Tumor-suppressor *lgl* in Drosophila: cell/organism and population aspects.

In the later sixties Gateff and Schneiderman (1969) provided the first clear evidence that inactivation of Drosophila tumor supressor gene *lgl* can directly lead to neoplastic transformation in vivo. This discovery opened a new field in studies in genetic basis of cancer (Gateff, 1978, 1994; Lorenzo, Mechler, Bryant 1999). More 50 Drosophila tumor supressor mutations have been described since that time. However, the *lethal (2) giant larvae*, or *lgl*, together with two similar tumor suppressors *dlg* and *scrib*, are of special interest for human cancer because these gene are needed for basic developmental processes, regulate cell polarity, tumor prevention.They are evolutionary conservative. The Lgl protein is described as a new type of tumor suppressor involved both in signal transduction and cell/tissue architecture (Lorenzo et al 1999; Humbert et al 2003).

Cell and developmental effects of *lgl* mutations.

The gene *lgl* is the most distant gene in the right arm of 2nd chromosome of *D. melanogaster*. It is located immediately after telomeric region.Thus, *lgl* deletions often delete neighboring telomeric sites (Mechler et al 1985; Golubovsky et al 2001, Mason et al 2003). The *lgl* encompasses 13.1 kb of DNA , includes 10 exons and produces 5.4 kb transcripts It codes a protein p127 of 1161 residues. Analysis of the spatial-temporal expression of the *lgl* transcripts and proteins has shown that they quite active during early embryogenesis.. At the later stages of embryogenesis *lgl* expression becomes restricted to tissues that do not show any phenotypic alteration in mutant animals. The Lgl protein exhibits two distinct intracellular localisation. It is prefentially found in the cytoplasm and in the inner plasma membrane to domains linking contiguous cells.

The p127 is a negative regulator of non-muscal cytosceleton myosin II. Mutations in *lgl* can specifically block cell migration processes during embryogenesis and oogenesis that dependent upon the contractile function of non-muscle myosin II protein. It is suggested that loss of Lgl product result in some tissues in increased cell motility and hence in metastasis. The *lgl* is the first example of monogenically controlled tumor, luckily rescued by means of P-element directed transgenosis.The development of lgl -/- animals having one additional insertional P-mediated lgl+ copy became normal.

The loss of *lgl* function leads to overgrowth of imaginal discs (benign tumor) and invasive malignant brain neuroblastoma, capable to unlimited cell growth or immortalisation. Fragments of *lgl*-/- brains transformed to the abdomen of the adult female host may be serially cultured in adults abdomen for many transfer generations. The product of *lgl* gene, p127 protein, is needed for both maternal and zygotic functions. It is also play a role in both germ-line and somatic cell development in oogenesis. The substitution of a serine by a phenylalanine at position 311 of Lgl protein produces the temperature sensitive ts-mutation. It has normal development under 22 degree C but causes drastic effects at 29 degree. Female become infertile , for mutation blocks egg chamber development at the onset of vitellogenesis. Thus, in addition to

tumor suppression, the *lgl* gene plays a critical function in oogenesis, regulating growth of the oocyte, follicle cell migration and architecture of palisadic epithelium (Lorenzo, Strand, Mechler 1999a,b).

Cell polarity, epithelial cell junctions and tumor

Loss of cell polarity and tissue architecture are typical for malignancy derived from epithelial tissues and are correlated with more aggressive and invasive cancers (Hanahan, Weinberg 2000; Humbert et al 2003). Cells in epithelial sheets are characterized by columnal or cuboidal shape, strong cell to cell adhesion and pronounced polarity (metaphor: as troop columns on the parade). Tumors of epithelial origin lose these features as they progress from benign growth to malignant carcinoma. The Lgl protein in close association with products of another two tumor supressor gene *dlg* and *scrib* regulates both epithelial structure and cell proliferation.

Epithelial cell are linkedby at least three type of multiprotein complexes: adherent junction, septate junction, and subapical complex. Adherent junctions form the zonula adherens, the structural belt that surrounds the apex. It includes cadherin and rwo kinds of catenin proteins. Lgl in complex with other two tumor-suppressor proteins Dlg and Scrib is involved in septate junction organization. The absence of human homologues of septate junction proteins is correalated to the more invasive types of gastric cancer Humbert et al 2003).

There is the molecular link between cell polarisation and tissue architecture and cell proliferation. In tissues where epithelial cells are integrated in a polarised cell monolayer, proliferation is prevented. But where polarity and cell linkages are lost, this change in cell shape may provide a signal to trigger proliferation. Many cell receptors are localised and activated at junctional complexes. Disruption of cell polarity due to mutations in genes lgl, dlg and scrib may disrupt receptor mediated signalling. Thus in Drosophila Dlg is associated with the EGF, the Epidermal Growth Factor receptor, involved in cell cycle control (review and discussion: Humbert et al 2003).

Recent finding showed another important association of septate junction complexes and oncogenic factors. The septate proteins are targeted by proteins of some oncogenic viruses including HTLV-1 and papilloma. This targeting leads to ubiquitinisation and subsequent proteasome-mediated degradation of the proteins, which constitute the septate junction Then viruses penetrate in the host cells (Lee et al 1997; Kijono, 1997; Gardiol et al 1999; Nakagawa et al 2000).

Evolutionary conservation and human lgl homologues

The product of *lgl* is evolutionary conservative. Its homologues have been found in diverse species, including yeast, C.elegans, mouse, human. There are two yeast homologues of *lgl –sop1* and *sop2*. The gene *sop1* affects salt tolerance. Cells deleted for SOP1 exhibited sensitivity to sodium NaCl stress but not to general osmotic stress. Double-mutant *sop1sop2* cells manifested increased sensitivity to Na(+) ions, partial

defects in cell polarity and actin and a cold-sensitive growth phenotype. Remarkably, these defects could be partially rescued by transgenosis of lgl+ Drosophila cDNA, indicating a functional relationship between the Sop protein and its novel function in cell homeostasis (Larsson et al 1998).

Humans have two Lgl homologue, Hugl-l and Llgl-2 . Both genes map at the chromosome 17, at the sites 17p11.2 and 17q24-25 where a cluster of potential cancer predisposition genes is observed (Strand et al 1995). As Lgl in Drosophila, human protein Hugl is a part of multiprotein cytosceleton network associated with non-muscle myosin II and a serine kinase. Recently It was found that Hugl-1 is lost in some human solid malignancies, supporting its role in humans as a tumor suppressor. Moreover, Hugl1 expression in homozygous Drosophila mutants lgl-/- is able to rescue larval lethality (Grifoni et al 2004). These data demonstrate that human Hugl-1 can act as a tumor suppressor in Drosophila. They highlight the usefulness of a Drosophila as a model system for investigation *in vivo* the mechanisms of cell polarity and cell proliferation in human cancers (Grifoni et al 2004).

Alleles of *lgl* are the most frequent 2nd chromosome lethals in wild populations

Due to permanent mutation process and diploidy a lot of hidden heterozygous abnormal alleles saturate the genepool of every species. Lethal alleles are regular part of the genepool, they may occur in hundred of autosomal loci . In natural Drosophila populations about 15-30% of each autosome carry one or more lethal mutation.

Long-term genetic monitoring showed that allelic content of the lethal genepool is drastically renewed every season of propagation. However, it turned out that against the backgroumd of constant allelic flow, distant populations keep polymorphism on lethal alleles of *lgl* tumor suppressor gene. The number of chromosomes carrying *lgl* lethal alleles appeared rather high. Thus, among 1210 2nd chromosomes containing lethals and extracted from distant populations of EuroAsia, Caucasus, Middle Asia and Far East 78 chromosomes carried alleles of *lgl* tumor suppressor (Golubovsky, 1978; 1980). Each wild fly in about 25-50 of them was heterozygous on a lethal *lgl* allele.

Molecular data unexpectedly showed that most of the *lgl*-natural lethals are deletions encompassing *lgl* gene and part of the adjacent telomeric region (Mechler et al 1985; Golubovsky et al 2001; Mason et al 2003). These findings raised the shocking possibility that the loss of function of *lgl* might be adaptive under some conditions in *lgl*-/+ heterozygotes. Indeed, we found that under optimal laboratory temperature conditions and 25 degree C most *lgl*-/+ animals had decreased viability in comparison with control genotypes. However *lgl*-/+ animals had revealed selective advantage under their development both in low (18 degree C) and higher (29 degree C) temperatures (Sokolova, Golubovsky 1979a,b). The suggestion was made that in heterozygotes one normal dosage of tumor supressor might promote to stress resistance.

Resistence of *lgl*-/+ animals to Picornavirus infection.

Each animal species in nature is under strict selective control of viral agents. Presence of viruses and resistance to viruses in *Drosophila* populations are component of the general polymorphism. Drosophila C virus (DCV) is frequent in most flies in laboratory stocks and in many natural populations of *D. melanogaster* (Brun and Plus, 1980; Thomas-Orillard 1995; Gravot et al 2000). This DCV virus is horizontally transmitted by injection of contaminated medium (feces or cadavers) or by contact between flies. It is similar to the Picornaviridae family , members of vertebrate enteroviruses. DCV drastically increases mortality rate.This virus is pathogenic after injection and is easy to bioassay on this host. There was tested the resistance to DCV of flies *lgl*-/+, heterozygous on *lgl*. The resistance was measured as a % of mortality observed after injection of the standard DCV suspension into 50 imagos. In normal control stock 90-100% of injected flies died during 2-3 days. The resistance various *lgl*-/+ ranged from weak to almost complete resistance (Plus, Golubovsky 1980). The percent of mortality was rather independent from the age of the injected flies (varied from young 1 day flies to the old 12 day).

We tested 15 *lgl* alleles extracted from distant populations ofCaucasus, Middle Asia and Far East. Most of lgl*/+ genotypes exhibited increased resistant. For instance, in condition when 100% of control +/+ flies died after injection through 2-3 days, mortality of *lgl432*/+ heterozygotes, which carried *lgl432* allele from Erevan population (Armenia) reached only 17-26%. Maternal component in virus resistance was observed in three series of reciprocal crosses:

Table 1
Maternal presence of *lgl* tumor suppressor increases virus resistance of F1 progeny

Crosses	% mortality in the F1 progeny		
	expr.1	exper. 2	exper.3
♀ ***lgl432* / Cy** X ♂ **+/+**	54 ± 5	42 ± 5	25 ± 4.5
♀ **+/+** X ♂ ***lgl432* /** Cy	80 ± 4	60 ± 3	33 ± 4

The F1 flies lgl/+ and Cy/+ expressed intermediate resistance. But maternal origin of tumor suppressor allele *lgl432* provided increased resistance in F1 progeny.Recent molecular findings that Lgl protein has cytoplasmic localization and regulates epithelial septate junctions targeted by oncogenic viruses gives a rationale of both virus resistance and its cytoplasmic maternal determination (Humber et al 2003).

These data and recent studies between Drosophila C virus and its host (Thomas-Orillard et al 1995) might be associated with studies on *lgl* cell and molecular biology. It is pertinent to mention here the maternal presence of Lgl protein, its cytoplasmic and cytosceleton localization and its disposition in the septate junctions targeted by viral proteins. If normal Lgl protein is the target for pathogenic virus attachment, then mutant state of *lgl* tumor suppressor or diminishing of its dosage in *lgl*-/+ animals might promote to host virus resistance .