkfz.de/signaling/jak-pathway/

TABLE 2

Functional groups classified by InterPro prediction and GO.

Functional Group†	N*
Signalling factors	17
Transcription factors	14
Protein modification and Metabolism	12
Cytoskeleton and Transport	7
All others	9

TABLE 2-continued

Functional groups classified by InterPro prediction and GO.

Functional Group†	N*
Predicted proteins classified as part of a	59
Predicted proteins without classification	31

Queries were performed with InterPro 8.0
†InterPro and GO results classified into one of functionally related groups. See Table 1 for complete list of genes, specific IPR domains and GO assigned within each group.
*Number of proteins identified with InterPro domains and/or GO found in 90 translated gene sequences.

SUPPLEMENTARY TABLE 3

Genetic interactions with hop^{Tuml} (1)

Exp	Genotype	Insert/ Allele	Tumours (%) None	Small	Large	n	z-score
I	y, w, hop^{Tuml}/+; +/+	OreR	31.0	50.6	18.4	358	−0.4(*)
II	y, w, hop^{Tuml}/+; +/+	OreR	31.0	43.8	25.2	445	−0.4(*)
II	y, w, hop^{Tuml}/+; +/+	w1118	23.9	31.2	44.9	356	0.6(*)
II	y, w, hop^{Tuml}/+; STAT92E/+	397	67.5	21.5	11.0	228	−5.3(2)
I	y, w, hop^{Tuml}/+; STAT92E/+	06346	68.6	26.1	5.3	283	−5.4(3)
II	y, w, hop^{Tuml}/+; STAT92E/+	06346	64.2	26.6	9.2	282	−4.9(3)
II	y, w, hop^{Tuml}/+; dBRWD3/+	05842	56.6	24.4	19.0	221	−3.8
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-EGFP	5a.2	19.9	35.1	45.0	151	1.2(*)
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-EGFP	6a.3	41.0	33.3	25.7	451	−1.7(*)
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-lacZ	BG4-1-2	25.8	26.4	47.8	341	0.4(*)
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	1b.2	46.5	27.7	25.7	101	−2.5
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	1b.2	46.5	29.1	24.3	230	−2.5
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	1a.3	22.6	28.8	48.6	177	0.8
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	1a.3	19.6	24.4	56.0	168	1.2
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	3a.3	35.8	28.5	35.8	165	−1.0
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fa	7a.3	16.4	36.1	47.5	61	1.6
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fc	1a.1	68.2	21.4	10.4	280	−5.4
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fc	2a.4	56.1	30.6	13.3	255	−3.8
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fc	2a.4	52.3	40.7	7.0	344	−3.2
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fc	2b.3	59.4	33.8	6.8	234	−4.2
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-ptp61Fc	2b.3	63.3	29.3	7.3	300	−4.7
II	y, w, hop^{Tuml}/+; cg-Gal4/UAS-dPIAS-GFP	26b.3	67.0	27.4	5.7	106	−5.2(4)
I	y, w, hop^{Tuml}/+; cg-Gal4/UAS-dPIAS-GFP	26b.3	63.1	33.6	3.3	122	−4.7(4)

Values shown represent percentage of 0-24 hr old female flies containing no, small or large tumours visible in abdomen or thorax. Table shows results from two independent experiments (first column) undertaken under identical conditions.
(*)'wild type' results used to calculate z-scores

References:

(1)Hanratty, W. P. & Dearolf, C. R. The *Drosophila* Tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. Mol Gen Genet 238, 33-7 (1993).
(2)Silver, D. L. & Montell, D. J. Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. Cell 107, 831-841 (2001).
(3)Hou, X. S., Melnick, M. B. & Perrimon, N. Marelle acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell 84, 411-9 (1996).
(4)Betz, A., Lampen, N, Martinek, S., Young, M. W. & Darnell, J. E. Jr. A *Drosophila* PIAS homologue negatively regulates stat92E. Proc Natl Acad Sci USA 98, 9563-8 (2001).

TABLE 4

Sequence and cytological information

dsRNA ID	Amplicon primer 1A	Ampilcon primer 2
HFA00627	TGC CTG TTT TCT GGA AAT ATG	CTC GCT GGG TTT CAT GGT
HFA11324	TCG AAC TCA CGT TCG AGT	ATC ATC TTC GGG ATG GAT AAC
HFA04919	GAG ATA CCC CGT GAT GAC A	CTT GGG AAT ACG CAC AAA GA
HFA16914	AGG TGC TGG TGG AAA AGA A	ACC CGT CAC CCG GAA AG

TABLE 4-continued

Sequence and cytological information		
HFA16596	TAT TTG CTG TCA GCC TCT G	TGG TCC GTC CTC AGC ATC
HFA14173	CGC CCT GAT CTT TGT GGG	GGA CGA GTA CAT CGC AAT G
HFA09691	GCA CCA CCT CGT TGA AGA	GGG CAG CCA CAT CGG T
HFA02102	GAA CTT CAT TTG GAA GCG TTT	CTT GCG CCG GAA CCA G
HFA09807	GCC GCC GGT ACC GTC	AAG TAG GTG GGC GAT TCC
HFA11648	CCG TGG CCA CAG GAA CA	CAG TCC TGT TCA TGT GGA AAT
HFA06070	TTG TCT GGC TGT GTC TGT C	GAC AAT CCT TGG CCC AAT AAC
HFA14317	ATG GCA TCC CCA GTA GTC A	GTG ACT TTG ATG ATC TGG ATT C
HFA19417	GCC GAC GAA CAG CCA AA	TCG CAC ACC TCG GGA C
HFA14478	TAA CGG TGA CGG AAC CCA	CCG AAT CCT CGA TGG GTT
HFA20970	GCC AAA ATC AAG CGA ATC AG	CTT AAT TGC CTG CAC CTC C
HFA19459	ATC GGC TGC GTG AGA AC	TTC GTT GGC CAA ACT TTA CA
HFA01920	GAT TGG ACG CTT CGC TTT GA	GTT GAA ACA TTG CTG GGT GA
HFA10017	TGG CTG CCA TGC AGA AG	CCA ATT TCG GCA CGG TAG
HFA04144	AGT GGC AGC GGA GGT G	GCC CTC GCA GTG GGT T
HEA14742	AAA ATA AAT GGA GTA ACT TCC CC	TAC GCC TCG CAC TCC A
HFA17927	CGC AAT GTG GAG GTG AAG	ATC GAA ATA CGA GCC GAT C
HFA17993	TTC GAG GGC CCA CAA TGT	TGG CAA GTC GCA ACT TTA C
HFA00432	CAA AGG CAC CTG GTT TGT G	CAG TAG CGC AGA CGT TG
HFA00449	GGT ATT ACT CTG TTC CGA TTG	CTT CCA GGT TTT TGT GTA TGT C
HEA15093	GGC AAA GAT CCC AAG CAG	GTT GAA GGT GCA GCA GAA G
HFA06577	CAG CCA TCG ATT GGA ACA G	CTC CAA GTG CCA GAA CAT AAA
HFA18090	GGC CAC AAG CAT GGT CG	CCT TGC CCT TGC ACT TCT
HFA18561	TCG CCC ATG GTG CTA GA	CGA TCC ACG GTG ATT ACA G
HFA02552	CAG ACT CCT ACC TCG TTT TG	AAC ATG CGC TCC AGA TAG T
HFA10258	GCC AAG AGA CGG AGA AGA	TAC GGA TGC TGG TTG ATG T
HFA02623	CCC AGG GCC ATT TGG ATT T	TCC TTT AAG CGC TGC ATG
HFA15304	GGG CAT GCC GTC ATT ACA	CGG CGA TAT TTG CTG GTC
HFA21006	GTG GCG CAC CGG AAA G	GAT GAA CTT CAT TGT TGT TGA AA
HFA06272	TGA CGA AGC ATA TAC AAG GAT A	TGG GTT TTT CTG GTG AAA CAA
HFA06935	GTT TGC ATC GGC CAA ACC	GTG TCA GAG AAA TTC ACT AAG TA
HFA00563	AAT ACG TTT CGG TCA CGA TT	GTA TCT GTA CTT GGT AGA GTA GT
HFA15507	CCC CGA GCT GAA TCC CA	CTT CAT GCG GTT GAT GAC TA
HFA15369	CGT AAG TGC TAG TTC CTC TG	TGC CGA GCG TCC CTT T
HFA16032	CCC ACG GAG CTG TTC TTT	AAA CGA CTA CCC AGG ACA TT
HFA15235	AGG CAT CTG CAG ATT CTC T	GAG GAA TGG GAA TGG ATG AAG
HFA00415	GTC ATG GGT CCC GGG ATG	TCG CTT GTC ACG ATT CTT T

TABLE 4-continued

Sequence and cytological information		
HFA09966	CCG CCA CAA TGA TAA CCA AC	CGC GTG CGT GAA GAG T
HFA19906	ATC TGT TGA ACG CCG AGG	GGT ATC GGT GAA GTT CTT CTC
HFA15470	TTG TCG CGA CCT TCC CA	ACT TCT TGG AGC AGA TCT TG
HFA10378	CGG ACA CCG GCT ATG TG	ATG TTC TTG GCC GAG TCA A
HFA10395	TAC TCA AGG ATC GCG ATA TC	GGC TGG GTG TGG GAG TG
HFA20930	GCA GGA CGT TCG GAA TAT C	TCC CAT TAC AGA CTT TTG ATT G
HEA19892	GGC GGC ACA TGT GCA TG	GCC GCT GCC CAT ATA CTT
HFA10420	TGT GGC TGT CGC TTA TCT T	AAA AAT ATA CAG CCG TTT CCT T
HEA19909	ACC CAG CTA AAT CCT ACA ATG	ACT CCA GAT GCT GGG TCA
HFA04488	TTG ACG GAT TGC CAC ATC T	GCC TCC GCG TCC AAG T
HFA15673	TGG GCT CGG CAG AGA TA	CAA GTA GAG GAG CCC GAT
HFA16036	TCT TTG TCA TCA AAT CGT ACT C	CAT CGG GCC CAT GCA TT
HFA10635	TTG AAC ATC GTG GCT TCT TT	CCT CGC AAA CTC GAT GC
HFA16145	CAA CAA CAT GCT GGG CTT C	CGA AGT TCG AGC CGA CA
HFA20054	GAG CGG GCG ATC ATC TT	CTC GGC GGC GAT CAC
HFA09675	GAT GAG AAG GAC GAG AAG AG	CTT GAT GCG GCA ATG GAC
HFA20148	ATA GGT TCA ACA CGA TCC CC	GAA GGC TGG TGT TAG TTT TG
HFA11946	ACT TGC GTG GAG GAA CTA A	ATG CGT AGA GTT CTT CGG T
HFA20230	AGC TCG AGG ACA ATC CAC	GGC TGA CTT TCA CAG TAG AC
HFA09995	CGT ACG ATG ATG CAC TGG	GAA CGG GCA GAA TGG TTG
HFA16617	GGC AGT GGG AGC TCT GA	CTC GGG TCC GGT GAA CT
HFA19583	CGT CTG CGC AGT GAT CC	TGG GCT CCG ATG GAT AGA
HFA08714	AGC GAC GAG GAA GAT GTG	TGA CAA ATG TGG CCT CTG G
HFA20983	TTG GAA AAT CGA GAG GAT TTA A	CAC ATT TTT CGA ATT CAA TTG TC
HFA04096	CGT CTA ATG AGG CAA AGA AAC	CCG TTT TTG CCA CTT TAA CC
HFA01091	TCG TGA TGG TGT TGG TGA C	TCC ACT GAA AGT GCT TTG GT
HFA11427	GGG CGA ATG CAC GGA AT	TGG CAT ACC TCG AAT AAC TG
HFA20340	TAA TCG A CG ATC AGG A AC AG	GTG TGG CCT CGG AGG TG
HFA00357	CGT CCC CCG GTT TTA CG	ATC AGC CAG TCT TGA ATA GTC
HFA04167	ATA AAA GGC GCC AAG GTG A	TCA CCT GCA TTC CCG TTT C
HFA07637	GAC GGG CTT CAA TTC CTA TG	GCG ACG AGG AGA GTG TG
HFA19450	TGC TGC GCA AGC GAC	CAT TTG CGT GGA AGA TGA CA
HFA16984	CAC AAA GCC GCT GAA CAG	TTC GTG GTT ACA CAC ACA GT
HFA07247	CCG CGC GAA CGA CTT	TGA TCG CTT ATC ATC GTA TAT TA
HFA15370	ACT AGT AGC AGT CAG TCC TC	GCG CCA GCG TTG CTA T
HFA20582	ACA GCA TTC GGG TGG TAA A	GCC ATC CGA AGT TGA TCG
HFA20357	AAC CAG AAC CAG AAT CAA AAT G	GTT TCC AGC GCG ATT ATT G

TABLE 4-continued

Sequence and cytological information

HFA03384	GGC TGG ATG GAG TTG TTT G	GGA CTT ATG GGC TGA TTG AAC
HFA15220	AGC GGG TGC AGG AGT TC	TTC TTA TTA CTG GCC ACA TCA T
HFA07660	CAC GTT CTG CGG TAG CC	GCT TGG GAT CGG CTA AAT C
HFA16795	TTG TGG GTA AAT TTT TAC AGA AG	CGA ATT CCC CGC AGT AGT
HFA16344	CGG ATC CGG AGC ACC C	GCG ATG GAG CTG CTG G
HFA08683	CTT GAC GCT GAA GAA CCC	CCT GGA ATT GGA TCG ATG C
HFA00777	GGC AAC CAC TCC ACG CA	TCC TGG CCA GCC GTG T
HFA00784	AGA GCC GCC GAA ACA AC	GGC TTG GTT TCA GTA GAG G
HFA02455	**CAG CAG TAA AGC ACT TTC AA**	**CCG ATT CCG GCA TGG C**
HFA20587	GAG TAC AAG CAT GTG TAC AAG	GTT CCT GGT GGA GGT AGT G
HFA16870	CTT GCC CAA AAC TAC AGT TAC	CGA CTG TGG GTG GAT TGT T
HEA11298	AAG GAA AGC GCA TTT CGT	AAA TCC ATA TCC ACT TCC TCA C
HFA11098	ATC CCT CAA ATC CCA GTT CC	AAA GTG GCG CTG TGG TG

dsRNA ID	No of efficient siRNAsB	Target gene (Symbol)	Cyto-logical location	SEQ ID NOs.
HFA00627	51/496	Art2	24E1	SEQ ID NOs. 266/267
HFA11324	61/489	asf1	76B9	SEQ ID NOs. 268/269
HFA04919	87/487	bin3	42A13--14	SEQ ID NOs. 270/271
HFA16914	60/496	bon	92F2--3	SEQ ID NOs. 272/273
HFA16596	81/496	Caf1	88E3	SEQ ID NOs. 274/275
HFA14173	139/494	CG10007	87A4	SEQ ID NOs. 276/277
HFA09691	72/484	CG10077	65D3--4	SEQ ID NOs. 278/279
HFA02102	103/497	CG10730	38B2	SEQ ID NOs. 280/281
HFA09807	63/495	CG10960	69E5--6	SEQ ID NOs. 282/283
HFA11648	51/242	CG11307	78E1	SEQ ID NOs. 284/285
HFA06070	85/459	CG11400	54A1	SEQ ID NOs. 286/287
HFA14317	28/312	CG11501	99B1	SEQ ID NOs. 288/289
HFA19417	64/486	CG11696	10C7	SEQ ID NOs. 290/291
HFA14478	78/498	CG12213	87A3	SEQ ID NOs. 292/293

TABLE 4-continued

Sequence and cytological information				
HFA20970	50/114	CG12460*	hetero-chromatin	SEQ ID NOs. 294/295
HFA19459	19/181	CG12479	12E2	SEQ ID NOs. 296/297
HFA01920	112/494	CG13243	35D4--5	SEQ ID NOs. 298/299
HFA10017	73/391	CG13473	70F3	SEQ ID NOs. 300/301
HFA04144	19/256	CG13499	58B1	SEQ ID NOs. 302/303
HEA14742	34/497	CG14247	97D1	SEQ ID NOs. 304/305
HFA17927	52/490	CG14434	6D7	SEQ ID NOs. 306/307
HFA17993	122/475	CG15306	9B7	SEQ ID NOs. 308/309
HFA00432	19/143	CG15418	24A2	SEQ ID NOs. 310/311
HFA00449	30/217	CG15434	24F3	SEQ ID NOs. 312/313
HEA15093	58/283	CG15555	100B9	SEQ ID NOs. 314/315
HFA06577	77/477	CG15706	52F11	SEQ ID NOs. 316/317
HFA18090	51/500	CG15784	4F10	SEQ ID NOs. 318/319
HFA18561	72/477	CG16903	2C10	SEQ ID NOs. 320/321
HFA02552	54/495	CG16975	34A7--8	SEQ ID NOs. 322/323
HFA10258	3/155	CG17179*	U	SEQ ID NOs. 324/325
HFA02623	71/486	CG17492	37B10--11	SEQ ID NOs. 326/327
HFA15304	78/475	CG18112	99C2	SEQ ID NOs. 328/329
HFA21006	50/114	CG18160*	U	SEQ ID NOs. 330/331
HFA06272	111/489	CG30069	50E2--3	SEQ ID NOs. 332/333
HFA06935	62/463	CG30122	55E3	SEQ ID NOs. 334/335
HFA00563	69/326	CG3058	24F1	SEQ ID NOs. 336/337
HFA15507	8/197	CG31005	100B8	SEQ ID NOs. 338/339
HFA15369	34/488	CG31132	95F12--13	SEQ ID NOs. 340/341
A16032	63/495	CG31132	95F12--13	SEQ ID NOs. 342/343

TABLE 4-continued

Sequence and cytological information				
HFA15235	112/488	CG31358	87A5	SEQ ID NOs. 344/345
HFA00415	28/159	CG31694	23B7--8	SEQ ID NOs. 346/347
HFA09966	68/477	CG32406	65A2--3	SEQ iD NOs. 348/349
HFA19906	39/495	CG32573	14F5	SEQ ID NOs. 350/351
HFA15470	66/500	CG3281	87A3	SEQ ID NOs. 352/353
HFA10378	70/482	CG3819	75E6	SEQ ID NOs. 354/355
HFA10395	53/484	CG4022	6B84--5	SEQ ID NOs. 356/357
HFA20930	199/540	CG40351	U	SEQ ID NOs. 358/359
HEA19892	97/480	CG4349	11D11	SEQ ID NOs. 360/361
HFA10420	56/481	CG4446	67B2	SEQ ID NOs. 362/363
HEA19909	55/496	CG4653	15A3	SEQ ID NOs. 364/365
HFA04488	75/482	CG4781	60D10	SEQ ID NOs. 366/367
HFA15673	105/492	CG4907	94C2	SEQ ID NOs. 368/369
HFA16036	102/487	CG6422	96B17	SEQ ID NOs. 370/371
HFA10635	39/148	CG6434	77B4	SEQ ID NOs. 372/373
HFA16145	118/468	CG6946	86F8--9	SEQ ID NOs. 374/375
HFA20054	33/452	CG7635	18A6	SEQ ID NOs. 376/377
HFA09675	56/481	CG8108	67C11--D1	SEQ ID NOs. 378/379
HFA20148	93/492	CG9086	15C5--6	SEQ ID NOs. 380/381
HFA11946	144/490	Ckllalpha	80D1	SEQ ID NOs. 382/383
HFA20230	27/152	Ckllbeta	10E3	SEQ ID NOs. 384/385
HFA09995	37/499	comm3	71E3--4	SEQ ID NOs. 386/387
HFA16617	30/254	CtBP	87D8--9	SEQ ID NOs. 388/389
HFA19583	39/480	dome	18D13--E1	SEQ ID NOs. 390/391
HFA08714	65/476	dre4	62B7	SEQ ID NOs. 392/393

TABLE 4-continued

Sequence and cytological information				
HFA20983	159/488	elF-4B	U	SEQ ID NOs. 394/395
HFA04096	84/487	enok	60B10	SEQ ID NOs. 396/397
HFA01091	48/220	HDCC1676	30D1	SEQ ID NOs. 398/399
HFA11427	105/477	HDC11198	77D4	SEQ ID NOs. 400/401
HFA20340	52/493	hop	10B5--6	SEQ ID NOs. 402/403
HFA00357	75/488	lpk2	21E2	SEQ ID NOs. 404/405
HFA04167	18/202	jbug	59A3	SEQ ID NOs. 406/407
HFA07637	10/228	kn	51C2--3	SEQ ID NOs. 408/409
HFA19450	38/496	l(1)G0084	18D8--11	SEQ ID NOs. 410/411
HFA16984	88/496	larp	98C3--4	SEQ ID NOs. 412/413
HFA07247	35/377	lig	44A4	SEQ ID NOs. 414/415
HFA15370	30/486	mask	95F3--5	SEQ ID NOs. 416/417
HFA20582	58/473	mst	20A1	SEQ ID NOs. 418/419
HFA20357	27/118	nonA	14B18--C1	SEQ ID NOs. 420/421
HFA03384	87/500	Nup154	32C5	SEQ ID NOs. 422/423
HFA15220	36/167	Obp93a	93C1	SEQ ID NOs. 424/425
HFA07660	60/324	par-1	56D9--11	SEQ ID NOs. 426/427
HFA16795	12/118	Pp1alpha-96A	96A5	SEQ ID NOs. 428/429
HFA16344	32/469	PP2A-B'	90E4--5	SEQ ID NOs. 430/431
HFA08683	72/495	Ptp61F	61F7--62A1	SEQ ID NOs. 432/433
HFA00777	28/244	Rab5	22E1	SEQ ID NOS. 434/435
HFA00784	98/487	Rrp1	23C3--4	SEQ ID NOs. 436/437
HFA02455	38/490	Socs36E	36E6	SEQ ID NOs. 438/439
HFA20587	31/359	sol	19F5	SEQ ID NOs. 440/441

TABLE 4-continued

Sequence and cytological information				
HFA16870	64/479	Stat92E	92F1	SEQ ID NOs. 442/443
HEA11298	114/481	Taf2	67D1	SEQ ID NOs. 444/445
HFA11098	53/319	TSG101	73D1	SEQ ID NOs. 446/447

A Complete amplicon informaion can be obtained at http://mai.dkfz.de
B Efficiency calculated based on Reynolds et al., (2004). All siRNAs with score of 6 or higher were counted as efficient.
*Annotation according to Release 2 of the Berkeley Drosophila Genome Project

TABLE 5

Human homologues of *Drosophila* genes with JAK/STAT phenotypes

Drosophila Gene	BLASTP	Identity [%]	Human Gene	RefSeq protein	RefSeq nucleic acid	SEQ ID Nos
Art2	1.60E−77	44.2	protein arginine N-methyltransferase 4	NP_062828.2	NM_019854	SEQ ID NO. 1/88
asf1	3.20E−68	61.7	ASF1 anti-silencing function 1 homolog A	NP_054753.1	NM_014034	SEQ ID NO. 2/89
bin3	8.80E−49	34.3	hypothetical protein FLJ20257	NP_062552.2	NM_019606	SEQ ID NO. 3/90
bon	3.60E−45	30.5	tripartite motif-containing 33 protein	NP_056990.2	NM_015906	SEQ ID NO. 4/91
Caf1	0	91.9	retinoblastoma binding protein 4	NP_005601.1	NM_005610	SEQ ID NO. 5/92
CG10007	8.80E−50	34.5	chromosome 2 open reading frame 18	NP_060347.2	NM_017877	SEQ ID NO. 6/93
CG10077	7.00E−171	67.7	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	NP_004387.1	NM_004396	SEQ ID NO. 7/94
CG10960	3.40E−78	36.9	solute carrier family 2, (facilitated glucose transporter) member 8	NP_055395.2	NM_014580	SEQ ID NO. 8/95
CG11696	5.10E−33	29.9	zinc finger protein 502	NP_149987.2	NM_033210	SEQ ID NO. 9/96
CG12460	1.80E−17	54.0	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	NP_005057.1	NM_005066	SEQ ID NO. 10/97
CG13473	3.90E−17	34.9	thioredoxin 2 precursor	NP_036605.2	NM_012473	SEQ ID NO. 11/98
CG15306	3.30E−27	45.1	microtubule-associated protein, RP/EB family, member 1	NP_036457.1	NM_012325	SEQ ID NO. 12/99
CG15418	1.40E−10	41.1	tissue factor pathway inhibitor 2	NP_006519.1	NM_006528	SEQ ID NO. 13/100
CG15434	1.60E−17	50.6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8 kDa	NP_002479.1	NM_002488	SEQ ID NO. 14/101
CG15706	6.60E−20	20.0	FLJ20160 protein	NP_060164.2	NM_017694	SEQ ID NO. 15/102
CG16903	2.00E−100	63.1	cyclin L1	NP_064703.1	NM_020307	SEQ ID NO. 16/103
CG16975	4.00E−123	49.4	l(3)mbt-like 2 isoform a	NP_113676.2	NM_031488	SEQ ID NO. 17/104
CG17492	0	48.3	zinc finger, ZZ type with ankyrin repeat domain 1	NP_543151.1	NM_080875	SEQ ID NO. 18/105
CG18112	1.80E−20	27.8	chromosome 14 open reading frame 133	NP_071350.2	NM_022067	SEQ ID NO. 19/106
CG30122	7.20E−42	40.9	E1B-55 kDa-associated protein 5 isoform a	NP_008971.2	NM_007040	SEQ ID NO. 20/107
CG3058	2.60E−80	95.8	thioredoxin-like 4	XP_499552.1	XM_499552	SEQ ID NO. 21/108
CG31005	9.00E−100	52.5	trans-prenyltransferase	NP_055132.2	NM_014317	SEQ ID NO. 22/109
CG31132	0	49.9	bromo domain-containing protein disrupted in leukemia	NP_694984.2	NM_153252	SEQ ID NO. 23/110
CG31358	2.10E−51	44.2	stomatin-like 3	NP_660329.1	NM_145286	SEQ ID NO. 24/111
CG31694	2.00E−66	36.3	interferon-related developmental regulator 2	NP_006755.3	NM_006764	SEQ ID NO. 25/112
CG32406	5.50E−15	37.8	C1 domain-containing phosphatase and tensin-like protein isoform 3	NP_938072.1	NM_198316	SEQ ID NO. 26/113
CG3281	4.00E−41	31.7	zinc finger protein 91	NP_003421.1	NM_003430	SEQ ID NO. 27/114
CG40351	2.20E−94	56.6	PREDICTED: KIAA1076 protein	XP_037523.9	XM_037523	SEQ ID NO. 28/115
CG4349	4.60E−35	45.2	ferritin, heavy polypeptide 1	NP_002023.2	NM_002032	SEQ ID NO. 29/116
CG4446	1.90E−66	47.2	pyridoxal kinase	NP_003672.1	NM_003681	SEQ ID NO. 30/117
CG4653	8.80E−23	30.7	protease, serine, 2 preproprotein	NP_002761.1	NM_002770	SEQ ID NO. 31/118
CG4781	2.00E−17	33.8	PREDICTED: similar to KIAA0644 protein	XP_379800.1	XM_379800	SEQ ID NO. 32/119
CG4907	1.10E−47	28.9	phosphatidylinositol glycan, class N	NP_036459.1	NM_012327	SEQ ID NO. 33/120
CG6422	1.30E−71	53.8	YTH domain family, member 1	NP_060268.2	NM_017798	SEQ ID NO. 34/121
CG6434	6.20E−69	71.2	retinoblastoma binding protein 5	NP_005048.2	NM_005057	SEQ ID NO. 35/122
CG6946	4.00E−39	46.2	heterogeneous nuclear ribonucleoprotein F	NP_004957	NM_004966	SEQ ID NO. 36/123
CG7635	2.90E−76	62.1	stomatin isoform a	NP_004090.4	NM_004099	SEQ ID NO. 37/124
CG9086	0	32.0	ubiquitin protein ligase E3 component n-recognin 1	NP_777576.1	NM_174916	SEQ ID NO. 38/125
CkIIalpha	4.00E−163	88.7	casein kinase II alpha 1 subunit isoform a	NP_001886.1	NM_001895	SEQ ID NO. 39/126
CkIIbeta	5.00E−107	89.2	casein kinase 2, beta polypeptide	NP_001311.3	NM_001320	SEQ ID NO. 40/127
CtBP	8.00E−152	72.4	C-terminal binding protein 2 isoform 1	NP_001320.1	NM_001329	SEQ ID NO. 41/128
dome	7.60E−15	28.2	sidekick 2	NP_061937.2	NM_019064	SEQ ID NO. 42/129
dre4	0	59.9	chromatin-specific transcription elongation factor large subunit	NP_009123.1	NM_007192	SEQ ID NO. 43/130
eIF-4B	4.70E−32	27.2	eukaryotic translation initiation factor 4B	NP_001408.1	NM_001417	SEQ ID NO. 44/131
enok	1.80E−94	33.4	MYST histone acetyltransferase (monocytic leukemia) 3	NP_006757.1	NM_006766	SEQ ID NO. 45/132

TABLE 5-continued

Human homologues of *Drosophila* genes with JAK/STAT phenotypes

Drosophila Gene	BLASTP	Identity [%]	Human Gene	RefSeq protein	RefSeq nucleic acid	SEQ ID Nos
HDC01676	2.80E−15	61.0	cholinergic receptor, nicotinic, alpha polypeptide 7 precursor	NP_000737.1	NM_000746	SEQ ID NO. 46/133
hop	1.60E−59	26.7	Janus kinase 2	NP_004963.1	NM_004972	SEQ ID NO. 47/134
Ipk2	1.80E−26	33.6	inositol polyphosphate multikinase	NP_689416.1	NM_152230	SEQ ID NO. 48/135
jbug	5.30E−45	27.6	filamin B, beta (actin binding protein 278)	NP_001448.1	NM_001457	SEQ ID NO. 49/136
kn	0	69.7	early B-cell factor	NP_076870.1	NM_024007	SEQ ID NO. 50/137
l(1)G0084	7.40E−28	31.5	PHD finger protein 10 isoform a	NP_060758.1	NM_018288	SEQ ID NO. 51/138
larp	2.00E−103	48.1	KIAA0731 protein	NP_056130.2	NM_015315	SEQ ID NO. 52/139
lig	5.50E−25	32.8	ubiquitin associated protein 2 isoform 2	NP_065918.1	NM_020867	SEQ ID NO. 53/140
mask	0	74.0	multiple ankyrin repeats, single KH-domain protein isoform 1	NP_060217.1	NM_017747	SEQ ID NO. 54/141
mst	7.00E−52	29.7	misato	NP_060586.2	NM_018116	SEQ ID NO. 55/142
nonA	1.80E−60	40.6	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	NP_005057.1	NM_005066	SEQ ID NO. 56/143
Nup154	0	32.6	nucleoporin 155 kDa isoform 1	NP_705618.1	NM_153485	SEQ ID NO. 57/144
par-1	0	54.1	MAP/microtubule affinity-regulating kinase 3	NP_002367.4	NM_002376	SEQ ID NO. 58/145
Pp1alpha-96A	8.00E−169	88.9	protein phosphatase 1, catalytic subunit, alpha isoform 1	NP_002699.1	NM_002708	SEQ ID NO. 59/146
PP2A-B'	0	78.9	delta isoform of regulatory subunit B56, protein phosphatase 2A isoform 1	NP_006236.1	NM_006245	SEQ ID NO. 60/147
Ptp61F	5.00E−32	37.9	hypothetical protein LOC9671	NP_055468	NM_014653	SEQ ID NO. 61/148
Rab5	4.90E−85	75.0	RAB5A, member RAS oncogene family	NP_004153.2	NM_004162	SEQ ID NO. 62/149
Rrp1	6.10E−82	55.2	APEX nuclease	NP_542380.1	NM_080649	SEQ ID NO. 63/150
Socs36E	4.80E−65	68.0	suppressor of cytokine signaling 5	NP_054730.1	NM_014011	SEQ ID NO. 64/151
Stat92E	6.40E−86	41.6	signal transducer and activator of transcription 5B	NP_036580.2	NM_012448	SEQ ID NO. 65/152
Taf2	0	52.5	TBP-associated factor 2	NP_003175.1	NM_003184	SEQ ID NO. 66/153
TSG101	4.30E−98	48.7	tumor susceptibility gene 101	NP_006283.1	NM_006292	SEQ ID NO. 67/154

Shown are human homologues of *Drosophila* genes with a BLASTP E value of 10^{-10} or less.

TABLE 6

Human disease homologues of *Drosophila* genes with JAK/STAT phenotypes

Drosophila Gene	BLASTP	Human Gene	RefSeq protein
bon	3.60E−45	tripartite motif-containing 33 protein	NP_056990.2
Caf1	9.90E−17	peroxin 7	NP_000279
CG10960	1.40E−39	erythrocyte/hepatoma glucose transporter	NP_006507
CG11696	2.60E−25	zinc finger protein 41	NP_006051
CG17492	4.80E−23	ankyrin, brain	NP_001139
CG31132	2.70E−17	Lissencephaly-1 Gene	NP_000421
CG31132	0	bromo domain-containing protein disrupted in leukemia	NP_694984
CG31358	7.40E−38	Podocin	NP_055440
CG32573	7.80E−47	Protein Kinase C, alpha	NP_002728
CG3281	2.00E−33	zinc finger protein 41	NP_009061
CG40351	2.10E−14	Androgen Receptor-Associated Coregulator 267	NP_071900
CG4349	4.60E−35	ferritin, heavy polypeptide 1	NP_002023.2
CG4349	3.50E−35	fth	NP_002023
CG4653	6.90E−22	Protease, Serine, 1	NP_002760
CG7635	2.90E−76	stomatin isoform a	NP_004090.4
CG7635	5.70E−62	Podocin	NP_055440
CkIIalpha	1.20E−23	serine/threonine protein kinase 9	NP_003150
CtBP	4.20E−24	3-phosphogylcerate dehydrogenase; 3pgdh	NP_006614
dre4	4.60E−46	Lipase A precursor	NP_000226
HDC01676	2.80E−15	cholinergic receptor, nicotinic, alpha polypeptide 7 precursor	NP_000737.1
hop	8.40E−52	Janus kinase 3	NP_000206
jbug	5.30E−45	filamin B, beta (actin binding protein 278)	NP_001448.1
jbug	5.30E−45	filamin B, beta (actin binding protein 278)	NP_001448.1
jbug	5.30E−45	filamin B, beta (actin binding protein 278)	NP_001448.1
jbug	6.00E−102	actin-binding protein 280; abp280	NP_001447
mask	5.30E−59	ankyrin, brain	NP_001139
par-1	5.30E−39	Oncogene Akt2	NP_001617
Ptp61F	7.80E−79	Protein phosphotyrosylphosphatase 1B	NP_002818
Rab5	8.90E−23	ras-associatad protein RAB27A	NP_004571
sol	2.80E−33	calcium-activated neutral protease 3	NP_000061

TABLE 6-continued

Human disease homologues of *Drosophila* genes with JAK/STAT phenotypes

Stat92E	6.40E−86	signal transducer and activator of transcription 5B	NP_036580.2
Stat92E	6.40E−86	signal transducer and activator of transcription 5B	NP_036580.2
TSG101	4.30E−98	tumor susceptibility gene 101	NP_006283.1

Drosophila Gene	RefSeq Nucleic acid	Disease	SEQ ID Nos
bon	NM_015906	Thyroid carcinoma, papillary(2)	SEQ ID NO. 4/91
Caf1	NM_000288	Refsum disease (1)	SEQ ID NO. 68/155
CG10960	NM_006516	Glucose transport defect, blood-brain barrier (1)	SEQ ID NO. 69/156
CG11696	NM_006060	Mental Retardation, X-linked nonsyndromic (1)	SEQ ID NO. 70/157
CG17492	NM_001148	Long QT syndrome 4 (1)	SEQ ID NO. 71/158
CG31132	NM_000430	Subcortical laminar heterotopia (1)	SEQ ID NO. 72/159
CG31132	NM_153252	Leukemia (3)	SEQ ID NO. 73/160
CG31358	NM_014625	Nephrotic syndrome, steroid-resistant (1)	SEQ ID NO. 74/161
CG32573	NM_002737	Pituitary Tumor, invasive (1)	SEQ ID NO. 75/162
CG3281	NM_007130	Mental Retardation, X-linked nonsyndromic (1)	SEQ ID NO. 76/163
CG40351	NM_022455	Sotos Syndrome, sporadic (1)	SEQ ID NO. 77/164
CG4349	NM_002032	Iron overload, autosomal dominant (2)	SEQ ID NO. 29/116
CG4349	NM_002032	Iron overload, autosomal dominant (1)	SEQ ID NO. 29/116
CG4653	NM_002769	Pancreatitis, hereditary (1)	SEQ ID NO. 78/165
CG7635	NM_004099	Stomatocytosis I (2)	SEQ ID NO. 37/124
CG7635	NM_014625	Nephrotic syndrome, steroid-resistant (1)	SEQ ID NO. 74/161
CkIIalpha	NM_003159	Rett Syndrome, atypical (1)	SEQ ID NO. 79/166
CtBP	NM_006623	Phosphoglycerate dehydrogenase deficiency (1)	SEQ ID NO. 80/167
dre4	NM_000235	Wolman disease (1)	SEQ ID NO. 81/168
HDC01676	NM_000746	Schizophrenia, neurophysiologic defect in (2)	SEQ ID NO. 46/133
hop	NM_000215	SCID, autosomal recessive, T-negative/B-positive type (1)	SEQ ID NO. 82/169
jbug	NM_001457	Atelostogenesis, type I (2)	SEQ ID NO. 49/136
jbug	NM_001457	Larson syndrome (2)	SEQ ID NO. 49/136
jbug	NM_001457	Spondylocarpotarsal synostosis syndrome (2)	SEQ ID NO. 49/136
jbug	NM_001456	Frontometaphyseal dysplasia (1)	SEQ ID NO. 83/170
mask	NM_001148	Long QT syndrome 4 (1)	SEQ ID NO. 71/158
par-1	NM_001626	Diabetes mellitus, type II (1)	SEQ ID NO. 84/171
Ptp61F	NM_002827	Insulin resistance, susceptibility to (1)	SEQ ID NO. 85/172
Rab5	NM_004580	Griscelli Syndrome (1)	SEQ ID NO. 86/173
sol	NM_000070	Muscular dystrophy, limb-girdle, type 2A (1)	SEQ ID NO. 87/174
Stat92E	NM_012448	Leukemia, acute promyetoyctic, STAT5B/RARA type (2)	SEQ ID NO. 65/152
Stat92E	NM_012448	Growth hormone insensitivity with immunodeficiency (2)	SEQ ID NO. 65/152
TSG101	NM_006292	Breast cancer (2)	SEQ ID NO. 67/154

Shown are the relevant diseases of the human homologues with a BLASTP E value of 10^{-10} or less as referenced in Homophila (Chien et al., 2002) and OMIM.
(1) data sets were downloaded from Homophila Version 2.1 (update 13 Apr 2005). Chien, S., Reiter, L. T., Bier, E. and Gribskov M., Nucleic Acids Research 30: 149-151 (2002)
(2) data sets from: Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
(3) information from: Kalla, C., Nentwich, H., Schlotter, M., Mertens, D., Wildenberger, K., Dohner, H., Stilgenbauer, S. and Lichter, P., Genes Chromosomes Cancer 42 (2): 128-143 (2005)

SUPPLEMENTARY TABLE 7

Expected and Observed Phenotype Frequency

Chromosome	No Genes*	%	Expected Phenotypes Pos	Expected Phenotypes Neg	Observed Phenotypes Pos	Observed Phenotypes Neg
X	2292	17%	11	4	16	0
2L	2444	18%	11	4	10	5
2R	2687	20%	12	5	5	7
3L	2612	19%	12	4	15	4
3R	3392	25%	16	6	15	8

SUPPLEMENTARY TABLE 7-continued

Expected and Observed Phenotype Frequency

Chromosome	No Genes*	%	Expected Phenotypes Pos	Expected Phenotypes Neg	Observed Phenotypes Pos	Observed Phenotypes Neg
4	82	1%	1	0	0	0
Unmapped					5	0

*Location according to Release 3.1 of the Berkeley *Drosophila* Genome Project

TABLE 7

Ranking	Gene Name: *D. melanogaster*	Gene Name: *Homo sapiens*	Accession Number	Associated Disease	SEQ ID NOs:
1	HDC01676	CHRNA7	NM_000746	Schizophrenia, neurophysiologic defect	46/133
2	CG4349	FTH1	NM 002032	Iron overload, autosomal dominant	29/116
3	TSG101	TSG101	NM_006292	Breast cancer	67/154
4	bon	TRIM33	NM_015906	Thyroid carcinoma, papillary	4/_9
5	mask	MLL3	NM_021230	Myeloid leukemia	83/170
6	enok	MYST3	NM_006766	Acute myeloid leukemia	45/132
7	Caf1	RBBP4	NM_005610	Refsum disease	68/155
8	Rab5	RAB5A	NM_004162		86/173
9	CG31694	IFRD2	NM_006764	Small cell lung cancer	25/112
10	sol	CAPN3	NM_000070	Muscular dystrophy, limb-girdle, type 2A	87/174
11	CG31132	BRODL	NM_153252	Leukemia	72/159
12	CG15434	NDUFA2	NM_002488	Muscular dystrophy, limb-girdle, 1A	14/101
13	CG3819	ENDOGL1	NM_005107	Carcinomas of lung, uterus, esophagus, kidney	207
14	CG31005	TPRT	NM_014317		22/109
15	Pp1alpha-96A	PPP1CC	NM_002710		59/146
16	CG10077	DDX5	NM_004396		7/_94
17	CG17492	LOC142678	NM_080875		71/158
18	kn	DKFZP667B0210	NM_024007		50/137
19	CG31132	C21ORF107	NM_018963		72/159
20	Pp1alpha-96A	PPP1CA	NM_002708		59/146
21	CtBP	CTBP2	NM_001329		80/167
22	PP2A-B'	PPP2R5D	NM_006245		60/147
23	Nup154	NUP155	NM_004298		57/144
24	mask	ANKHD1	NM_017747		83/170
25	Art2	HRMT1L4	NM_019854		1/_88
26	CG18112	C14ORF133	NM_022067		19/106
27	I(1)G0084	PHF10	NM_018288		51/138

TABLE 8

Gene name *Drosophila melanogaster*	Gene Name *Homo sapiens*	Accession Number	z-score [Dmel-screen]	STAT3 activity [induction SOCS3]	STAT1 activity [induction GBP1]	Associated Disease
HDC01676	CHRNA7	NM_000746	−2.3	0.6	1.0	Schizophrenia, neurophysiologic defect in (2)
CG4349	FTH1	NM_002032	−4.1	0.4	1.3	Iron overload, autosomal dominant (2)
TSG101	TSG101	NM_006292	3.1	2.0	1.2	Breast cancer (2)
bon	TRIM33	NM_015906	5.6	0.4	1.3	Thyroid carcinoma, papillary (2)
mask	MLL3	NM_021230	−2.3	1.2	0.5	Myeloid leukemia (6)
enok	MYST3	NM_006766	3.0	0.8	2.3	Acute myeloid leukemia (5)
Caf1	RBBP4	NM_005610	3.0	1.1	2.0	Refsum disease (1)
Rab5	RAB5A	NM_004162	2.1	2.8	1.9	
CG31694	IFRD2	NM_006764	−2.8	2.2	2.4	Small cell lung cancer (4)
sol	CAPN3	NM_000070	−2.5	2.5	2.0	Muscular dystrophy, limb-girdle, type 2A (1)
CG31132	BRODL	NM_153252	−2.8	1.1	0.6	Leukemia (3)
CG15434	NDUFA2	NM_002488	−2.5	3.8	3.7	Muscular dystrophy, limb-girdle, 1A (2)
CG3819	ENDOGL1	NM_005107	−2.3	0.9	1.9	Carcinomas of lung, uterus, esophagus, kidney (7)
CG31005	TPRT	NM_014317	−2.3	0.5	0.4	
Pp1alpha-96A	PPP1CC	NM_002710	3.0	0.4	2.1	
CG10077	DDX5	NM_004396	2.8	0.8	3.9	
CG17492	LOC142678	NM_080875	2.5	2.4	2.4	
kn	DKFZP667B0210	NM_024007	−2.4	2.0	1.3	
CG31132	C21ORF107	NM_018963	−2.8	0.5	0.5	
Pp1alpha-96A	PPP1CA	NM_002708	3.0	0.8	3.2	
CtBP	CTBP2	NM_001329	−2.9	0.5	0.8	
PP2A-B'	PPP2R5D	NM_006245	2.6	1.9	1.6	
Nup154	NUP155	NM_004298	2.9	1.6	2.0	
mask	ANKHD1	NM_017747	−2.3	0.6	1.9	
Art2	HRMT1L4	NM_019854	−2.9	0.6	2.9	

TABLE 8-continued

Gene name *Drosophila melanogaster*	Gene Name *Homo sapiens*	Accession Number	z-score [Dmel-screen]	STAT3 activity [induction SOCS3]	STAT1 activity [induction GBP1]	Associated Disease
CG18112	C14ORF133	NM_022067	2.1	1.2	1.7	
I(1)G0084	PHF10	NM_018288	−2.1	0.5	0.7	

References:
(1) data sets were downloaded from Homophila Version 2.1 (update 13 Apr 2005). Originally published: Chien, S., Reiter, L. T., Bier, E. and Griboskov M. Homophila: human disease gene cognates in *Drosophila*. Nucleic Acids Research 30: 149-151 (2002)
(2) data sets from: Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
(3) information from: Kalla, C., Nentwich, H., Schlotter, M., Mertens, D., Wildenberger, K., Dohner, H., Stilgenbauer, S. and Lichter, P. Translocation t (X; 11)(q13; q23) in B-cell chronic lymphocytic leukemia disrupts two novel genes. Genes Chromosomes Cancer 42 (2): 128-143 (2005)
(4) information from: Latif F., Duh, F. M., Bader, S., Sekido, Y., Li, H., Geil, L., Zbar, B. Minna, J. D. and Lerman, M. I. The human homolog of the rodent immediate early response genes, PC4 and TIS7, resides in the lung cancer tumor suppressor gene region on chromosome 3p21. Hum Genet 99 (3): 334-341 (1997)
(5) information from: Borrow, J., Stanton, V. P., Jr., Andresen, J. M., Becher, R., Behm, F. G., Chaganti, R. S. K., Civin, C. I., Disteche, C., Dube, I., Frischauf, A. M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A. E., Housman, D. E. The translocation t(8; 16)(p11; p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nature Genet. 14: 33-41 (1996)
(6) information from: Ruault, M., Brun, M. E., Ventura, M., Roizes, G., De Sario, A. MLL3, a new human member of the TRX/MLL gene family, maps to 7q36, a chromosome region frequently deleted in myeloid leukaemia. Gene 284: 73-81 (2002)
(7) information from: Daigo, Y., Isomura, M., Nishiwaki, T., Tamari, M., Ishikawa, S., Kai, M., Murata, Y., Takeuchi, K., Yamane, Y., Hayashi, R., Minami, M., Fujino, M. A., Hojo, Y., Uchiyama, I., Takagi, T., Nakamura, Y. Characterization of a 1200-kb genomic segment of chromosome 3p22-p21.3. DNA Res. 6: 37-44 (1999)

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20090186815A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. A method for identifying a compound capable of modulating the activity of the JAK/STAT pathway, comprising

a contacting a compound with at least one target molecule selected from

i nucleic acid molecules, comprising

ii a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

iii a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

iv a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

v a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3); and

ii polypeptide molecules

iii encoded by the nucleic acid molecules of (i) and/or

iv having the sequences as shown in SEQ ID NOs. 1-87, and

b determining the degree of modulation of the at least one target molecule by the compound.

2. The method according to claim **1**, wherein the compound is selected from compounds capable of directly and/or indirectly inhibiting or activating the transcription or translation of a nucleic acid molecule of (i).

3. The method according to claim **2**, wherein the compounds capable of directly and/or indirectly inhibiting or activating the transcription or translation of a nucleic acid molecule of (i) comprise polypeptides such as proteins, enzymes, antibodies, polypeptide inhibitors, polypeptide activators, agonist, antagonists, mimetics, low molecular weight substances, antisense molecules, RNAi molecules and ribozymes.

4. The method according to claim **1**, wherein the compound is selected from compounds capable of directly and/or indirectly inhibiting or activating a polypeptide molecule of (ii).

5. The method according to claim **4**, wherein the compounds capable of directly and/or indirectly inhibiting or activating a polypeptide molecule of (ii) comprise polypeptides such as proteins, enzymes, antibodies, polypeptide inhibitors, polypeptide activators, agonist, antagonists, mimetics, oligopeptides, low molecular weight substances and cofactors.

6. The method according to claim **1**, wherein the compound is an antibody or fragment thereof and wherein the antibody or fragment thereof is directed against a polypeptide molecule of (ii).

7. The method according to claim **1**, wherein the compound is an antisense molecule and wherein the antisense molecule is directed against a nucleic acid molecule of (i).

8. The method according to claim **1**, wherein the compound is an RNAi molecule.

9. The method according to claim **1**, wherein the degree of modulation of the at least one target molecule by the compound is determined by measuring the amount and/or expression rate of the nucleic acid molecule of (i).

10. The method according to claim **1**, wherein the degree of modulation of the at least one target molecule by the compound is determined by measuring the amount and/or activity of the polypeptide molecule of (ii).

11. The method according to claim **1**, wherein the method is a molecular based assay.

12. The method according to claim **1**, wherein the method is a cellular assay.

13. Use of at least one molecule selected from

i nucleic acid molecules, comprising

ii a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

iii a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

iv a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

v a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3); and

ii polypeptide molecules

iii encoded by the nucleic acid molecules of (i) and/or

iv having the sequences as shown in SEQ ID NOs. 1-87,

as a target for the modulation of the activity of the JAK/STAT pathway.

14. A method for modulating the activity of the JAK/STAT pathway comprising contacting a cell with at least one molecule selected from

i nucleic acid molecules, comprising

ii a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

iii a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

iv a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

v a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

vi polypeptide molecules

vii encoded by the nucleic acid molecules of (i) and/or

viii having the sequences as shown in SEQ ID NOs. 1-87, and

ii effector molecules of (i) and/or (ii).

15. The method according to claim **14**, wherein the effector molecules of (i) and/or (ii) are selected from antibodies or fragments thereof which are directed against a polypeptide molecule of (ii), antisense molecules which are directed against a nucleic acid molecule of (i) and RNAi molecules.

16. A pharmaceutical composition comprising as an active agent at least one molecule selected from

i nucleic acid molecules, comprising

ii a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

iii a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

iv a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

v a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

vi polypeptide molecules

vii encoded by the nucleic acid molecules of (i) and/or

viii having the sequences as shown in SEQ ID NOs. 1-87, and

ii effector molecules of (i) and/or (ii).

17. The pharmaceutical composition according to claim **16**, wherein the effector molecules of (i) and/or (ii) are selected from antibodies or fragments thereof which are directed against a polypeptide molecule of (ii), antisense molecules which are directed against a nucleic acid molecule of (i) and RNAi molecules.

18. The pharmaceutical composition according to claim **16**, optionally containing pharmaceutically acceptable carriers, diluents and/or adjuvants.

19. The pharmaceutical composition according to claim **16** for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder.

20. The pharmaceutical composition according to claim **16**, wherein the JAK/STAT pathway associated disorder is selected from the group consisting of papillary thyroid carcinoma, Refsum disease, blood-brain barrier glucose transport defect, X-linked nonsyndromic mental retardation, long QT syndrome 4, subcortical laminar heterotopia, leukemia, steroid-resistant nephrotic syndrome, invasive pituitary tumor, sporadic Sotos syndrome, autosomal dominant iron overload, hereditary pancreatitis, stomatocytosis I, atypical Rett syndrome, phosphoglycerate dehydrogenase deficiency, Wolman disease, neurophysiologic defect in schizophrenia, autosomal recessive SCID (T-negative/B-positive type), atelostogenesis (type I), Larson syndrome, spondylocarpotarsal synostosis syndrome, frontometaphyseal dysplasia, diabetes mellitus (type II), susceptibility to insulin resistance, Griscelli Syndrome, limb-girdle muscular dystrophy (type 2A), growth hormone insensitivity with immunodeficiency and breast cancer.

21. A method for the diagnosis, prevention or treatment of a JAK/STAT pathway associated disorder comprising administering

nucleic acid molecules, comprising

ii a nucleotide sequence as shown in SEQ ID NOs. 88 to 265;

iii a nucleotide sequence which is complementary to a nucleotide sequence of (i.1);

iv a nucleotide sequence which has an identity of at least 65% to a nucleotide sequence of (i.1) or (i.2); and/or

v a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of (i.1), (i.2) or (i.3);

vi polypeptide molecules

vii encoded by the nucleic acid molecules of (i) and/or

viii having the sequences as shown in SEQ ID NOs. 1-87, or

ii effector molecules of (i) and/or (ii).

22. A method according to claim **21**, wherein the effector molecules of (i) and/or (ii) are selected from antibodies or fragments thereof which are directed against a polypeptide molecule of (ii), antisense molecules which are directed against a nucleic acid molecule of (i) and RNAi molecules.

23. Use according to claim **21**, wherein the JAK/STAT pathway associated disorder is selected from the group consisting of papillary thyroid carcinoma, Refsum disease,

blood-brain barrier glucose transport defect, X-linked non-syndromic mental retardation, long QT syndrome 4, subcortical laminar heterotopia, leukemia, steroid-resistant nephrotic syndrome, invasive pituitary tumor, sporadic Sotos syndrome, autosomal dominant iron overload, hereditary pancreatitis, stomatocytosis I, atypical Rett syndrome, phosphoglycerate dehydrogenase deficiency, Wolman disease, neurophysiologic defect in schizophrenia, autosomal recessive SCID (T-negative/B-positive type), atelostogenesis (type I), Larson syndrome, spondylocarpotarsal synostosis syndrome, frontometaphyseal dysplasia, diabetes mellitus (type II), susceptibility to insulin resistance, Griscelli Syndrome, limb-girdle muscular dystrophy (type 2A), growth hormone insensitivity with immunodeficiency and breast cancer.

* * * * *

专利名称(译)	通过全基因组RNAI筛选鉴定JAK / STAT途径调节基因		
公开(公告)号	US20090186815A1	公开(公告)日	2009-07-23
申请号	US11/917653	申请日	2006-06-14
[标]申请(专利权)人(译)	布特罗斯MICHAEL ZEIDLER MARTIN MULLER PATRICK		
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[标]发明人	BOUTROS MICHAEL ZEIDLER MARTIN MUELLER PATRICK		
发明人	BOUTROS, MICHAEL ZEIDLER, MARTIN MUELLER, PATRICK		
IPC分类号	A61K38/14 G01N33/566 G01N33/50 C12Q1/68 G01N33/536 C12Q1/02 C07H21/04 C07K14/47 C12N5/06 C12N15/87 A61K31/711 C12N15/11 C12N15/113		
CPC分类号	C07K14/47 C12N15/111 Y10T436/143333 C12N2310/14 C12N2320/10 C12N15/113		
优先权	2005012934 2005-06-15 EP		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及鉴定能够调节JAK / STAT途径活性的化合物的方法，以及使用不同的JAK / STAT途径组分作为调节JAK / STAT途径活性的靶标。此外，本发明涉及调节JAK / STAT途径活性的方法。此外，本发明涉及药物组合物以及其用于制备用于诊断，预防或治疗JAK / STAT途径相关病症的此类组合物的不同JAK / STAT途径组分和/或其效应分子的用途。