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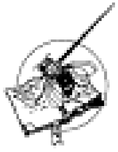

Gene combinations inducing neoplasms in *Drosophila*.

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Abstract

Drosophila mutant Malignant Brain Tumor carries mutations both in proliferative genes and developmental genes. Mutant proliferative genes break the restriction of the cell cycle, induce somatic pairing gaps of polytene chromosomes, allow replication and genome instability. Neoplasms are coupled to a proliferative gene defect. Tumor suppressor activity maps to 85cM on the third chromosome and was identified as *tolloid* allele. Transgenic Malignant Brain Tumor with addition of wild type *tolloid* partially rescue tumor formation: neuroblasts regain their ability to differentiate and lose malignancy. Still hyperplasia occurs, indicating that the tumor suppressor mutation adds to tumor formation, but that the initial tumor forming mutation, loss of cell cycle control, is not due to tumor suppression. *Toll* and *pelle* alleles induce tumor formation *in trans* over Malignant Brain tumor as oncogenes. Oncogenes or tumor suppressor are unable to induce overgrowth of cells, they give rise to tissue specificity of tumor formation. Neoplasms can be induced by a minimum of one dominant proliferative gene defect, two recessive proliferative gene defects, one proliferative gene defect over one oncogene defect, one proliferative gene defect over one tumor suppressor gene defect, or one proliferative gene defect over one deficiency.

Introduction

Both in humans and in *Drosophila*, malignant tumor formation is due to a multigenic process. Proto-oncogenes which can change their activity to become dominant cell growth promoting genes, are instrumental in developmental processes such as cell communication, signal transduction and regulation of gene expression. A number of human cancers are associated with mutation or loss of both copies of a tumor suppressor gene. Their protein products keep in check an otherwise uncontrollable ability of cells to proliferate (Bishop, 1982; Levine *et al.*, 1991; Weinberg, 1995). Cancer causing alleles of tumor suppressors are usually recessive, whereas oncogenic alleles can act in a dominant fashion to promote cell overgrowth. Tumors in humans are rarely associated with single gene defects (Foulds, 1958; Nowell, 1976; Peto, 1977). Two major features are altered in tumor cells. First they lose control over the cell cycle and proliferate in an unregulated fashion. Second they lose or alter differentiation. It is not yet completely understood which gene defects are involved in tumor induction, promotion and progression. Although general tumor suppressor genes are known (Levine *et al.*, 1991; Kamb *et al.*, 1994; Nobori *et al.*, 1994), no model system has been established so far, which can distinguish between single effects on differentiation, proliferation, and malignancy.

The *Drosophila* line Malignant Brain Tumor (MBT) has been subject to genetic analysis. This has shown the existence of proliferative genes involved in tumor formation (Riede, 1996). Mutant proliferative genes break the restriction of the cell cycle and induce somatic pairing gaps of polytene chromosomes (Riede, 1997). They induce cell overgrowth in different tissues and polytenization of brain cells, allow replication. Proliferative genes do not induce a brain tumor; accordingly a different

class of genes induces differentiation defects, causing undetermined brain cells which are competent to induce the cell cycle.

The early events during *Drosophila* embryogenesis, from the view of a single cell, involve a modulation of two features. First, the expression pattern of the genes active in the G1 or G2 phase, as well as the functions of the cell within the organism has to be defined. This type of modulation determines the cell fate and differentiates the tissue. Second, a programmed number of cell cycles has to be driven. Differentiation and proliferation of cells require an equilibrium. Excessive proliferation will lead to more cells than programmed, leading to hyperplasic growth. Premature differentiation of several cells, before the programmed number of cell cycles is driven, will lead to missing cells: organs or structures are incomplete. A lack of differentiation in addition with activity of the cellular program to proliferate will lead to an overgrowth of undifferentiated tissue and, accordingly, to a tumor.

The establishment of the basic features of the dorsal-ventral embryonic pattern requires specifically the action of maternal effect genes (Anderson and Nüsslein-Volhard, 1984). Signal transduction is triggered on the ventral side of the *Drosophila* embryo through the binding of an extracellular ligand to the *Tl* (*Toll*) receptor (Stein and Nüsslein-Volhard, 1992). *Tl* encodes a transmembrane protein, homologous to the human Interleukin-1 Receptor (Hashimoto *et al.*, 1988). *spz* (*spätzle*) acts immediately upstream of *Tl* in the genetic pathway and represents a component of the extracellular signaling pathway (Morisato and Anderson, 1994; Chasan and Anderson, 1993). Downstream of *Tl*, the action of *pll* (*pelle*) encoding a protein kinase, is required (Shelton and Wassermann, 1993). The product of *tube* (*tub*) is a protein that disrupts the interaction of *Tl* with pll (Letsou *et al.*, 1991, 1993). pll leads to dissociation of the *dorsal* (*dl*)-*cactus* (*cact*) complex and to *dl* nuclear import (Roth *et al.*, 1991). dl is a member of the rel family of transcription factors (Steward, 1987). Accordingly, proto-oncogenes are members of the dorsal-ventral patterning pathway. The gene activities of *spz*, *Tl*, *pll* and *tub* result in a ventral to dorsal gradient of dl protein in the nuclei of the syncytial blastoderm of the embryo. This gradient gives rise to region specific expression of zygotic genes. One of them, *tld* (*tolloid*), is transcribed by nuclei in the dorsal-most 40 % of the blastoderm embryo.

tld is homologous to Human Bone Morphogenetic Protein 1 (Shimell *et al.*, 1991). The protein consists of an amino terminal metalloprotease domain followed by an interaction domain including complement protein repeat motifs and epidermal growth factor type three domains. Two domains of the protein contribute to its genetic interaction with *decapentaplegic* (*dpp*), a member of the TGFß family (Childs and O'Connor, 1994). This family of extracellular factors can stimulate or inhibit cell growth or differentiation, depending on the cell type involved (Massagué, 1990).

Here I show that *tld* appears as the tumor suppressor gene in MBT. In MBT tumor formation, the role of tld^{+} lies in differentiation of neuroblasts. Another mutant gene, spz^{MBT}, belongs to the pathway inducing dorso-ventral polarity of the embryo. Out of the pathway, *Tl* and pll alleles are able to induce brain tumor formation *in trans* over MBT. The oncogenes *Tl* and pll and the tumor suppressor gene *tld* encode proteins that determine cell differentiation: developmental genes. Mutations in developmental genes alone do not break the restriction of the cell cycle. Distinct combinations of oncogenes, tumor suppressor, proliferative genes and deficiencies induce neoplasm formation in *Drosophila*.

Results

Mapping of tumor suppressor activity to vicinity of tld

To avoid interference with mutant genes causing lethality and not being involved in tumor formation, the third chromosome of MBT was recombined twice with wild type. From fifty

Table 1. *tld* Alleles over MBT

Mutant allele	Allele strength *a	Amino acid change *b	Strength over MBT *c
10F102	strong	Intron 4 splice	strong
10E95	strong	Ser267Phe	strong
6P41	strong	Tyr272Asn	strong
7M89	weak	Arg235His	strong, Tumor
9D36	weak	Asp728Tyr	intermed., (Tumor)
5H56	weak		weak, Tumor
7H41	strong	Gln478TAG	weak,(Tumor)
6P117	strong	Gln440TAG	weak
9Q19	intermed.	Glu517Lys	weak

*a: Alleles that exhibit moderate to severe phenotypes include 6P41, 6P11, 8L38, null like behavior is exhibited by 10F102, 7H41, 10E95 (Ferguson and Anderson, 1992).
*b: according to Childs and O'Connor, 1994.
*c: MBT was crossed over *tld* alleles at 29°C. A strong phenotype is defined if more than 80% heterozygote embryos die. A weak phenotype allows formation of pupae, sometimes adults which are always sterile. Brain tumor formation was observed in more than 20 %, or less than 20 % (brackets) of the heterozygote L3 larvae. Neither *tld* allele induces brain tumor over wild type. *tld* alleles 6B69, 7O47, 8L38, 9B66, 9K88, 9Q74 and T show a weak phenotype over MBT.

recombinants one strain was selected, MBT*, which was able to induce brain tumor as heterozygote over MBT. To map tumor suppressor activity, MBT* was recombined with *ru st e ca*. Recombinants were tested for their ability to induce brain tumor formation as heterozygotes over MBT. Out of 280 recombinants, 21 *ru st e* and 22 *ca* marked recombinants induced brain tumor formation over MBT. This localizes tumor suppressor activity to 85cM on the third chromosome. Independently, the same crosses were performed with markers *st e tx*. Tumor formation in 7% of MBT/recombinant MBT tx, based on 200 recombinants, indicates, that the tumor suppressor locus is 7 cM proximal from *tx*, *i.e*., at 84cM.

Interactions of tld alleles and MBT

Deficiencies Df(3R)X18E(tld^-) and Df(3R)XTA1(tld^-), that cover region 96B (85cM) are lethal over MBT. Df(3R)$tld^{68\text{-}62}$(*tld-*) deletes about 4 kb within *tld* (Shimell *et al*., 1991), and does not complement MBT. Tumors are induced in 20% of Df(3R)X18E/MBT and in 30% of Df(3R)XTA1/MBT. The primary *tld* alleles have been ordered into an allelic series based on complementation behavior and phenotype (Ferguson and Anderson 1992) (Table 1). Several alleles have been sequenced, and the interaction domains with *dpp* have been identified. Two domains of *tld* contribute to its genetic interaction with *dpp*, one was identified within the metalloprotease domain, and one in the first CUB repeat (Childs and O'Connor 1994). *tld* alleles were crossed over MBT and the hybrids screened for lethality and tumor

Table 2. Transformation rescue of MBT strains with *tld*+

Strain	Mutations	Brain *a	Lethality *b	rescue with *tld*+ *a	rescue with *tld*+ *b
MBT-III	*mali tld yeti spz*	tumor	100% L3	hyperplasia	100% P
tld-1	*tld yeti*	hyperplasia	90% P	normal	30% P
tld-2	*tld yeti spz*	tumor	100% L3	hyperplasia	60% P

Parents from three independent crosses were shifted to the restrictive temperature 29°C. The F1 generation was analyzed.
*a: Thirty L3 larvae were screened for brain tumor or hyperplasia formation, or wild type size of the optic lobes. A tumor contains undifferentiated tissue, ARD is not accordingly expressed. In hyperplasia the brain is enlarged but accurately differentiated. The neuroblasts of a hyperplasic brain do not invade into the ventral ganglion like neuroblasts from a brain tumor.
*b: Six independent vials were screened for lethality. Larval stage 3 (L3) or pupal lethality (P) occurs in the percentage of animals indicated.

induction. tld^{5H65}, tld^{7M89}, tld^{7H41} and tld^{9D36} are able to induce brain tumor formation over MBT (Table 1). None of the *tld* alleles analyzed does complement MBT, indicating that tld^{MBT} represents a strong allele. The strong alleles 10E95, 10F102 and 6P41 show a strong phenotype over MBT. The strong alleles 7H41 and 6P117 exhibit weak phenotypes over MBT. The weak allele 7M89 shows strong interaction in MBT. Thus, any allele being mutant in region around amino acid 250 shows strong interrelation in MBT. This region confers one of the *tld-dpp* interaction domains, within the metalloprotease region of *tld*.

tld-1 and mali-2 are recombinants of MBT containing tld^{MBT} in different genetic environment (Riede 1996). They were crossed over tld^{5H56}. In hybrids *tld* phenotype should occur, additional recessive defects should be complemented by the heterozygote wild type function of *tld*5H56. Hybrids exhibit rough eyes at the permissive temperature. At the restrictive temperature 30% die as pupae. The optic lobes are not hyperplasic, brain cells are differentiated but do not adhere. Wild type brain cells adhere. Non-adhesion of the upper and the lower surface of the wings occurs, resulting in haltere-like wings of adults. Thus, cell adhesion is affected by *tld* in MBT. Adhesion of cells helps them to communicate and is a precondition for contact inhibition.

Rescue with tld+ transgene

pMBO1366 contains DNA en-coding for the 3.5 kb transcript of tld^{+}. Chromosome 1 or 2 with pMBO1366 were combined accordingly with the third chromosome of MBT (MBT-III) (Table 2). tld^{+} induces growth disadvantage at the permissive tempera-ture. MBT-III is viable and sterile. The addition of tld^{+} leads to 80% pupal lethality, to a three days longer generation time and to defects in eclosed flies, these move very slowly and are unable to fly. Accordingly tld^{+} is crucial in the speed of cell division and differentiation of MBT. At the restrictive temperature, MBT exhibits unsegmented optic lobes with undifferentiated malignant neuroblasts. The enlargement of the brain is reduced by tld^{+}, the ventral ganglion is elongated. Ingrowth of tumor tissue into the ventral ganglion was not observed. Thus, cells have lost their ability to invade. Hyperplasic growth of the tissue was not completely rescued. 80% of the transgenes' brains are two to three times larger than wild type brains. tld^{+} transgenic MBT regain wild type pattern of ARD expression in the brain, indicating that tld^{+} drives differentiation of neuroblasts. *tld* is expressed in larval wild type brain. Expression occurs in the segment where cells undergo cell divisions before they differentiate (Finelle *et al.*, 1995; Nguyen *et al.*, 1994). *tld*-1 induces brain hyperplasia formation, which could be rescued by recombinant tld^{+}. *tld*-2 exhibits brain tumor formation, which was

Table 3. *Tl* and *pll* are proto-oncogenes.

	Allele	Brain tumor	MBTx	xMBT
		*a	*b	*b
Tl^{r632}	recessive	80%	weak	strong
Tl^{r444}	strongly dorsalized	30%	weak	strong
$Tl^{1\text{-RXA}}$	revertant	5%	strong	weak
pll^{019}	D1	50%	weak	strong
pll^{078}	S1	30%	weak	weak
pll^{312}	S	50%	weak	weak
pll^{628}	D2	50%	strong	weak
wild type		0%	+	+

*a: Brain tumor induction: 30 minutes old embryos were shifted to 29°C. L3-larvae were screened for brain tumor. Given is the percentage of animals that induce brain tumor formation (two independent crosses).
*b: MBT virgins (MBTx) or males (xMBT) were crossed with *Tl* or *pll* alleles at the restrictive temperature. A strong phenotype indicates lethality of heterozygotes. Weak response: up to 50% eclose. In addition other alleles were tested: r26, 5BRXV and 9QRE1 induce strong responses over MBT in both directions. rm9, rm10, 1-RXD, 1-RXH, 5BREQ and 9QRE induce strong responses in crosses with MBT virgins and weak responses with *Tl* virgins. *pll* alleles 316 and rm8 induce weak responses in both directions, 385 and 74 strong responses with MBT virgins and weak responses with *pll* virgins. +: complementation

partially rescued.

Oogenesis and Embryogenesis are temperature sensitive periods for brain tumor induction in MBT.

Three mutant genes coopera-tively induce brain tumor for-mation in MBT: *tld*, *yeti* and *spz*. In 100% of the L3 larvae brain tumor formation is induced, when parents were shifted to the restrictive temperature 24 hours prior to egg deposition. Temperature sensitive period for *spz* is the oogenesis and the first hour of embryogenesis. *tld* temperature sensitivity encompasses 4 to 6 hours of embryogenesis (Lindsley and Zimm 1992). Oogenesis at the permissive temperature reduces the rate of tumor formation to 30% of the larvae, thus *spz* contributes to tumor formation. If the period for the *tld* effect occurs at the permissive temperature, 70% of the larvae induce brain tumor. Thus, both oogenesis and embryogenesis are temperature sensitive periods for MBT, both, *spz* and *tld*, add to tumor formation in MBT which includes temperature sensitivity in both cases.

Tl and pll promote tumor formation.

Tl and *pll* are members of the signalling pathway that is initiated by *spz*. Deficiencies comprising *Tl* and *pll* are complemented by MBT and *spz*-1, accordingly both genes are not expected to be mutant in MBT. Several alleles were found interrelating with MBT (Table 3). Seven alleles are able to induce tumor formation over MBT, indicating oncogenic potential of this pathway. *Tl*r632 and *pll*^{078}, both recessive alleles, are able to induce malignant brain tumor over MBT. This defines *Tl* and *pll* as proto-oncogenes.

In *pll*^{628}/MBT a second eye on one or both sides occurs in 50% of the flies. The same phenotype exhibits in *pll*^{628}/*spz*-1, but not in *pll*^{628}/+ or MBT/+. Other *pll*^{628}/*spz*-1 reveal asymmetries at the front head, leading to tissue outgrowth. This indicates, that *pll*^{628} might further destabilize the head and brain development, already disorganized by *spz*-1.

Table 4. Interactive alleles

	srn^{88}	*efe*^{79}	*efe*^{89}	Df(3R)*mbt*^{P}	MBT
spz^{67}	P	+	+	+	E
Tld^{7M89}	+	+	(P)	(P)	P
Tl^{632}	P	+	+	+	L3
Tl^{26}	+	+	+	+	P
pll^{019}	(L3)	nd	nd	E	(P)
pll^{078}	(P)	+	(P)	(P)	(P)
tub^{238}	(P)	+	+	+	P
dl^{2}	+	+	+	(P)	(P)
dl^{Q10}	(P)	+	+	(P)	(P)
cact^{VQ}	+	+	+	(P)	P
cact^{99}	P	+	E	(P)	(P)
Ser^{RX106}	P	P	+	(P)	P
N^{55e11}	+	+	+	P	(P)
Dl^{6B}	+	+	+	(P)	+
neu^{12H56}	+	+	+	(P)	L1-P
gro^{E75}	P	+	+	(P)	L1-P
wild type	+	+	+	+	+
Df(3R)*ro*^{82b}	P	P	P	P	P
MBT	L3	(P)	(P)	P	L3
	56cM	92cM	92cM	92cM	multiple

P/(P): (Partial = more than 80%) pupal lethality. Eclosed adults show defects of the abdominal segmentation pattern and are always sterile. E: embryonic lethality. L3: lethality as third instar larvae. +:

Proliferative alleles interact with developmental genes over srn

Mutations in proliferative genes break the restriction of the cell cycle, induce melanotic tumors in 100% of stage three larvae and somatic pairing gaps in salivary gland chromosomes. Lethality of homozygous animals occurs at different developmental stages. Adult viability of heterozygotes is reduced. Over MBT, *Aus*^{9}, *mer*^{14} and *srn*^{88} induce lethality and brain tumor formation. *efe* alleles over MBT induce brain hyperplasia and partial pupal lethality (Riede, 1997). Developmental genes *spz* and *tld* are mutant in MBT, *pll* and *Tl* act as oncogenes over MBT. The genetic interlink between the

proliferative genes and the signal transduction cascades can be identified, by crossing proliferative alleles over alleles in differentiation genes and screening the hybrids for lethality and growth aberrations. MBT does not complement a number of developmental genes (Table 4). Alleles interfering with MBT were crossed over proliferative alleles. srn^{88} reveals as a major interactor. Full lethality occurs over alleles of *spz*, *Tl*, *cact*, *Ser,* and *gro*. Partial lethality was observed over *pll*, *tub*, and *dl* alleles. mbt^{P} interacts with several alleles of differentiation genes. Complete lethality of heterozygotes is rare, and is seen only with *pll* and *N* alleles. srn^{88} does not complement efe alleles. As srn^{88} interferes with differentiation genes and with proliferative genes, it represents the genetic link between both.

Neoplasm formation (Tables 5 -7)

Several combinations of MBT recombinant strains, differentiation genes, deficiencies and proliferative alleles are lethal. More than 30 combinations induce aberrant cell proliferation that is manifested as neoplasic growth. Tables 5-7 summarize the phenotypic manifestations of gene combinations with oncogenic potential. From that, the minimal gene defects can be defined that is necessary for neoplasic growth of a tissue:

One strong proliferative gene defect induces neoplasm for-mation

Proliferative alleles show tumor formation as melanomes. The strong proliferative alleles Aus^{9}, srn^{88} and mer^{14} induce lethality and melanome for-mation in homozygous and heterozygous animals. Variable expression shows that individual factors influence the phenotypic expression of the genotype. 50% of the heterozygote Aus^{9} larvae show melanomes. Accordingly, one dominant proliferative mutation gives rise to melanomes and is sufficient for tumorformation.

Table 5. Defined alleles over MBT strains

	N^{55e11}	Ser^{Rx106}	neu^{12H56}	gro^{E75}	tld^{T}	tld^{7M89}
MBT	(P)	P	E	(P),T1	(P),T1	(P)T2,4
spz-1	(P)	+	E/P	(P),T1	+	(P)
spz-2	E	(P)	(P)	(P)	(P)T4,8	+
tld-1	P	(P)	E	(P),T4	nd	P
mali-2	(E),T8	+	(P)	(P)	(P)	P
wildtype	+	+	+	+	+	+

	tld^{9D36}	spz^{67}	Tl^{632}	pll^{628}	pll^{078}
MBT	L3,T2	(P)T4	L3,T2	P,T2	(P),T4
spz-1	(P)	P	(P)	(P),T3	(P)
spz-2	(P)	E	P	(P),T6	(P),T2,4
tld-1	E	(P)	nd	+	+
mali-2	(P)	(P)	(P)	+	(P)
wildtype	+	+	+	+	+

	srn^{88}	mer^{14}	btx^{P}	rig^{P}
MBT	L3,T2	L1-3,T2	E	E-P
spz-1	(P)	(P)	nd	+
spz-2	P,T2	P	nd	+
tld-1	P,T2	P	(P),T3	+
mali-2	+	P	nd	nd
wildtype	+	+	(P)	+

Females carrying alleles were crossed to MBT and MBT recombinant males. +: complementation; (incomplete = 80%) lethality as P: pupae, L1-3: larvae stage 1-3, E: embryos. nd: not determined.
Neoplasms (T) are indicated, if more than 30% of the animals reveal overgrowth of a tissue. T1: ubiquitous neoplasm/melanomes (no tissue specificity), T2: brain tumor, T3: front neoplasm, T4: eye neoplasm, T5: höcker neoplasm (thorax), T6: wing neoplasm, T7: leg neoplasm, T8: terminal (anal plate) neoplasm. At least two independent crosses were performed.

Two recessive proliferative gene defects induce neoplasm formation

All recessive alleles of proliferative genes, when homozygous, induce melan-

Table 6. Deficiencies over MBT strains

Df(3R)	roeST1	T32	XTA1	X18E	8D06	R1	*mbt*^{P}	wildtype
MBT	(P), T4	E	P, T1	P, T2	E	(P)	P	+
spz-1	nd	P	nd	nd	nd	nd	+	+
spz-2	(P)	E	(P)	(P)	(P), T7	(P)	P	+
tld-1	(P), T5	E	P	E	(P), T7	(P), T1	(P), T4	+
mali-2	(P), T4	+	(P)	nd	nd	nd	+	+
wildtype	+	+	+	+	+	+	+	+

Females carrying deficiencies were crossed to MBT and MBT recombinant males. Classification as in Table 5.

omes. Two recessive mutations *in cis* induce neoplasm formation. *efe*^{89} is a recessive proliferative allele, mutant in a gene at 92cM on the third chromosome far from *srn*^{88} (56cM). *efe*^{89} does not complement *srn*^{88}, all hybrids die as third instar larvae with melanomes. Ac-cordingly, two reces-sive alleles in trans induce melanome for-mation.

One proliferative gene defect over one oncogene defect in-duces neoplasm for-mation

pll^{628}/*spz*-2, *pll*^{078}/*spz*-2, *mer*^{14}/ *pll*^{628} and *rig*^{P}/*pll*^{628} induce neoplasms. Accordingly, the com-bination of an onco-gene over a proliferative allele *in trans* induces cell overgrowth. The tissue most frequently involved is oncogene specific, while proliferative alleles do not exhibit tissue specificity.

One proliferative gene defect over one tumor suppressor gene defect induces neoplasm formation

tld^T/*spz*-2 and *srn*^{88}/*tld*^{7M89} induce neoplasms. Accordingly, *trans* heterozygotes of proliferative alleles over tumor suppressor genes induce cell overgrowth. Heterozygotes for alleles of the tumor suppressor gene *tld* and a wild type allele induce neoplasm formation if a proliferative mutation is present.

One proliferative gene defect over one deficiency induces neoplasm fo-rmation

8D06/*spz*-2, 8D06/*tld*-1, R1/*tld*-1, *btx*^{P}/8D06 induce neo-plasms. Accordingly, het-erozygotes of a proli-ferative allele over a deficiency in cis induce cell overgrowth. roeST1/ *tld*-1, roeST1/*mali*-2, *mbt*^{P}/*tld*-1 and *mer*^{14}/8D06, proliferative alleles over deficiencies in trans, induce neoplasms.

Identified oncogenes or tumor suppressor genes are unable to induce neoplasms

Alleles *tld*^{7M89}, *pll*^{078}, *pll*^{628} and *Tl*^{r632} were screened for neoplasms as homozygotes, heterozygotes inter se, heterozygotes over wild type or heterozygotes over deficiencies *in cis*. None of these combinations induces a neoplasic growth of cells.

Table 7. Proliferative Genes over differentiation genes and deficiencies.

	efe^{11}	*srn*^{88}	*mer*^{14}	*btx*^{P}	*rig*^{P}	wildtype
tld^{7M89}	+	(+), T1	(P)	L2	(L2)	+
pll^{628}	+	(P)	(L3), T8	+	(L2), T8	+
pll^{078}	+	(P)	(P)	(P)	(L2)	+
neu^{12H56}	+	(+)	(L1)	P	+	+
Ser^{Rx106}	+	P	+	+	+	+
XTA1	nd	+	(L3)	E	(P)	+
8D06	nd	+	(P), T8	(P), T8	+	+
mbt^P	P	P	+	(+)	(L3)	+

Females carrying proliferative alleles were crossed to males of differentiation alleles or deficiencies. Classification as in Table 5. Very frequent in these combinations is a phenotype, allowing 20% escapers to eclose as adults, 80% of the hybrids die.

Neoplasms without proliferative gene defect

pll^{628}/*spz*-1 and *gro*^{E75}/*spz*-1 induce neoplasm formation. Other gene combinations with oncogenic potential like *spz*^{67}/*pll*^{078}, *pll*^{628}/*spz*^{67}, *spz*67/*pll*^{628}, *spz*^{67}/*pll*^{019} do not induce neoplasms. Thus, neoplasm formation induced

by gene combinations without identified proliferative gene defect is coupled to *spz*-1. This strain has a complete somatic pairing of the giant chromosomes (Riede, 1997).

Discussion

A tumor cell circumvents the restrictions of the cell cycle. Mutations in proliferative genes change the chromatin structure, allow replication of DNA and lead to hyperplasic growth of tissue. Thus, a proliferative gene mutation is thought to be the primary initiative event in tumor formation. A lack of differentiation provides a cell the competence to divide and migrate. Differentiation genes lead to determination of the tissue. Mutations of *tld* and *spz* in MBT and the oncogenic potential of *pll* and *Tl* alleles over MBT show that the determination process is destabilized in tumor formation. Mutation of *tld* in MBT is a secondary event: the addition of wild type *tld* in transgenes reduce vitality of MBT at the permissive temperature. Thus, tumor suppression is a secondary event, an adaptive mutation which suppresses lethality induced from a primary defect in a proliferative gene.

A human autosomal disorder, nevoid basal cell carcinoma syndrome, that predisposes to both cancer and developmental defects, is associated with mutants of the human homolog of *Drosophila patched* (Hahn *et al.*, 1996). *patched* plays a role in segment polarity, and interferes with TGFß gene family members (Hooper and Scott, 1989). *tld*, the identified tumor suppressor gene of MBT interferes as well with TGFß family members. Thus, tumor predisposition and tumor suppression can act on similar biochemical levels.

The differentiation genes and proliferative genes interact genetically. To identify genetic interactors, proliferative alleles were screened for lethality over differentiation genes. One allele, srn^{88}, exhibits most interactive potential. This allele does not complement a number of proliferative alleles (Riede, 1997). Accordingly, it represents the genetic link between proliferative alleles and differentiation genes. All alleles of the proliferative genes show an unusual feature: the somatic pairing of the chromosomes is incomplete. Shorter or longer stretches of the chromosomes are involved, depending on the allele. srn^{88} frequently induces somatic pairing defects of long distances, up to half a chromosome.

Mutations in human *BRCA1* are responsible for about 10% of breast cancers and ovarian cancers. Its protein associates with Rad51, a member of a protein family mediating homologous pairing (Scully *et al.*, 1997). *BRCA1* breast tumors are characterized by a high degree of genome plasticity (Marcus *et al.*, 1996). Proliferative genes in *Drosphila* induce somatic pairing gaps and replication initiation errors (Riede 1997, 1998). Accordingly, the phenotype of *Drosophila* reflects the molecular interaction of *BRCA1*: defect chromosome pairing and genome plasticity. This reflects, that *BRCA* belongs to the class of proliferative genes. Proliferative genes are the only genes that are causally related to cell proliferation in cancer formation. Developmental genes only add to the event by changing the differentiation pattern of the cells.

Materials and Methods

Genetics of MBT strains

The temperature sensitive (ts) *Drosophila* mutant line Malignant Brain Tumor (MBT) forms malignant neuroblasts in the brain of larvae. It carries interrelative mutant genes: $höcker^{MBT}$ $hedera^{MBT}$ (second chromosome) $mali^{MBT}$ tld^{MBT} $yeti^{MBT}$ spz^{MBT} (third chromosome) (Riede, 1996). Of the list, *mali* and *yeti* are proliferative genes, *i.e.* induce cell overgrowth and somatic pairing gaps of polytene chromosomes. Not proliferative is *spz*, this gene defect alone does not induce cell proliferation or

somatic pairing gaps. The polygenic defect in MBT was analyzed by recombination analysis. In principle, first phenotypes had been identified and reference strains exhibiting this phenotype were obtained. Second, the phenotypes were mapped. Third, the phenotype had to appear with the deficiencies of the region in question over MBT and the reference strain carrying the mutation. Fourth alleles of suspected genes had to react with MBT and the reference strain carrying the gene defect. With defined deficiencies and alleles all other strains were tested, to evaluate the genotype of all strains. $mali^{MBT}$ has been localized to 87B (Riede, 1997), $yeti^{MBT}$ to 96F (Riede, 1996). One of the loci being involved in lethality has been localized to 97F by the P-element insertional deletion Df(3R)mbt^{P} (Wismar *et al.,* 1995). MBT/Df(3R)mbt^{P} hybrids are 100% temperature sensitive pupal lethal. In MBT/Df(3R)mbt^{P} larvae grown at 29ºC, no brain tumor is observed. MBT was recombined with Df(3R)mbt^{P}. If the deficiency would cover a mutation causing 100% temperature sensitive lethality, wild type recombinants should not appear. One per cent of the recombinants eclose. Therefore, this deficiency does not harbor the tumor suppressor gene causing 100% temperature sensitive lethality.

To obtain tld^{MBT} stocks, MBT and MBT* was recombined with *ru st e ca.* Recombinants were screened for temperature sensitivity over tld^{5H56}, tld^{7M89}, Df(3R)X18E and Df(3R)XTA1. Strains were selected that exhibit different phenotypes: *tld*-1 (two identified mutant genes inducing ts pupal lethality and hyperplasia formation of the brain *ru st e* tld^{MBT} $yeti^{MBT}$), *tld*-2 (*ru st e* tld^{MBT} $yeti^{MBT}$ spz^{MBT}, brain tumor formation) and mali-2 (two identified mutant genes inducing partial ts lethality, $mali^{MBT}$ tld^{MBT} *ca*). All induce brain tumor formation over MBT. Partial pupal lethals (*spz*-1, one identified mutant gene, *ru st e* spz^{MBT}) and pupal lethals inducing hyperplasia formation of the brain (*spz*-2, two identified genes, *ru st e* $yeti^{MBT}$ spz^{MBT}) have been isolated. spz^{MBT} has been identified at 92cM, *i.e.* region 97 (Riede, 1996). *spz*-1, *spz*-2 and MBT do not complement spz^{67}.

Genes and fly stocks

Three strong proliferative recessive lethal alleles were selected for this study (56cM): Aus^{9}, srn^{88} and *merlin* (mer)^{14} (Riede, 1997). They have been induced with EMS, give rise to brain tumor over MBT and reveal long unpaired chromosome regions. Lethality *in trans* over many proliferative alleles define them as interactors. srn^{88} over proliferative alleles of *amanda*, *drache*, *efendi* (*efe*) or *Aus* are not viable. *efe* (92cM) is a proliferative gene that does not express a mutant phenotype in MBT; *efe* alleles are recessive. They exhibit weak interactive potential, as they complement, in part, each other. A number of P-element insertions are lethal over MBT and disrupt the somatic pairing process. Two P-element insertions of this kind were chosen for this study. They have non expressed *bellatrix* (btx)^{P} and *rigel* (rig)^{P} (Cooley *et al.*, 1988).

Within the embryo, the neurogenic ectoderm is fixed by ventral-laterally located cells. Within the segmented germ band, the neurogenic ectoderm becomes subdivided, the neuroblasts segregate and proliferate in a defined manner (Campos-Ortega 1993). Products of proneural genes and neurogenic genes, such as *Delta* (*Dl*), are involved in the determination process that includes the proliferation and differentiation of cells. *Notch* (*N*), *neuralized* (*neu*), *E*(*Spl*)-*C* and *gro* prevent neural hyperplasic growth. *N* is an embryonic tumor suppressor gene that acts through a lateral inhibition of neuroblasts (Gateff 1994). *Serrate* (*Ser*) is involved in the control of cell proliferation (Speicher *et al.*, 1994). Deficiencies roeST1 (84A6B1;84D4-9, tub^{-}), T32 (86E2-4;87B9-10, mali-), XTA1 (96A22-23B1;96D2-3, tld^{-}), X18E (96A17-20;96C1, tld^{-}), 8D06 (96E10-12;97A3-4, $yeti^{-}$ gro^{-}), R1 (96F2;96F12-14, $yeti^{-}$ gro^{-}), ro82b (96F11-14;97F3-11 $yeti^{-}$ gro^{-} Tl^{-} pll^{-} spz^{-} efe^{-}) and mbt^{P} (97F, efe^{-}) induce lethality over MBT. Stocks were obtained from E. Gundelfinger, Magdeburg (Df(3R)XTA1, Df(3R)X18E,

Df(3R)*Ser*$^{+82f24}$) and M. B. O'Connor, Irvine (*tld*+ transgene stocks 1366-67, 1366-68, Df(3R)*tld*$^{68-62}$ (Shimell *et al.*, 1991)). *spz*^{67}, *cact*, *dl*, *tub*, *pll*, *tld*, *neu*, *N*, *Dl*, and *Tl* mutants were obtained from C. Nüsslein-Volhard and I. Koch, Tübingen, SerRX106 (Thomas *et al.,* 1991) from U. Thomas, Magdeburg, *gro*^{E75} (Preiss *et al.*, 1988) and gro- deficiencies from A. Preiss, Hohenheim. All other stocks were obtained from the Bloomington Stock Center. The balancer chromosomes, deficiencies and markers are described (Lindsley and Zimm, 1992).

Brain whole mounts, ARD stain

The non-ligand binding structural subunit of nicotinic acetylcholine receptor, ARD, is expressed in the ventral ganglion and widely distributed in neuropiles of the optic lobes. The expression of this subunit is a marker for appropriate differentiation of the neuropiles. ARD like immunreactivity in larval brains was obtained according to Schuster *et al.* (1993). Parents were placed on fresh medium and shifted immediately to the restrictive temperature of 29ºC. The F1 generation was analyzed. Same size L3 larvae were dissected in Ringer`s solution. A hyperplasic brain (at least twice the volume of wild type brain) shows wild type segmentation of the optic lobes. The cells of the optic lobes are small, ingrowth of the tissue into the ventral ganglion is not observed. A brain tumor shows no signs of segmentation, ARD is not expressed in neuropiles. Brain tumor cells are small and large and do not adhere. Malignancy is defined as ingrowth of cells into the ventral ganglion.

Transformation rescue of tld^{MBT}

Two stocks containing pMBO1366 with recombinant *tld* $^{+}$ (Shimell *et al.*, 1991) on the first or the second chromosome were crossed with appropriate marker/balancer strains. The F1 was subsequently crossed with MBT or *tld*^{MBT} containing recombinants. The F2 generation containing pMBO1366 on the first or second chromosome and a TM6B,*Tb* balanced third chromosome of MBT, *tld*-1 or *tld*-2 was shifted to 29ºC. L3 larvae, homozygous for the third chromosome were analysed. As control, in parallel the same crosses were performed with a *w* marked first or a *Pm* second chromosome without pMBO1366.

Screening for neoplasm formation

The F1 generation of at least two independent crosses was analyzed for complementation, *i.e.,* the occurrence of eclosed hybrids. Adults were screened for growth abnormalities or neoplasm formation. In case of lethality, percentage and stage were determined. If brain tumor was suspected, L3 larvae were dissected in PBS and the brains were screened for brain tissue overgrowth. All crosses were maintained routinely at 20ºC. For crosses with the temperature sensitive lines MBT, MBT recombinants, *tld* ^{9D36}, *Dl* ^{6B}, *spz*^{67}, *Tl* ^{r632} and *Tl* ^{r26} virgins were collected and kept at 18ºC over night, prior to the addition of males and immediate shift to 29ºC.

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