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Meta-analysis of expression of l(3)mbt tumor-associated germline genes supports the model that a soma-to-germline transition is a hallmark of human cancers

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Evidence is starting to emerge indicating that tumorigenesis in metazoans involves a soma-to-germline transition, which may contribute to the acquisition of neoplastic characteristics. Here, we have meta-analyzed gene expression profiles of the human orthologs of *Drosophila melanogaster* germline genes that are ectopically expressed in *l(3)mbt* brain tumors using gene expression datasets derived from a large cohort of human tumors. We find these germline genes, some of which drive oncogenesis in *D. melanogaster*, are similarly ectopically activated in a wide range of human cancers. Some of these genes normally have expression restricted to the germline, making them of particular clinical interest. Importantly, these analyses provide additional support to the emerging model that proposes a soma-to-germline transition is a general hallmark of a wide range of human tumors. This has implications for our understanding of human oncogenesis and the development of new therapeutic and biomarker targets with clinical potential.

Cancer Genetics

Neoplastic disease occurs when cells acquire altered biological capabilities, ultimately enabling them to form potentially lethal tumor colonies capable of evading intrinsic and extrinsic tumor suppressing activities.[1] However, individual tumors are a complex, mixed population of cells subjected to ongoing genetic and epigenetic changes and it seems likely that specific subgroups of cells within the tumor make differential contributions to the tumor hallmarks.[2,3] Defining the acquired changes of tumors on a whole tumor scale provides a global insight into the characteristics of the tumor as a defined biological entity and increases the likelihood of identifying clinically important biomarkers. Recent work from a range of organisms has revealed that tumors may acquire a more germline-like state.[4–9] Moreover, it has been demonstrated that the germline gene expression has the potential to be oncogenic in *Drosophila melanogaster*[4] and is associated with clinically more aggressive tumors in humans.[8] Janic *et al.* recently demonstrated that *l(3)mbt* tumors in *D. melanogaster* ectopically expressed a broad range of germline genes, with up to a quarter of ectopically expressed genes being associated with a known germline function.[4] Inactivation of some of these germline genes results in suppression of tumorigenesis, indicating that they play an essential role in tumor development.[4] *l(3)mbt* encodes a transcriptional repressor component of the dREAM-MMB and LINT complexes[10,11] and inactivation of other components of the dREAM-MMB complex also results in germline gene expression[12]; however, some mutations of dREAM-MMB component genes do not result in laval brain tumors suggesting that the LINT complex plays an important tumor suppressing function.[10] A similar activation of a germline-like transcriptional programme has been observed in *Caenorhabditis elegans* strains mutated for dREAM-MMB related functions, suggesting a conserved functional relationship between tumorigenesis and germline gene expression in metazoans.[13,14]

Key words: soma-to-germline transition, gene expression meta-analysis, cancer, cancer hallmarks, meiosis
Additional Supporting Information may be found in the online version of this article.

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What's new?

Although individual tumors are a complex mosaic of cells, subject to ongoing genetic and epigenetic change, evidence suggests that a general hallmark of human cancer is the development of tumors from a soma-to-germline transition. This meta-analysis supports that idea, revealing that human genes that are orthologues of the oncogenic germline drivers of brain tumors in *Drosophila melanogaster* are activated in a wide range of human cancers. The findings have implications for the understanding of cancer and for the development of new therapeutic and diagnostic tools.

In humans, there is a group of genes with expression restricted to testicular cells which are also aberrantly expressed in various tumor types, the so called cancer-testis (CT) genes, which has led to the suggestion that a soma-to-germline transformation may also occur in human cancers.[5,9,15] The immunological privilege of the testis[16] makes the antigens encoded by CT genes promising candidates for a wide range of novel immune-directed therapeutic and monitoring tools for clinical applications.[17,18]

As ectopic expression of some germline genes is required for *l(3)mbt* brain tumor growth in *D. melanogaster*,[4] it is important to investigate the expression pattern of germline genes in cancerous and healthy human tissues. Recently, a novel cohort of CT genes was identified by meta-analyzing the expression profiles of human orthologs of mouse meiotic genes.[19] This and other recent works[8] support the proposal that a generalized hallmark of human tumors is a soma-to-germline transformation.[9] Here, we provide further support for this proposal by investigating the expression of the orthologs of the *D. melanogaster* germline genes ectopically expressed in *l(3)mbt* tumors in human cancerous and normal tissue.[4] We meta-analyzed a range of human tumor profiles[19,20] to demonstrate that most of the *D. melanogaster* genes for which human orthologs were identified were also up-regulated in human cancers. Moreover, 19 of these have normal expression restricted to tissues residing in immunological privilege (brain, placenta and testis) indicating they may be CT genes and thus may make excellent cancer-specific therapeutic targets.

Material and Methods

Human homologs of the *Drosophila* germline genes

We assigned the 49 germline genes ectopically expressed in *l(3)mbt* tumors in *D. melanogaster* (3) to their human orthologs using the databases Flybase,[21] Homologene[22] and Ensembl[23] as well as literature search (Supporting Information Table S1). We could identify human orthologs for 28 genes, resulting in 46 human genes due to human paralogs. Enriched gene ontology (GO) terms for the human homologs were determined using the functional annotation tool DAVID.[24]

EST meta-analysis

Forty-three of the forty-six human homologs could be mapped to Unigene IDs. A comprehensive EST expression profile across 36 tissues was constructed for these genes based on a methodology developed for a previous study.[19] Briefly, all ESTs of a given tissue type t available from the Unigene database (Unigene Build #230)[25] were merged to a meta-library, excluding ESTs from normalized and subtracted cDNA libraries or deriving from uncharacterized, mixed or embryonic/fetal tissues. Meta-libraries with an EST count below 10,000 were excluded to assure significance, resulting in cancer and normal meta-libraries for 36 tissue types. For each Unigene cluster the global expression profile in cancerous and healthy tissues is computed by EST counting, following the concept of the Unigene EST profiles.[25] The expression profiles in cancerous and healthy tissues were normalized by calculating the transcripts per million ($tpm_{t,c}=m_{t,c}/n_t \cdot 10^6$), where $m_{t,c}$ is the number of ESTs for a given cluster c and for a given tissue type t, and n_t is the total number of ESTs for that given tissue type t. Genes with expression restricted to the testis, brain and placenta as well as limited expression in one or two tissues were selected to be testis- or testis/brain restricted. The significance of upregulation in cancer was calculated using the Fisher's exact test.[26] Genes with a p value < 0.05 or with expression in cancerous meta-libraries but not in the corresponding healthy meta-libraries were considered to be upregulated or ectopically expressed, respectively, in these cancer types. To visualize the analysis results, Circos plots[27] and bar charts were created.

Single and meta-analysis of microarray studies

Forty-one of the forty-six human homologs could be mapped to Affymetrix array indices for the HG-U133 Plus 2 array and thus could be evaluated for their differential expression in 13 cancer types by means of a meta-analysis approach developed for a previous study.[19,20] Genes with a meta-log twofold change >1 or a confidence interval that does not span 0, and a meta-p value <0.05 were considered as potentially significant. To visualize the analysis results, Circos plots[27] and forest plots[28] were created.

Implementation

The meta-analysis pipelines described above were implemented using: R 2.12.1 (available at: http://www.cran. r-project.org)[29]; the Bioconductor package (available at: http://www.bioconductor.org)[30]; MySQL 5.0.77 (available at: http://www.mysql.com) and Perl 5.8.8 (available at: http://www.perl.org).

Results

Identification of human homologs of the *Drosophila l(3)mbt* tumor up-regulated germline genes

Janic *et al.* reported 49 *Drosophila* germline genes to be overexpressed in *l(3)mbt* tumors.[4] We could map 28 of these

Table 1. Human orthologs of 28 *Drosophila* germline genes overexpressed in *l(3)mbt* tumors[1]

Drosophila gene	Human orthologs
AGO3	***PIWIL4, PIWIL2, PIWIL3, PIWIL1***
aub	***PIWIL4, PIWIL2, PIWIL3, PIWIL1***
bgcn	*YTHDC2*
BicC	*BICC1*
bol	***DAZ1, DAZ2, DAZ3, DAZ4, BOLL, DAZL***
c(3)G	*SYCP1*
dhd	*TXN, TXNL1*
fus	*ESRP1, ESRP2*
hdm	***C16orf73***[2]
krimp	***TDRD1, RNF17***
loki	*CHEK2*
mael	***MAEL***
mia	*TAF6, TAF6L*
mre11	***MRE11A***
nos	*NANOS1*, ***NANOS3, NANOS2***
orb	***CPEB1***[2], *RP11-152F13.10*
ovo	***OVOL1, OVOL2***, *OVOL3*
piwi	*PIWIL4, PIWIL2, PIWIL3, PIWIL1*
png	*NEK1, NEK5, NEK3*
RpS19b	*RPS19*
RpS5b	*RPS5*
shu	*FKBP6*
spn-E	***TDRD9***[2]
tej	***TDRD7***
vasa	***DDX4***
vis	*TXN, TXNL1, TGIF2LX, TGIF2LY*
zpg	*TGIF2*
γTub37C	***TUBG1***

Bold = human orthologs with germline GO designation (also see Supporting Information Table S2). None embolden genes have GO designations that are not directly associated with germline or development programmes (although this does not exclude them from having a germline function).
[1]A full list of all *Drosophila* germline genes over expressed in *l(3)mbt* tumors along with Ensemble/Flybase ID and sources is given in Supporting Information Table S1.
[2]Human genes with germline GO terms are associated with these genes using AmiGO (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi); no GO designations were identified by DAVID at the time of analysis (see Supporting Information Table S2).

genes to their human orthologs, resulting in expansion to 46 human genes due to human paralogs (Table 1 and Supporting Information Table S1). In support of this, the top enriched gene ontology (GO) terms show that many are linked to germline functions in humans as well, with 56.5% (26/46) being associated with germline functions (Table 1 and Supporting Information Table S2).

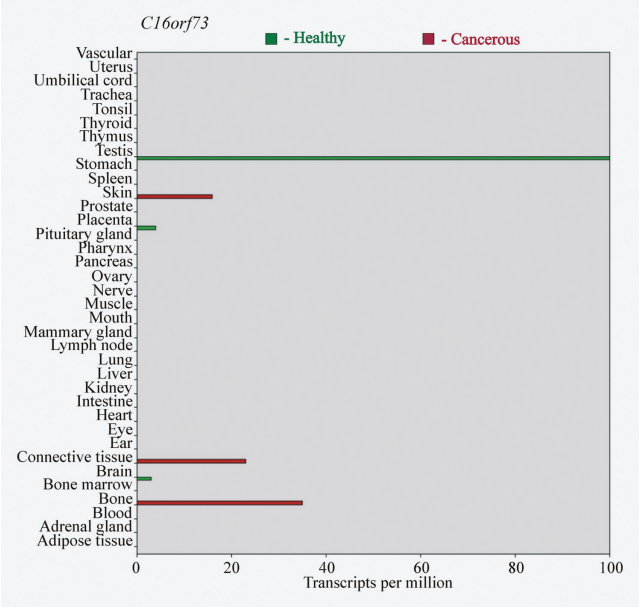

Figure 1. Example of a bar chart showing the integrated expression profile of the *C16orf73* gene. *C16orf73* exhibits expression restricted to the brain, placenta and testes, but is aberrantly expressed in melanoma DNA sarcomas of the bone and the connective tissue. Gene expression is given as transcripts per million (tpm). Further examples of expression profiles are given in Supporting Information Figure S1.

EST meta-analysis

We investigated 43 human orthologs for their cancer expression, cancer marker potential and tissue-specificity based on the construction of a comprehensive expression profile (of the original 46 human orthologs identified 3 could not be mapped to Unigene IDs and so only 43 were taken forward). Briefly, if genes show expression only in immunologically privileged tissues and in not more than two other healthy tissues, the genes are considered as testis- or testis/brain restricted. Nineteen genes exhibit such an expression profile (Supporting Information Table S3) including the previously characterized CT genes, *SYCP1*,[31] *TDRD1*[32] and *PIWIL2*.[33] *MAEL*, also a previously characterized CT gene,[34] shows expression in three normal tissues. Furthermore, 12 of these 19 genes exhibit ectopic cancer expression in at least one cancer type; for example, the gene *C16orf73* is expressed in testis, brain and placenta as well as ectopically expressed in melanoma and sarcomas of the bone and of the connective tissue (Fig. 1; further examples are given in Supporting Information Fig. S1). In total, 35 of 43 human homologs exhibit ectopic expression or are up-regulated in a wide range of cancers (Fig. 2 and Supporting Information Fig. S2), consistent with an extensive soma-to-germline transformation in a range of tumor types.

Single and meta-analysis of microarray studies

We evaluated the differential expression for 41 human orthologs (five genes are not present on the arrays) based on a

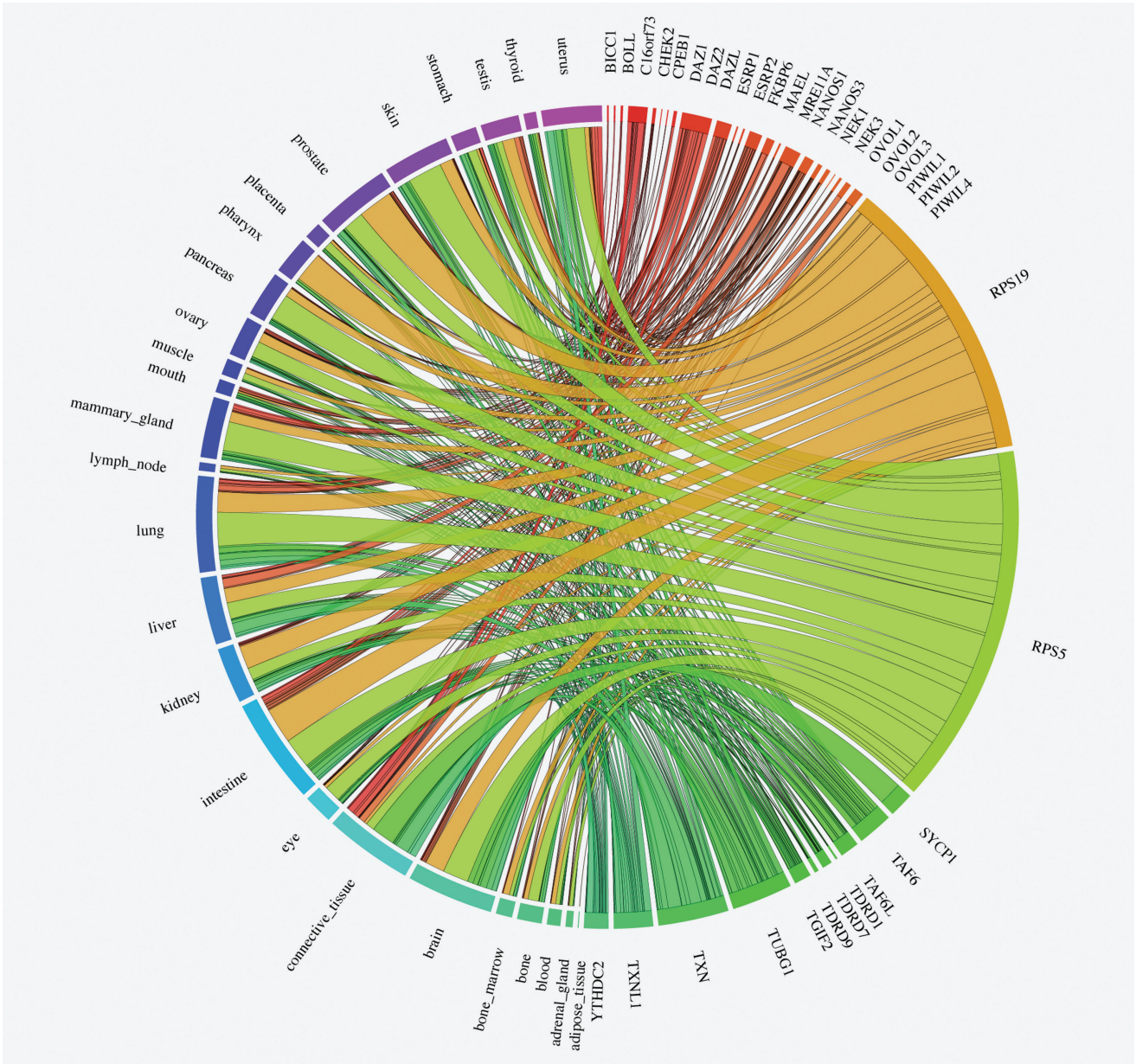

Figure 2. Circos plot showing the gene expression in relation to the corresponding cancer types for the 43 human orthologs based on the EST meta-analysis. Thirty-five of these forty-three human genes present in the Unigene database exhibit ectopic expression or are up-regulated in a wide range of cancers according to the EST meta-analysis. Each connection between a gene and a cancer type indicates found expression in cancer. The magnitude of the connection corresponds to the transcripts per million (tpm) for the given gene in a given tissue. A Circos plot showing the EST meta-analysis for testis-restricted alone is given in Supporting Information Figure S2.

microarray meta-analysis approach across 13 cancer types. Thirty-one of the forty-one human orthologs are significantly up-regulated in 11 distinct cancer types (Fig. 3, Supporting Information Fig. S3 and Table S4). Nine of the nineteen testis- or testis/brain-restricted genes were found to be significantly up-regulated, in particular in ovarian and brain cancer. For example, the gene *RNF17* shows up-regulation in ovarian, prostate and brain cancer (Supporting Information Fig. S4 and Table S4).

Furthermore, analysis of differential expression in 80 individual microarray studies provides evidence that even 39 of the total 41 genes and 14 of the 19 testis- or testis/brain-restricted are up-regulated in specific cancers (Supporting Information Fig. S5).

Cancer expression of human germline genes

Combining the results of the EST meta-analysis of the single microarray analysis as well as that of the microarray meta-analysis can provide a comprehensive picture of the cancer expression of the human germline genes investigated. In addition to the 4 previously characterized CT genes (*SYCP1*, *TDRD1*, *MAEL* and *PIWIL2*), 36 other germline genes show

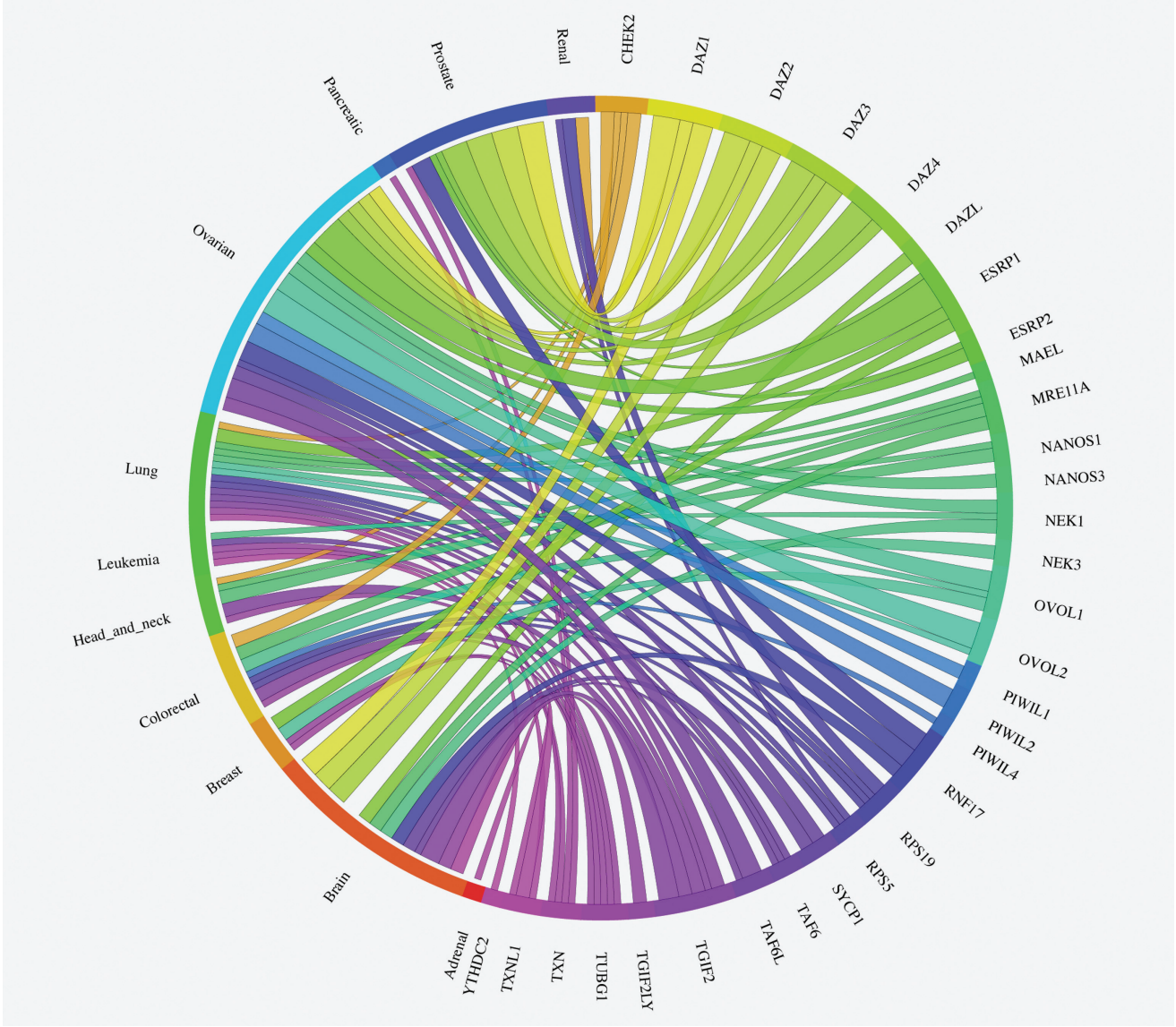

Figure 3. Circos plot showing the meta-change in gene expression in relation to corresponding cancer types for the 41 human homologs based on the microarray meta-analysis. Thirty-one of these forty-one human genes covered by the arrays exhibit an up-regulation according to the microarray meta-analysis. Each connection between a gene and a cancer type indicates a statistically significant mean up-regulation for that cancer type derived from a number of combined array studies for cancer *versus* normal tissue. The weight of the connection corresponds to the magnitude of the meta-change in gene expression. A Circos plot showing the microarray meta-analysis for testis-restricted alone is given in Supporting Information Figure S3.

evidence for ectopic expression or up-regulation in various cancer types (Supporting Information Table S5). Because of likely gene duplication events there are a number of human paralogs to orthologs *Drosophila* gene, resulting in the inclusion of gene families in the human analysis; for example, the *PIWIL* family genes *PIWIL1* and *PIWIL2*. Differential expression in cancer of the family member genes *PIWIL3*, *TGIF2LY* and *OVOL3* could not be evaluated, as these genes are not present on the arrays investigated (Supporting Information Table S5). Thus, these genes need to be further investigated to determine their cancer expression.

New CT candidates

Fifteen genes exhibit a testis- or testis/brain-restricted expression pattern according to the EST meta-analysis as well as showing evidence for aberrant cancer expression based on the results of the different expression analyses (Supporting Information Table S5). These include the three known CT genes *SYCP1*, *TDRD1* and *PIWIL2* and mainly members of the gene families mentioned above. A further four genes (*NANOS2*, *PIWIL3*, *TGIF2LX* and *NEK5*) have a testis- or testis/brain-restricted expression profile, but no cancer expression could be detected to date.

Discussion

Expression of human germline genes in cancers

Here, we show that the *Drosophila* germline genes ectopically expressed in *l(3)mbt* tumors are also aberrantly expressed or overexpressed in a wide range of human cancers. Fifteen of these genes also exhibit a testis- or testis/brain-restricted expression pattern, which makes them potential CT gene candidates. We have used the results of the EST meta-analysis, of the single microarray analysis as well as of the microarray meta-analysis to construct a comprehensive picture of their expression. Combining studies can enhance reliability and generalizability of the results, as meta-analyses are generally accepted to compute a more precise and reliable estimate of gene expression.[35]

Although most known CT genes are encoded on the X chromosome,[15] most of the 15 CT gene candidates are autosomally encoded. In general, almost all human homologs we have investigated are autosomally encoded (Supporting Information Table S5). We found mainly germline gene family members to be ectopically expressed or up-regulated in cancer. Most of these family members are thought to be involved in meiosis or spermatogenesis such as the *NANOS* or *DAZ* family genes.[36,37] At least 11 genes produce proteins that are associated with meiosis, and a total of 14 gene products may function in spermatogenesis (Supporting Information Table S2). Consistent with this, we have recently identified a cohort of CT candidate genes involved in meiotic spermatogenesis by analyzing the expression of human homologs of meiotic mouse genes, many of which are also autosomally encoded.[19]

We also found several genes to be down-regulated in a range of cancer types such as the genes *CPEB1* and *ESRP1*. This is not surprising as several genes are not germline-specific. Here, the loss of their functions could drive the malignant state of cancer cells. *CPEB1* and *ESRP1*, for example, are both potential tumor suppressor genes.[38,39]

A soma-to-germline transformation in human cancers

Tumorigenesis in humans may involve a soma-to-germline transformation, which in turn may support the acquisition of malignant attributes such as rapid proliferation, undifferentiated phenotype and immortality. Such transformations have not only been reported in *Drosophila* with mutations in *l(3)mbt* (dREAM-MMB/LINT pathways),[4,10,12] but also in *C. elegans* strains with mutations in the homologs of the dREAM-MMB complex[14] and in members of the nucleosome remodeling and histone deacetylase (NuRD) complex,[13] which are chromatin regulators of the SynMuv pathway.[40,41] Many SynMuv proteins and their antagonistic SynMuv suppressor proteins have been associated with histone modification, nucleosome remodeling as well as transcriptional repression and play a role in germline-soma distinction.[40,42–44] These data suggest that genes functioning in particular in the retinoblastoma pathway are responsible for repression of germline expression in somatic cells and thus mutations in this pathway may initialize a soma-to-germline transformation. Many of these genes are conserved in mammals[40] and the human retinoblastoma pathway is disrupted in virtually all cancer types, which is known to promote cellular proliferation.[45,46] Few *Drosophila* germline genes are already known orthologs of human CT genes; for example, *SYCP1* is the human homolog of the *Drosophila* germline gene *c(3)G*.[31] In human cancer, the expression of numerous CT genes may reflect the occurrence of such a soma-to-germline transformation. Also in humans, it has been suggested that cells become altered in genes that control germline gene expression, which could lead to an induction of a gametogenic programme in cancer.[9,15] Here we provide evidence that 40 of 46 human homologs of the *Drosophila* germline genes ectopically expressed in *l(3)mbt* tumors are also ectopically expressed or up-regulated in a wide range of human cancers, not just germline tumors, which supports the proposal that human cancer cells undergo a similar soma-to-germline transformation. Many of the hallmarks of cancer have recently been delineated[1] and collective evidence, including that presented here, points to a soma-to-germline transformation being a key additional hallmark of a wide range of human tumor types.[5,8,9,15,19]

Soma-to-germline transformation: meiotic genes as oncogenic drivers

The ectopic expression of a few testis-specific factors, which act as epigenetic and transcriptional regulators, could further drive the soma-to-germline transformation.[9] The expression of meiotic genes is tightly regulated and mostly restricted to germline cells. The expression of meiotic genes, in particular those with chromosome modulating potential, in mitotic cells could lead to perturbation of the mitotic process and thus could result in inappropriate recombination events, leading to oncogenic changes such as translocations, aberrant chromosome segregation and aneuploidy,[9,15,19,47] which in turn are hallmarks of cancer.[1] Kalejs *et al.*, for example, reported the up-regulation of meiosis-specific genes in tumor cells, which appears to be associated with arrested mitosis and polyploidy.[48] A key example of how germline genes might play a fundamental role in oncogenesis comes from the finding that PIWI proteins have been implicated in tumorigenesis.[49] Many cellular events are controlled by small RNA molecules and PIWI proteins regulate small germline micro RNAs known as piRNAs.[49] Quite how PIWI proteins might influence tumor formation and progression has been poorly studied to date, but one key suggestion that has been postulated proposes that PIWI proteins can contribute to the unprogrammed silencing of tumor suppressor genes, thus fostering oncogenesis.[49] A number of the human germline genes we investigated here are also associated with meiosis. Not only may ectopic expression of germline genes be important as a key oncogenic driver, but the normal immunological privilege of the gene products make these excellent targets for the development of new therapeutic and clinical biomarker strategies.

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