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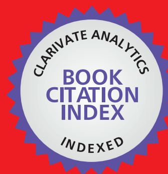

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11

The Tumor Suppressor Function of LGI1

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1. Introduction

The Leucine rich Glioma Inactivated 1 (LGI1) gene has been related to different pathologies such as epilepsy and cancer. The aim of this chapter is to present the evidences on the tumor suppressor function of LGI1. Initially LGI1 gene was identified at the brake point of a balanced chromosome translocation in a glioblastoma cell line (Chernova et al., 1998). The observation that this rearrangement abolished expression of LGI1 and that expression of LGI1 is absent in most high-grade gliomas lead to the hypothesis that it may function as tumor suppressor.

1.1 Evidences for the tumor suppressor function

The tumor suppressor function was sustained by the re-expression of LGI1 in glioblastoma cells, lacking endogenous expression, LGI1 inhibited cell proliferation and migration (Kunapuli et al., 2003). The role of tumor suppressor was further supported by the identification of several mutations in LGI1 gene associated with gliomas (Barnholtz-Sloan et al., 2008). The tumor suppressor function of LGI1 was supported also by the results of LGI1 expression in neuroblastoma and adenocarcinoma cells, in which endogenous expression is very low or absent. The expression of LGI1 in these cancer cells not only produced a potent inhibition of cell proliferation, as in the case of glioblastoma cells, but also impaired cell survival (Gabellini et al., 2006; Gabellini & Masola, 2009). Moreover a study on malignant esophageal tumors showed a significant downregulation of LGI1 (Peng et al. 2008).

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1.2 Induction of apoptosis

Investigations on the mechanism of spontaneous cell death caused by the expression of LGI1 in neuroblastoma cells showed the induction of intrinsic apoptosis, produced by an unbalance of important regulator of mitochondrial membrane permeability, namely of the anti-apoptotic B-cell lymphoma 2 gene (BCL2) and of the pro-apoptotic B-cell lymphoma 2-associated X protein gene (BAX). These studies also pointed out a possible involvement of LGI1 in the negative regulation of essential signaling pathways supporting cell proliferation and survival.

1.3 Regulation of signaling pathways

Consistently with the evidence that LGI1 triggered a mitochondrial pathway of apoptosis, the phosphoinositide 3-kinase (PI3K/AKT) pathway, important regulator of BCL proteins,

was blocked by the expression of LGI1 (Gabellini & Masola, 2008). In the case of glioblastoma cells the inhibition of cell proliferation and migration exerted by LGI1 was determined by the inhibition of the extracellular signal-regulated kinase subgroup of the mitogen-activated protein kinases (ERK/MAPK pathway), resulting in the downregulation of matrix metalloproteinase (MMP) genes (Kunapuli et al., 2004). These observations pointed out the possibility that LGI1 might impair trophic signaling because the PI3K/AKT and the ERK/MAPK pathways are activated downstream of tyrosine kinase receptors (RTK) in response to growth factors. This possibility is supported by the homology of the leucine-rich repeats flanked by cysteine rich regions (LRR domains) present in the N-terminal region of LGI1 with the region of mammalian Trk receptors involved in neurotrophin binding (Kobe and Deisenhofer 1994; Kalachikov et al. 2002), however, evidences for LGI1 interaction with RTK receptors are lacking presently.

1.4 Suggested functions

The domain architecture of LGI1 protein includes a leucine-rich repeats flanked by cysteine rich regions (LRR domains) and a seven-bladed beta-propeller domain located in the C-terminal half of the protein (Scheel et al., 2002; Staub et al., 2002). Both LGI1 domains appear to be involved in protein-protein interaction and investigation in this direction revealed important aspects on the function of LGI1. LGI1 was shown to be a ligand of the trans-membrane receptor ADAM metallopeptidase 22 (ADAM22), this receptor is highly expressed in brain, but precise information on its function and signaling are limited at the moment (Fukata et al., 2006). It was also shown that LGI1 forms complexes with voltage gated potassium channels (Kv1.1), which are critical regulators of synaptic transmission (Schulte et al., 2006). Their activity is well characterized and is supposed to control several functions in a variety of cell types. In particular, it was shown that binding of LGI1 prevented channel inactivation by Kv1β and that LGI1 mutations associated with Autosomal Dominant Lateral Temporal Epilepsy (ADLTE) produced a modification of the inactivation properties of the channels that might promote epileptic activity.

1.5 Regulation of potassium channels and apoptosis

The role of LGI1 in the regulation of (Kv1.1) is also in relation with the tumor suppressor function of LGI1, since these channels are important regulator of cell survival. In particular the blockage of channel inactivation determined by binding of LGI1 to regulatory subunit Kv1β, results in an increase of K^+ permeability, which has been shown to enhance apoptosis (Yu et al., 1997). Thus in addition to the inhibition of pro-survival pathways such as the PIK/AKT, LGI1 might induce apoptosis by interfering with Kv channels inactivation. Thus the tumor suppressor function of LGI1 might be related to its pro-apoptotic activity, and the dowregulation or inactivation of LGI1 gene often observed in glioblastoma and other cancer cells might be necessary to suppress apoptosis.

2. LGI1 gene

The first isolation LGI1 gene was achieved by positional cloning of the region rearranged by a balanced translocation t(10;19)(q24;q13) in a glioblastoma cell line (Chernova et al., 1998). LGI1 gene covers 40,274 bp of chromosome 10 in the region q23.33. It consists of 8 exons (Fig. 1). The 5′ UTR preceding the codon for the start methione is located in exon 1 sequence.

Exon 8 includes two stop codons alternatively utilized in two spliced isoforms carrying 3'UTRs of different legth. A minimal promoter region is placed immediately upstream of the Transcriptional Start Site (Sommerville et al., 2000).

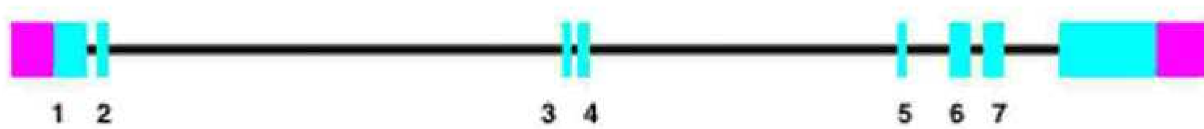

Fig. 1. Organization of LGI1 gene. The coding regions of exons 1-8 are depicted in blue, the untranslated regions (UTR) in pink, introns are shown by black bars.

Processing of the primary LGI1 transcript produces two main isoforms showing tissue specific expression (Chernova et al., 1998; Morante-Redolat et al., 2002). The full length LGI1 mRNA is composed of 2290 bases and corresponds to isoform 1 (RefSeq: NM_005097). Alternative splicing produces the small isoform 2 consisting of 1456 bases including a shorter exon 8.

3. LGI1 protein

The full length LGI1 protein comprises 557 amino acids (64 KDa) and corresponds to isoform 1 (UniProtKB/Swiss-Prot ID: 095970). Isoform 2 is composed of 291 amino acids (Isoform ID: O95970-2); it includes an amino acid sequence variation of residues 280-291 and lacks the C-terminal amino acids stretch (292-557). The domain composition of the full-length protein is shown in Fig.2.

Fig. 2. LGI1 protein (isoform 1).

The N-terminal sequence of LGI1 precursor starts with a cleavable signal peptide (SP, AA: 1-34); the predicted molecular size of the mature protein is 60 KDa. A hydrophobic stretch (amino acids 288-309) was pointed as a possible trans-membrane segment, suggesting that LGI1 might be anchored to the membrane (Chernova et al., 1998). The domain composition of the N-terminal half of LGI1 protein includes three leucine-rich repeats (LRR; AA: 90-113, 114-137 and 138-161) flanked by cysteine-rich leucine-rich repeats (N-terminal LRRNT AA: 41-71 and C-terminal LRRCT AA: 173-222). The C-terminal portion of LGI1 is characterized by the presence of 7 repeats, termed Epilepsy Associated Repeats (EAR; Scheel et al., 2002) or Epitempin (EPTP; Staub et al., 2002) denoted EAR/EPTP repeats (AA: 224-267, 270-313, 316-364, 365-415, 418-462, 463-506, and 509-552).

3.1 Homology

LGI1 is part of a protein family comprising four members (LGI1, LGI2, LGI3, and LGI4) with high sequence homology. The Leucine-Rich repeats flanked by Leucine-rich cysteine-rich domains of LGI protein share the highest identities with that present in cell adhesion proteins and receptors. The structure of LRR domains of LGI1 protein is related to that of

Drosophila protein slit, involved in the development central nervous system for its role in neuronal growth-cone guidance and migration (Battye et al., 2001). The LRR domain of LGI1 is related also to that identified in the extra-cellular region of mammalian Trk receptors, specifically in the region binding neurotrophin (Kobe and Deisenhofer 1994; Kalachikov et al. 2002), suggesting that LGI1 might interfere with ligand binding of Trk receptors. The structure of the EAR/EPTP domain is similar to the ß-propeller domains found in several intracellular and secreted proteins. It was identified by homology among the four paralogous genes LGI1-4, and with the large G protein coupled receptor MASS1/VLGR1, which is mutated in a murine form of audiogenic epilepsy (McMillan et al. 2002). The presence of a similar domain in the integrin α chain receptors (Xiong et al. 2001; Springer 2002) suggests a possible role in cell adhesion. The role suggested for both LRR and EAR/EPTP domains is to provide an interface for protein–protein interaction.

3.2 Cellular localization

The presence of a cleavable signal peptide suggests that LGI1 protein might be addressed to endoplasmic reticulum and localized in the membrane or secreted. Although the presence of the hydrophobic segment initially suggested the it might be associated with the membrane, subsequent studies based on heterologous transfection of the full-length LGI1 cDNA in 293T cells showed the LGI1 protein is secreted (Senechal et al., 2005). However, it was shown also that the short isoform, probably corresponding to isoform 2, is retained within the cell (Sirerol-Piquer et al., 2006).

4. Expression

Northern blot analysis with human tissues detected LGI1 mRNA only in brain and skeletal muscle; two mRNA species were identified (2.4 kb and 1.6 kb) suggesting expression of at least two isoforms. RT-PCR analysis detected LGI1 mRNA also in heart, liver and pancreas although at lower levels (Chernova et al., 1998). High resolution *in situ* hybridization on adult mice brain suggested that LGI1 is primarily expressed in neurons located in specific brain regions. Strong labeling was observed in granule cell layer of the dentate gyrus and in the CA3-CA1 pyramidal cell layers of the hippocampus; weak hybridization signals were observed in the neocortex and limbic region. LGI1 expression is consistently more elevated in the regions implicated in ADLTE (Kalachikov et al., 2002; Herranz-Pérez et al., 2010; Senechal et al., 2005). Subsequent experiments with transgenic mice carrying the GFP reporter gene under the control of regulatory regions of LGI1 gene supported high levels in neurons and revealed LGI1 expression also in glial cells. Strong expression was also identified in the prostate gland, tubules and sympathetic ganglia of the kidney, as well in several glandular tissues as in skin sebaceous glands, in pancreatic islets of Langerhans, endometrium, ovary and testes (Head et al., 2007). The expression profile of LGI1 determined by DNA microarrays, SAGE and EST number (reported by ECgene and Gene Expression Atlas) is in agreement the reported data but extended the expression to pituitary gland, peripheral nerve, B-lymphocytes, eye, lung.

4.1 Expression in tumors

Immunohistochemistry and mRNA analysis showed that LGI1 levels are very low or completely absent in most tumor tissues and in several cancer cell lines including,

glioblastoma, neuroblastoma, melanoma and breast, furthermore the decrease of LGI1 expression shows a significant correlation with the grade of malignancy of astrocytic gliomas (Rossi et al., 2005; Besleaga et al., 2003). DNA microarrays, SAGE and EST number suggest low or absent expression in the majority of tumor tissues (see Gabellini, 2007).

5. Glioma

A number of germ line and somatic mutations involved in the initiation and progression of human gliomas have been identified. The most frequent mutations are associated with the tumor suppressor protein p53, which induces cell cycle arrest and apoptosis, with the negative regulator of the cell cycle RB1 and with oncogenic components of the PI3K pathway (Parson et al., 2008; Barnholtz-Sloan et al., 2008). Typical chromosomal alterations occurring in malignant gliomas consist of an increased chromosome 7 copy number, resulting in the amplification of the proto-oncogene for the tyrosine kinase receptor (EGFR; Henn et al. 1986). Other recurrent events are related to the loss of one copy of chromosome 10 and often also the second copy especially of the region 10q23-q26. Loss of heterozygosity in this region is observed in about 80% of glioblastoma multiforme tumors, suggesting the presence of multiple tumor suppressor genes (Karlbom et al., 1993). Indeed this region of chromosome 10 includes the Phosphatase and Tensin Homolog gene (PTEN; 10q23), important tumor suppressor with pro-apoptotic function antagonizing the PI3K pathway (Blanco-Aparicio et al., 2007); the Deleted in Malignant Brain Tumors 1 gene (DMBT1; 10q25-q26; Mollenhauer et al., 1997) and the Leucine-rich, Glioma-Inactivated-1 gene (LGI1; 10q24).

5.1 Alterations of LGI1 gene

The interruption of LGI1 gene by a balanced chromosomal translocation t(10;19)(q24;q13) in the T98G glioma cell line results in the complete loss of LGI1 expression (Chernova et al., 1998). Similar alterations occur also in the A172 glioma cell line and glioblastoma tumors. However, fluorescence *in situ* hybridization study showed that alterations of LGI1 gene are absent in some glioblastoma cell lines (Krex et al., 2002).

5.1.1 Point mutations

The occurrence of point mutations in LGI1 gene has been shown by DNA sequence analysis of brain tumors versus normal brain samples (Barnholtz-Sloan et al., 2008). This study revealed several missense and nonsense tumor-specific mutations, mostly located in the region encoding the N-terminal portion of LGI1 protein including LRR domains and flanking cystein-rich regions (Fig.2). Heterozygous mutations in the human LGI1 gene have been associated with Autosomal Dominant Lateral Temporal Epilepsy (ADLTE), a disorder characterized by partial seizures typically preceded by auditory symptoms (Kalachikov et al., 2002; Morante-Redolat et al., 2002; Michelucci et al., 2009). However, the incidence of malignant brain tumors appears to be unvaried in ADLTE families carrying LGI1 mutations (Brodtkorb et al., 2003), supporting the notion that multiple genetic alterations are necessary for malignant transformation.

5.1.2 Downregulation

Also the downregulation of LGI1 gene expression might be important for malignant transformation. A gradual decline in the expression of LGI1 gene accompanies the

malignant progression of gliomas (Besleaga et al., 2003). Northern blot, RT-PCR and immunohystochemistry of various brain tumor samples revealed that in the majority of low-grade tumor such as pilocytic astrocytomas and astrocytomas, defined by the World Health Organization (WHO) grade II, the expression of LGI1 was similar to that of normal brain; in anaplastic astrocytomas (WHO grade III) the levels of LGI1 was consistently lower than in normal tissue, whereas LGI1 expression was absent in 80% of glioblastoma multiforme samples (WHO grade IV). Genetic alterations of the promoter region and epigenetic factors such as methylation of the control regions might determine the differences of LGI1 expression.

5.1.3 Epigenetc factors

The results of tiling array assays (MAUD assay) for the identification of methylated or unmethylated DNA sequences, which can accommodate the entire mouse brain transcriptome, revealed that LGI1 is one of the genes presenting dual methylation pattern of the regulatory regions determining monoallelic expression (Wang et al., 2010). Although mutations of tumor suppressor genes are usually inherited as recessive traits, because both alleles must be inactivated to eliminate gene function, in case of monoallelic expression heterozygous mutations of the ummethylated allele might be sufficient to abolish expression. This explains the dominant inheritance of ADLTE in the presence of heterozygous mutations, furthermore the process of random inactivation of one of the two alleles by methylation accounts for the characteristic incomplete penetrance of this disease.

5.2 Re-expression

The first study supporting the role of tumor suppressor for LGI1 was based on the results of forced expression of LGI1 in glioblastoma cells (Kunapuli et al., 2003). Cells stably expressing LGI1 were obtained by selection of neomycin G418 resistance brought by the plasmid vector in which LGI1 was incorporated. In this study the LGI1 cDNA used for transfection was modified in the region encoding the C-terminal end of LGI1 by the addition of a flag epitope, to allow the detection of the expressed protein in the absence of specific antibodies, which were in preparation. The LGI1 cDNA construct was transfected in the glioblastoma multiforme derived cell line T98G characterized by a chromosomal rearrangement interrupting LGI1 gene t(10;19)(q24;q13) and in the A172 glioma cell lines carrying a reciprocal chromosomal translocation t(10;19)(q26;q13.4), which break up the WDR11 gene (10q25-26) encoding a member of the WD repeat protein family. Both T98G and A172cells lack endogenous LGI1 expression. In addition the U87 cells derived from an astrocitoma and naturally expressing LGI1 were transfected with the same LGI1 construct. T98G and A172 yielded only few clones of stably transfected cells, suggesting that expression of LGI1 affected cell vitality. In contrast cell clones derived from U87 cells grew normally. Variable levels of LGI1 mRNA and protein were detected among the different cell clones, however expression was more elevated in U87 than in T98G and A172 derived cells.

5.2.1 Decline of malignant features

A quantitative cell proliferation assay indicated that the proliferation rate of T98G and A172 cells stably transfected with LGI1 was reduced in comparison with control cells transfected

with empty vector; inhibition of cell growth was proportional with the levels of LGI1 expression. In contrast the proliferation rate of U87 derived cells overexpressing LGI1 was similar to that of control cells, suggesting that selection for increased proliferation during malignant transformation operated independently of LGI1 in U87 cells. Also the invasion ability, another feature associated with malignancy, was analyzed in this study. The capability of cells to pass through pores of 8 μm of a coated membrane was evaluated. The majority of the T98G and A172 cell clones stably expressing LGI1 had completely lost their original migration ability; whereas U87 derived cell clones retained the capacity of passage, which was similar to that of control cells. A third characteristic of malignant cells is their capacity to grow without being anchored to a substrate. The ability of cells to grow in an anchorage independent manner was evaluated by counting the number of colonies formed on soft agar. The ability to grow on soft agar was strongly impaired in cell clones expressing LGI1 in comparison with cell transfected with empty vector or with parental T98G and A172 cells whereas the number of colonies formed by U87 cells expressing LGI1 was similar to that of control cells. The results of this study showed that cell proliferation was reduced (≥40%), invasion ability and anchorage-independent growth were almost abolished when LGI1 was re-expressed in glioblastoma cells lacking endogenous LGI1 expression. These results provide a possible explanation for the frequent occurrence of chromosome 10 rearrangements during the transition from low to high-grade gliomas, which might reflect the need of eliminating the tumor suppressor function of LGI1 to allow tumor progression.

6. Neuroblastoma

The function of LGI1 was studied also in a neuronal cell model represented by human neuroblastoma cells. These embryonic cancer cells are pluripotent precursors of the sympathoadrenal nervous system cells deriving from the neuronal crest (see Schwab et al., 2003). The typical alterations of chromoseme 10 observed in glioblastomas are not present in neuroblastoma; instead loss of the chromosome 1p region is frequent in neuroblastomas, as well as the amplification of the proto-oncogene MYCN and alterations of neurotrophin receptors levels (see Schwab, 2004). However, the results of RT-PCR analysis with a series of neuroblastoma cell lines suggested that LGI1 expression was either absent or very weak in the vast majority of the cases, suggesting that LGI1 gene is often downregulated in neuroblastoma cells as well (Rossi et al., 2005). Our results of RT-PCR analysis with two human neuroblastoma cell lines (SH-SY5Y and SK-NBE) employed for transfection experiments described below showed negligible levels of LGI1, especially when compared with expression in human whole brain, which was estimated about 30-fold greater.

6.1 Over-expression in neuroblastoma cells

Human neuroblastoma cells SH-SY5Y and SK-N-BE were transfected with the full-length LGI1 cDNA inserted in the expression vector pcDNA3 and with the empty vector as control (Gabellini et al., 2006). Neomycin G418 selection was performed to obtain stably transfected cell clones. During the procedure it became apparent that cells transfected with LGI1 grew more slowly than control cells. In addition only few cell clones stably transfected with LGI1 came across selection, whereas transfection with pcDNA3 generated a greater number of cell clones, suggesting that expression of LGI1 affected cell survival. A semiquantitative RT-PCR

analysis of LGI1 mRNA on cell clones transfected with LGI1 was performed to evaluate the levels of LGI1 expression, a segment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified in parallel reactions to evaluate the relative mRNA content of the samples (Fig. 3).

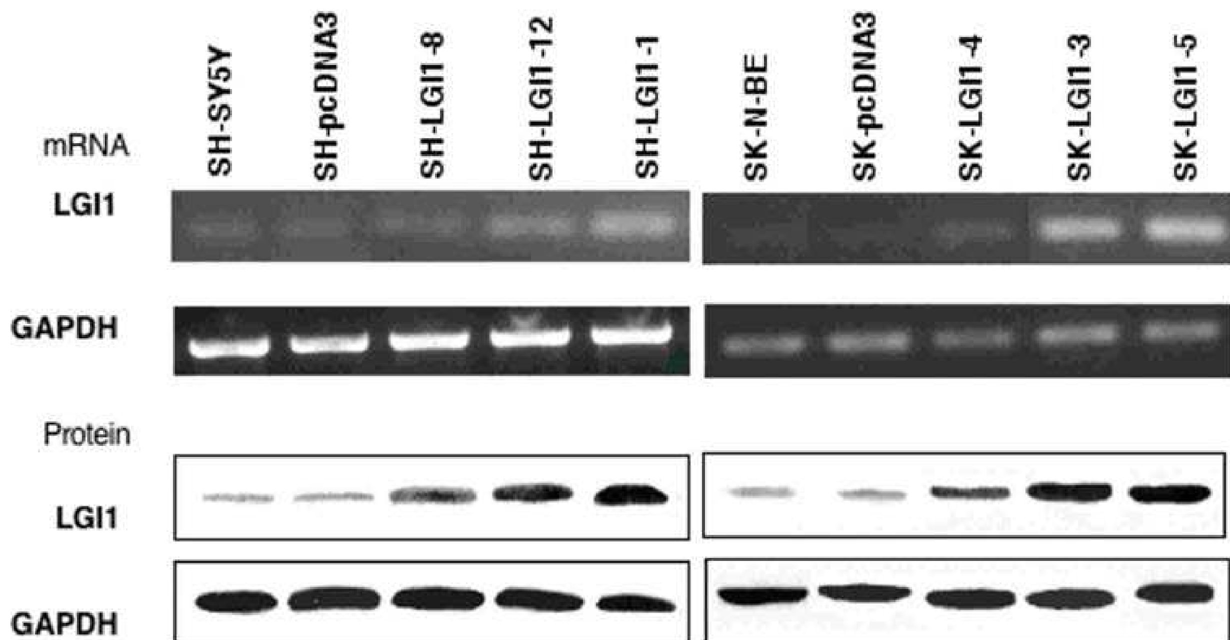

Fig. 3. Evaluation of LGI1 mRNA and protein in the neurobalstoma cells

Three cell clones derived from SH-SY5Y cells, designated SH-LGI1-8, SH-LGI1-12, and SH-LGI1-1, showed LGI1 mRNA levels slightly higher than original or pcDNA3-transfected cells, the latter showed barely detectable levels of LGI1 mRNA. The densitometry values of ethidium bromide stained PCR products were normalized to the corresponding values of GAPDH. The results showed on average 5-, 10- and 20-fold, increase in cell clones SH-LGI1-8, -12, -1, respectively. The levels of LGI1 mRNA in three cell clones SK-LGI1-3, -4, -5, derived from SK-NB-E were 4-, 6- and 8-fold greater than those estimates in control cells. The evaluation of LGI1 protein was performed by Western blot analysis of whole cells using an antibody directed to the N-terminal peptide of LGI1 (Fig. 3). Blots were re-probed with antibodies to GAPDH to normalize the protein content of the samples. The normalized densitometry values of the LGI1 protein (60 KDa) were in good agreement with the mRNA data. Because it was shown that LGI1 protein was secreted in the medium of transfected 293T cells (Senechal et al., 2005), the conditioned medium of neuroblastoma cell clones was concentrated and analyzed by Western blotting, nevertheless LGI1 protein was detected only in the cell lysates. It is possible that in neuroblastoma cells LGI1 protein might be anchored to the extra-cellular site of the plasma membrane, in agreement with the presence of a signal peptide and of a putative trans-membrane segment emerged from its primary structure, and with its immune-localization in secretory vesicle (Morante-Redolat et al. 2002).

6.1.1 Inhibition of cell proliferation

The proliferation rate of neuroblastoma cells over-expressing LGI1 was measured by the BrDU incorporation two days following plating (250 cells/mm^2). The results showed a

significant decrease of cell proliferation in all LGI1-transfected cells in comparison with original neuroblastoma cells or with pcDNA3 transfected cells, which showed similar rates. The proliferation of SH-LGI1-8, SH-LGI1-12 and SH-LGI1-1 cell clones was 40%, 70% and 90%, lower than that of control cells, respectively.

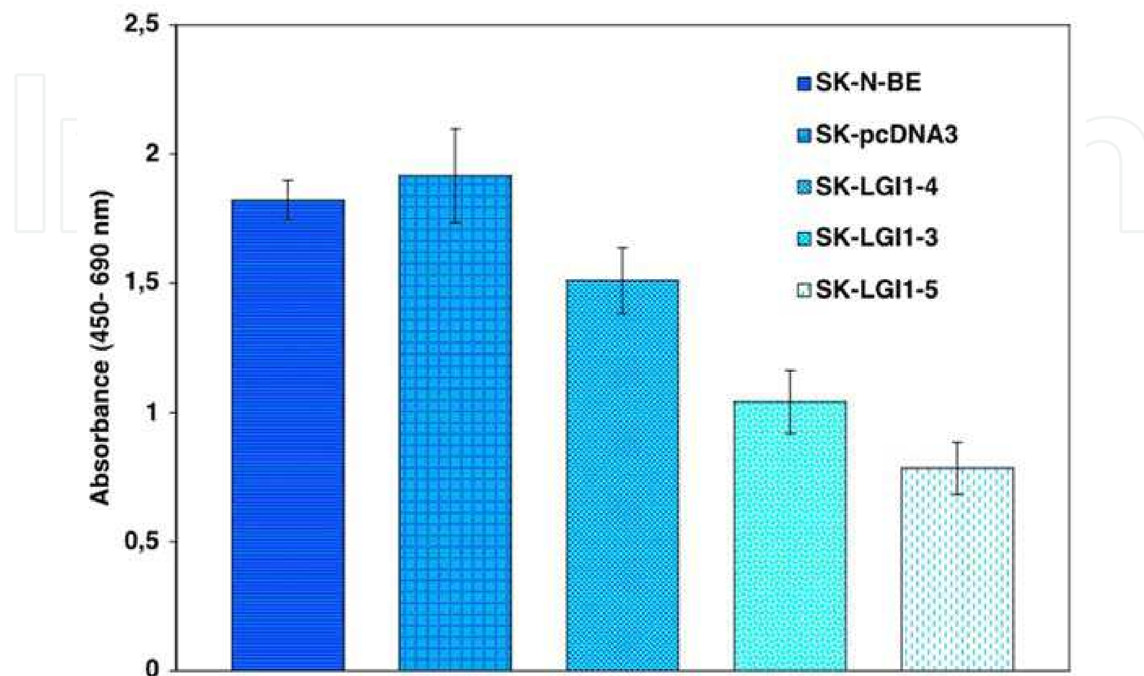

Fig. 4. Decline of cell proliferation

Similarly, the proliferation of LGI1 cell clones derived from SK-N-BE was consistently inhibited when compared to that of control cells. The proliferation rate of SK-LGI1-4, SK-LGI1-3 and SK-LGI1-5 was decreased on average by 20%, 50% and 60%, respectively (Fig. 4). The proliferation rates of SK-LGI1 cell clones and that of pcDNA3 were significantly different from that of control pcDNA3 cells or parental cells (mean ±SD; n=9-12; Student's t test $p<0{,}0004$). The study revealed a striking correlation between the extent of growth inhibition and the level of LGI1 expression, similarly to what occurred when LGI1 was re-expressed in glioblastoma cells lacking endogenous expression (Section 5.2).

6.1.2 Impaired survival

The viability of SH-SY5Y cell clones was assessed by a cytotoxicity assay based on tetrazolium salt (WST-8). The assays measures dehydrogenase activity of living cells, thus giving an indication of both cell proliferation and cytotoxicity. The results showed that the number of viable SH-LGI1 cells was consistently lower than that of control cells. Four days after plating (250 cells/mm^2) viability of SH-LGI1-8, -12 and -1 cell was 29%, 46% and 60% lower than that of control cells, respectively. In line with the results of BrDU incorporation, this assay showed a direct relation between the levels of LGI1 expression and the decrement of cell viability. The cytotoxic effect of LGI1 became more pronounced when cells were plated at high density (1000/mm^2). In these conditions death of LGI1 cells became morphologically evident already after the second day of culture, while after four days the majority LGI1 cells were dead. In contrast, only a relatively modest decrease of cell viability was measured with control cells transfected with pcDNA3 or parental SH-SY5Y cells,

indicating a strong cyotototoxic effect of LGI1 (Gabellini et al., 2006). The lactate dehydrogenase assay (LDH) was also employed to measure effective cell death. The fraction of LDH released in the medium as a consequence of cell death was calculated as percentage of the total LDH activity. The assay was performed 2- and 3- days following plating (1000/mm²), at these time points the WST8 assay indicated extensive death of LGI1 cells. In accord, the results of LDH assays showed that the percentage of cytoxicity of all LGI1 cell clones was substantially greater than that of control cells. The percentage of SH-LGI1-8 and SH-LGI1-1 cell death was considerably greater than that of pcDNA3 or parental cells (Fig. 5; mean ±SD, n=12-20; Student's t test p<0,001). Significant differences between the percentage of cytoxicity of SK-LGI1 and SK-pcDNA3 cells were also detected. Cell death increased in parallel with the levels of LGI1 expression suggesting that the elevated levels of LGI1 affected cell survival. This observation might explain the marked downregulation of LGI1 gene expression observed in neuroblastoma cells.

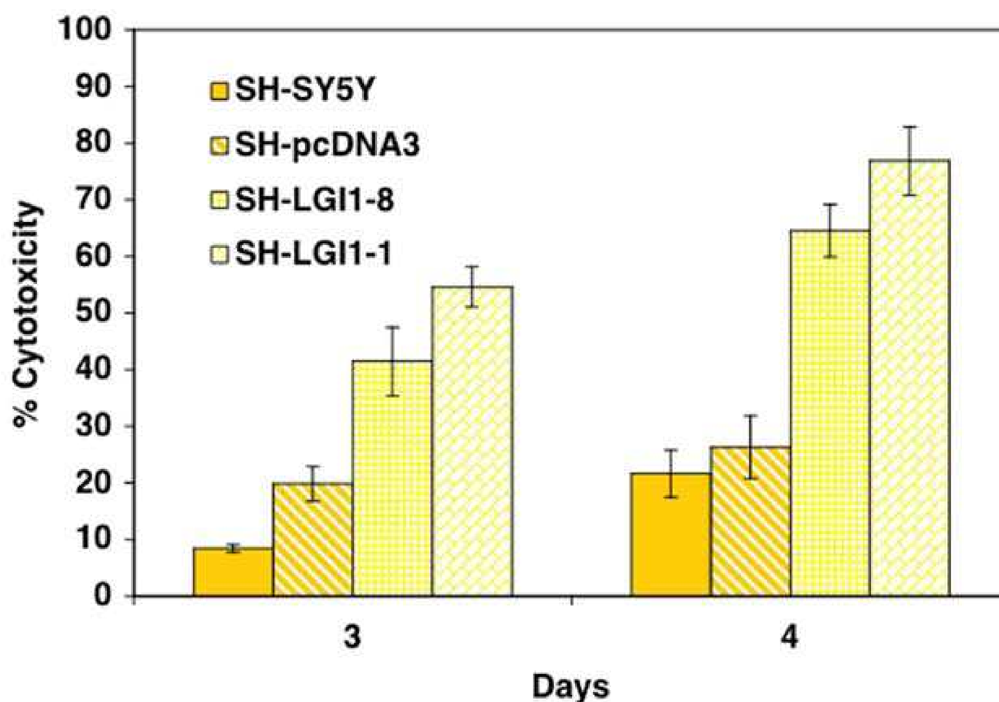

Fig. 5. Increased cell death.

6.1.3 Glioblastoma cell death

Although in the case of glioblastoma cells a quantification of cell death was not performed, it was observed that T98G and A172 stably transfected with LGI1 produced only few cell clones (Kunapuli et al., 2003). Since the levels of LGI1 expression measured in both stably transfected glioblastoma and neuroblastoma cell clones were relatively low, it might be suggested that high levels of LGI1 are not be compatible with the existence of both types of cancer cells.

6.2 Apoptosis

The mode of neuroblastoma cell death induced by the elevation of LGI1 levels was investigated. The evaluation of several apoptotic markers such as activation of caspase-3/7,

alteration of the BCL-2 to BAX ratio, release of cytochrome c and AIF from mitochondria, oligonucleosomal DNA fragmentation, phosphatydil serine (PS) exposure and nuclear morphology (Gabellini et. al., 2006). The data reported in the following sections suggests that LGI1 impairs survival of neuroblastoma cells by inducing intrinsic apoptosis. Although inhibition of cell proliferation similarly occurs in glioblastoma and neuroblastoma cells, up to now induction of apoptosis in glioblastoma cells have not been not reported. The results of this study strengthen the tumor suppressor function of LGI1 and outline the possibility that the downregulation of LGI1 gene expression observed in several cancer cells might be necessary to suppress apoptosis.

6.2.1 Activation of caspases–3 -7

The proteolytic activity of caspase-3 and -7, important effectors of apoptosis in mammalian cells (see Hengartner, 2000), was determined by the relative fluorescence (RFLU) emitted by the specific substrate (Z-DEVD-R110) upon cleavage. The RFLU values of SH-LGI1 cells, control SH-SY5Y and SH-pcDNA3 cells were determined each 24 hrs during a period of 4 days after plating 250, 500 or 1000 cells/mm^2. The average numbers of living cells determined in replicated samples by cytotoxicity assays was used to correct each RFLU value, with the aim to normalize variations of basal caspase activity resulting from variable cell proliferation rates. The caspase-3/7 activity of all cell types increased progressively during the time course at each cell density, however, the RFLU values of all LGI1 cells were significantly higher than those of pcDNA3 cells or original SH-SY5Y cells, (mean ±SD, n=12; Student's t p< 0,0001).

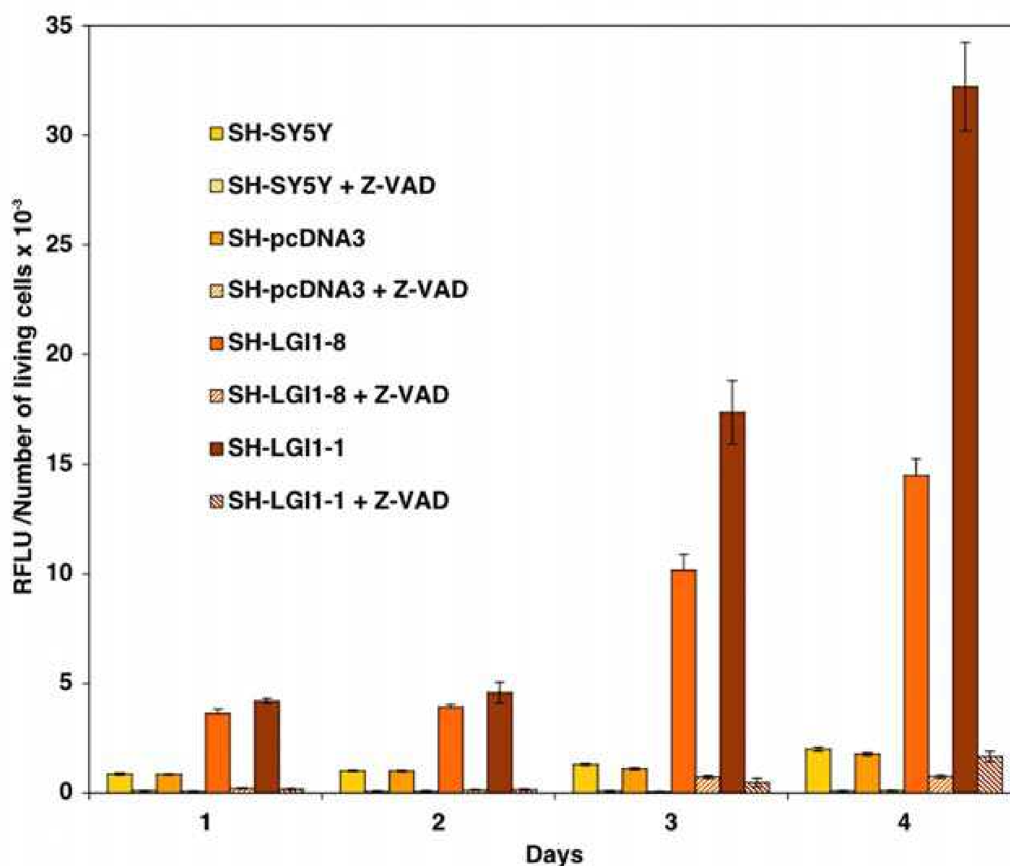

Fig. 6. Enhancement of caspase –3 –7 activity and inhibition by Z-VAD-FMK.

In the conditions of high cell density (1000 cells/mm^2), which showed the greatest cytotoxicity (Fig. 5), caspase-3 -7 activity was on average 4 - 5 fold greater in SH-LGI1 cells than in control cells during the initial two days; and increased up to 10- to 20-fold during the following 2 days in coincidence with the appearance of extensive cell death (Fig. 6). The broad-range caspase inhibitor Z-VAD-FMK blocked caspases -3 -7 in all cell types (~ 90%). The activity of caspase -3 -7 correlated with the extent of cytotoxicity and with the levels of LGI1 expression, suggesting that increased LGI1 levels triggered apoptosis of neuroblastoma cells.

6.2.2 Intrinsic apoptosis

The involvement of caspases linked to death receptor stimulation in the initiation of the apoptotic stimulus was investigated by measuring cytotoxicity and cell proliferation in the presence of the broad caspase inhibitor Z-VAD-FMK. The percentages of cytotoxicity of SH-LGI1 cells were similar to those determined in the absence of the inhibitor, whereas SK-LGI1 cells showed a small decrease. Thus the inhibition of proteases by Z-VAD-FMK substantially failed to prevent cell death. This suggested that apoptosis of neuroblastoma cells might be initiated in the absence of caspase activation, thus involvement of the extrinsic pathway of apoptosis linked to caspase-8 activation by death receptors is unlikely. These evidences suggest that apoptosis of LGI1 cells most likely involves the activation of the intrinsic mitochondrial pathway of apoptosis.

6.2.3 Decreased ratio of BCL-2 to BAX

The relative proportion of the anti-apoptotic BCL2 and of the pro-apoptotic BAX gene products is crucial to control apoptosis. These members of the BCL protein family play an important role in the regulation of mitochondrial outer membrane permeability determining the release of death factors that initiate intrinsic apoptosis (see Reed, 1998; Cory and Adams, 2002). Beside the release of cytochrome c, which stimulates formation of the apoptosome complex and caspase-3 activation, the proteins of the BCL family also control the release of the caspases-independent apoptotic effectors AIF and endonuclease G (Susin et al., 1999; Li et al., 2001). Thus to substantiate the evidence that LGI1 might initiate a mitochondrial pathway of apoptosis we evaluated the expression of BCL-2 and BAX by RT-PCR and Western blot (Fig. 7). The densitometry values of BCL2 and BAX were normalized to the GAPDH value of each sample. The results of the semiquantitative RT-PCR analysis showed that the levels of BCL-2 mRNA were reduced by 30% to 50% in neuroblastoma cells expressing LGI1 when compared with control cells, on the contrary the levels of BAX mRNA increased 2- to 3-fold. The results of Western blot showed that also BAX protein increased in all LGI1 cells (Fig. 7). The amount of BAX protein was directly proportional to that of LGI1 protein, reaching the highest levels in SH-LGI1-1 cells (~10-fold). At the opposite in this cell clone expressing the maximum levels of LGI1, the amount of BCL-2 protein was lowest.

The results indicated that an increase of LGI1 expression even if modest strongly influenced the balance between pro- and anti-apoptotic BCL2 proteins, in favor of pro-apoptotic factors. Because the pro-apoptotic function of BAX is prevented by its heterodimerization with BCL2, the ratio of BCL2 to BAX determines the execution of apoptosis in various cancer cells

including neuroblastoma (Lombet et al., 2001). Thus LGI1 appears to stimulate the intrinsic pathway of programmed cell death through the alteration of BCL2 to BAX ratio.

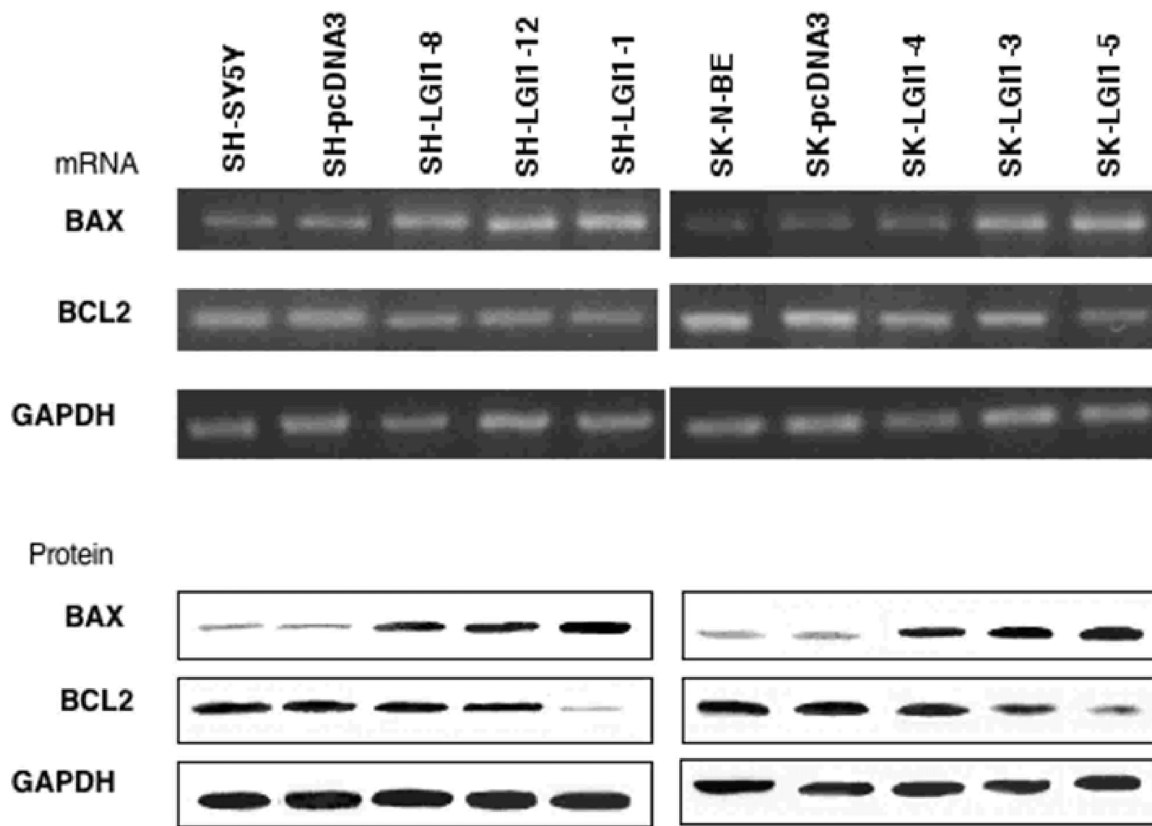

Fig. 7. Alteration of BCL2 and BAX levels by expression of LGI1 in neuroblastoma cells

6.2.4 Release of apoptosis-inducing factors from mitochondria

The shift of BCL-2 to BAX ratio suggested that LGI1 cell death might be triggered by the mitochondrial pathway of apoptosis by the release of cytochrome c and apoptosis inducing factor (AIF); (see Kroemer and Reed, 2000; Martinou and Green, 2001). The amount of cytocrome c released in the cytosol was determined by ELISA assays of cytosolic fractions isolated from neuroblastoma cells cultured for 3 days at high cell density, at what time massive death of LGI1-cells and large activation of caspase 3/7 occurred (Fig. 5, Fig. 6). The amount of cytochrome c in the cytosolic fraction of LGI1 cells was significantly greater than that present in fractions from control cells. A 2.4- to 3.5-fold increase of cytosolic cytochrome c was detected in SH- and SK-LGI1 cells. Consistently with the notion that cytosolic cytochrome c activates caspase-3, the greatest activation of caspases 3/7 in LGI1 cells coincides with the release of cytochrome c. The death effector AIF translocates to the nucleus following transition of mitochondrial membrane permeability, where it induces chromatin condensation and high molecular weight DNA fragmentation ending with apoptosis, independently on caspases activation (Susin et al., 1999; Cregan et al. 2002). The amount of AIF in nuclear fractions of the neuroblastoma cell clones was determined by Westen blot in the same experimental conditions used to determine the release of cytocrome c. The nuclear content of AIF was on average 2- to 4- fold greater in LGI1 cells than in control cells. In agreement with the failure of the caspase inhibitor Z-VAD-FMK to increase

cell survival, the observed translocation of AIF in the nucleus might support the apoptotic program, even in the absence of caspases activation.

7. Adenocarcinoma

HeLa cells derive from a human cervical adenocarcinoma and have been shown to contain several copies of the human papillomavirus HPV-18 integrated in their genome (Inagaki et al., 1988). In particular HeLa cells express the viral oncoprotein E6, which in complex with a cellular protein interacts with the tumor suppressor protein p53, to promote its ubiquitin-dependent degradation (Scheffner et al., 1993). Consequently, the physiological function of p53 sustaining cell cycle arrest and apoptosis is inhibited. The results of RT-PCR and Western blotting performed to assed expression of LGI1 revealed the absence of LGI1 mRNA and protein in HeLa cells (Gabellini and Masola, 2009). This finding is in agreement the results of Expressed Sequence Tags (ESTs) in human uterine tumors as well as in normal uterine tissue (information from UniGene). The absence of LGI1 expression has been reported also in mouse uterus (Head et al., 2007). Furthermore, the analysis by a proteomic approach of several Barrett's-related adenocarcinomas compared to normal mucosa samples showed 100% downregulation of LGI1 (Peng et al. 2008).

7.1 Expression in HeLa cells

The robust growth and survival ability together with the absence of endogenous LGI1 expression render HeLa cells an appropriate system to examine the tumor suppressor function of LGI1. We performed stable transfection of HeLa cells with LGI1 cDNA and with empty vector pcDNA3 using the same procedure adopted for neuroblastoma cells. The characterization of stably transfected cell clones expressing LGI1 clearly showed inhibition of cell proliferation and increased of cell death, as in the case of neuroblastoma cells. Again expression of LGI1 modified the balance of BCL2 to BAX in favor of the pro-apoptotic factor (Gabellini and Masola, 2009). Consistently with the downregulation of LGI1 reported in Barrett's-related adenocarcinomas this study supports the role of tumor suppressor of LGI1 in adenocarcinoma-derived cells (Peng et al., 2008), in addition to neuroblastoma and glioblastoma.

7.2 Induction of apoptosis

The activity of the apoptosis effectors caspase-3 and -7 was measured to investigate the mode of cell death caused by the expression of LGI1. The caspase-3 and -7 activity of HeLa cells expressing LGI1 was consistently greater than that of control cells. To strengthen the evidence on the induction of apoptosis of HeLa cell expressing LGI1, the relative amount of BCL2 and BAX expression was assessed by semi-quantitative PCR analysis and Western blotting. The levels of the anti-apoptotic BCL2 decreased whereas those of pro-apoptotic BAX systematically increased in all cell clones expressing LGI1 when compared with control cells, the variations correlated with LGI1 levels. The decreased ratio of BCL2/BAX supports the activation of the intrinsic pathway of apoptosis by the release of apoptogenic molecules from mitochondria of HeLa cells as it occurs in various cancer cell types (Xiong et al., 2003; Lombet et al., 2001; Raisova et al., 2001). In the case of HeLa cells the activity of LGI1 seems to antagonize that of the HPV oncoprotein E6. The latter impedes apoptosis by blocking p53

transcriptional regulation activity, which enhance the expression of BAX and repress that of BCL2 (Scheffneret al., 1993; Miyashita and Reed 1995; Wu et al., 2001). In other words LGI1 might destabilize the equilibrium of BCL2 and BAX in favour of apoptosis. The evidence that LGI1 interferes with central pro-survival pathway phosphoinositide 3-kinase (PI3K)/AKT, discussed in the next section, supports this suggestion because this pathway directly regulates BCL2 and BAX expression (Brunet et al., 2001).

8. Inhibition of the phosphoinositide 3-kinase pathway in neuroblastoma cells

The observation that neuroblastoma cells overexpressing LGI1 required daily changes of growth medium raised the possibility of an increased necessity of fresh serum to sustain survival. Thus the possibility that essential signaling pathways mediating survival stimuli conveyed by serum growth factors might be inhibited by LGI1 was investigated. The PI3K/AKT pathway is a central signaling pathway activated downstream of growth factor receptors. The activation of receptor tyrosine kinase stimulates PI3K to produce the second messengers phosphatidylinositol-3, 4,5-trisphosphate (PIP3), this activates the serine-threonine protein kinase AKT (protein kinase B), which directly regulates key cellular functions including apoptosis. AKT inactivates death signals by phopshorylating pro-apoptotic proteins such as forkhead transcription factors, caspase-9, Bad (Fukunaga et al. 2005; Clerkin et al., 2008). At the same time AKT up-regulates the expression of anti-apoptotic proteins such as BCL-XL and prevents the translocation of BAX to mitochondria (Tsuruta et al., 2002). AKT also up-regulates vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF-1), thus enhancing angiogenesis, tumor growth and metastasis (Jiang and Liu, 2009). Furthermore, AKT prevents apoptosis determined by loss of cell-matrix interactions (anoikis), thus favoring metastasis formation (Wang, 2004).

8.1 Failure of PI3K inhibitors to increase cell death

The possibility that the elevation of LGI1 levels might inhibit trophic signaling through the PI3K/AKT pathway was investigated by measuring the fraction of dead cells in the presence of increasing amounts of the PI3K inhibitors wortmannin (Fig. 8) or LY294002. The results of LDH assays showed that the fraction of control cells death increased proportionally with the concentration of inhibitors to reach a maximum of about 30% at 50 nM wortmannin or 50 μM LY294002.

By contrast, the PI3K inhibitors failed to increase significantly dead of LGI1 cells, which even in the absence of inhibitors was similar to maximum values determined in the presence of the inhibitors with control pcDNA3 cells. The failure of the inhibitors to significantly increase cell death suggested that PI3K pathway was already inhibited by LGI1. This was particularly evident with SH-LGI1-12 cell clones, in which the effects of the inhibitors were completely absent.

8.2 Inhibition of AKT phosphorylation

The blockage of PI3K in LGI1 cells was further investigated by the evaluation of the activating AKT phosphorylation. For this purpose we performed an ELISA assays with two antibodies: one directed to AKT phospho-serine 473 and one to the total AKT protein. Cells were stimulated with 10% serum for 30 minutes to induce AKT phosphorylation, following

16 hours of serum starvation. The results of the quantitative analysis showed a sharp enhancement of AKT phosphorylation in control pcDNA3 cells (about 5-fold), which was abolished by inhibition of PI3K with wortmannin (50 nM), however, significant phosphorylation of AKT was absent in both LGI1 cell clones (Fig. 9).

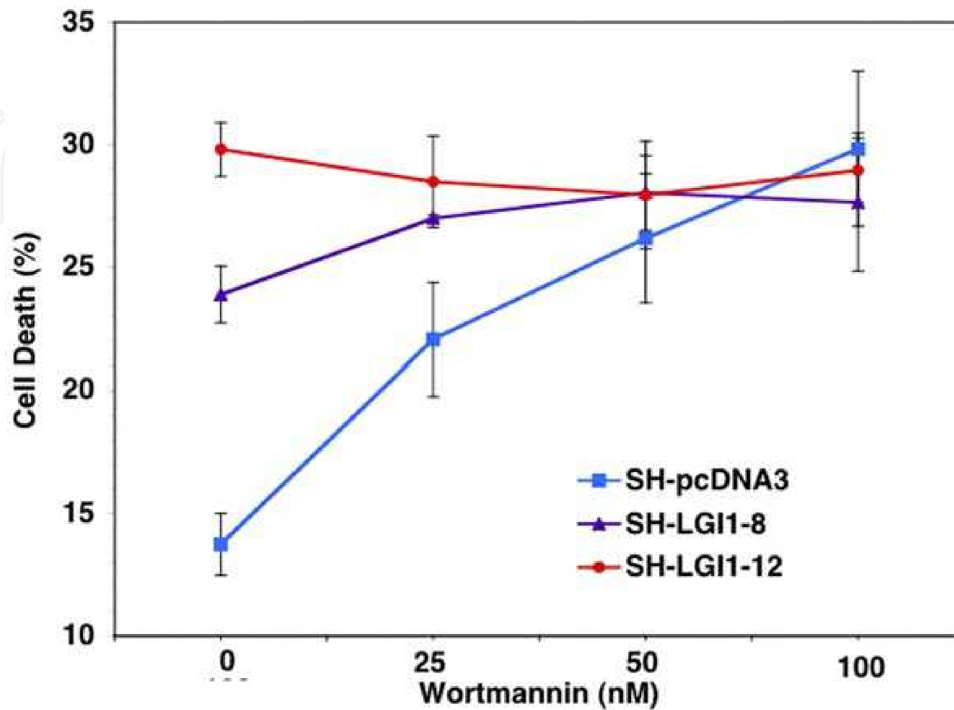

Fig. 8. Effects of PI3K inhibition on neuroblastoma cell death

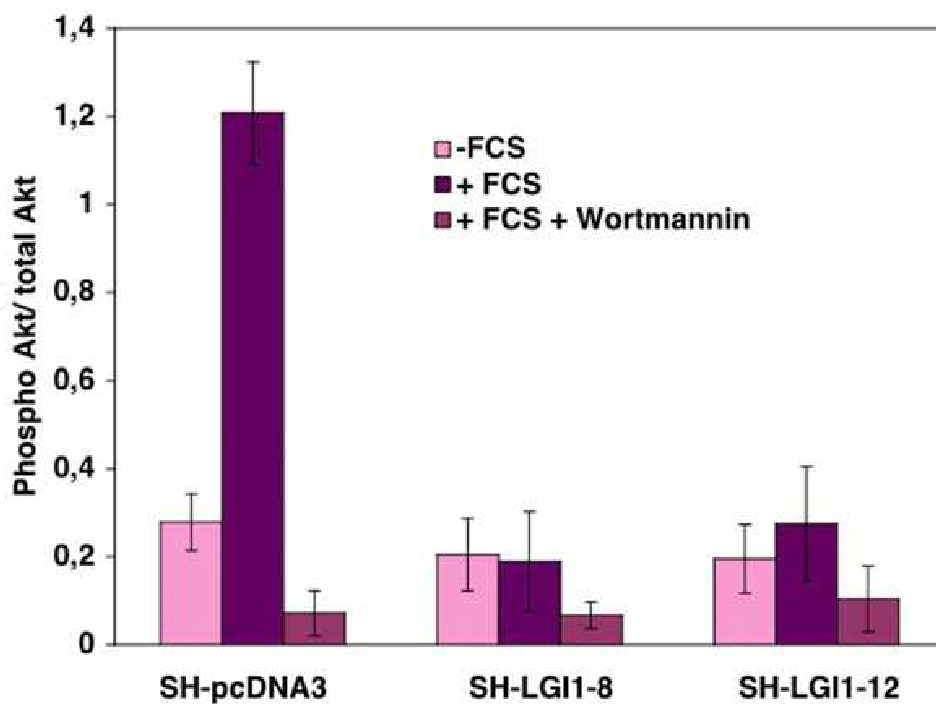

Fig. 9. Quantitative analysis of AKT phosphorylation induced by serum (FCS) in the presence or absence of PI3K inhibitor wormannin

The blockage of the downstream target of PI3K in LGI1 cells was supported by Western blotting of phospho-AKT and total AKT in the same experimental conditions used for ELISA assays. Following serum stimulation phopho-AKT Ser-473 was observed only in control pcDNA3 cells, which was consistently prevented in the presence of the inhibitor wortmannin. AKT failed to be phosphorylated in both LGI1 cell clones in response to serum (Fig. 10).

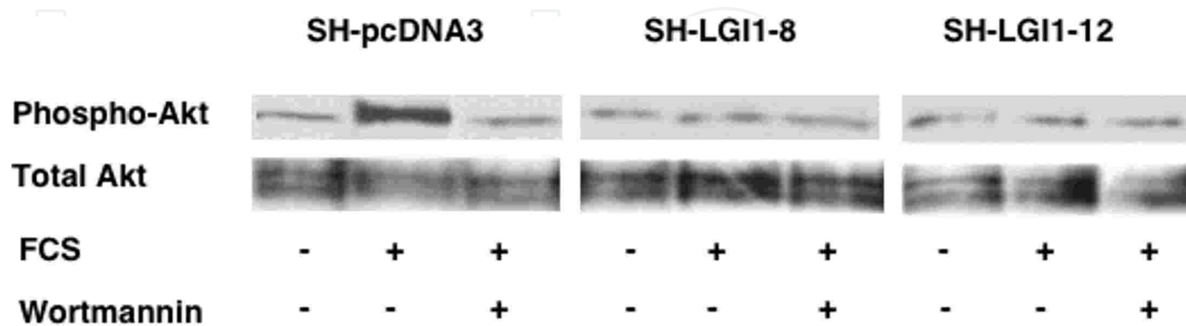

Fig. 10. Western blot analysis of total AKT and phospho-AKT induced by serum (FCS) in the presence or absence of PI3K inhibitor wormannin

The inhibition of the PI3K/AKT pathway in LGI1 cells is in agreement with the evidence that LGI1 triggers an intrinsic pathway of apoptosis, because deficiency of AKT activity shift the balance of mitochondrial factors in favor of apoptosis (Section 6.2.3).

9. Blockage of the MAPK/ERK pathway in glioblastoma cells

Also the mitogen-activated protein kinases (MAPKs) pathway can be activated downstream of tyrosine kinase receptors. This central signaling pathway controls a number of crucial cellular responses such as proliferation, migration and apoptosis. It is divided in at least four segments: the ERK pathway, which is activated downstream of growth factor receptors, and the JNK, p38 and ERK5 pathways, which are activated by stress and also in response to growth factors. Modifications of the MAPKs signaling determined by mutations or altered regulation of the components of this pathway, such as Ras or epidermal growth factor receptor, play an important role in cancerogenesis and metastasis formation (Roberts & Der 2007). In particular, the amplification of the epidermal growth factor receptor (EGFR) is one of the typical genetic alterations occurring in gliomas (Henn et al. 1986). The state of this signaling pathway was investigated in glioblastoma cells re-expressing LGI1, in view of the evidences on the inhibition of cell proliferation and migration ability emerged by the re-expression of LGI1 in glioblasoma cells (section 5.2.1).

9.1 Downregulation of matrix metalloproteinases

Matrix metalloproteinases (MMPs) are involved in the degradation of the extracellular matrix. Their physiological role is particularly important in development, tissue repair, and angiogenesis. MMPs are highly expressed in malignant brain tumors and have been implicated in the malignant developmemt of gliomas (Chintala et al. 1999; VanMeter et al., 2001). The increase of MMPs expression in tumors facilitates metastasis formation and neovascularization. Gene expression profiling of glioblastoma T98G cells re-expressing LGI1 compared with T98G control cell clones lacking LGI1 expression was performed to

identify differentially expressed genes and to achieve some indications on the signaling pathway affected by LGI1 (Kunapuli et al. 2004). Oligonucleotide microarray analysis revealed that several genes for extracellular matrix proteins were downregulated in cells re-expressing LGI1. In particular the expression of matrix metalloproteinase –1 and -3 (MMP-1 and MMP-3) showed a significant downregulation, which was substantiated by RT-PCR analysis.

9.2 Inhibition of ERK1/2 phosphorylation

The influence of LGI1 on the activation MAPKs signaling pathways that were shown to control transcription of MMP genes (Westermarck et al., 2001) was investigated in the T98G glioblastoma cell clones (Kunapuli et al. 2004). In particular the phosphorylation status of the extracellular signal protein kinases ERK1/2 was analyzed by Western blot. The levels of phosphorylated ERK1/2 were lower in T98G cells expressing LGI1 than in control cells. Pharmacological treatment with MEK1 inhibitors significantly decreased the levels of ERK1/2 phosphorylation and inhibited the expression of MMP-1/-3 in T98G control cells. Also inhibitors of the p38 MAPK pathway interfered with the expression of MMP-1/-3, however, p38 phosphorylation was not modified significantly by the expression of LGI1. Thus the ERK1/2 pathway was identified as the specific target of inhibition by LGI1, resulting in the reduction of MMPs expression and consequently of the invasive potential of glioblastoma cells (Kunapuli et al. 2004).

9.3 Type-II tumor suppressor

The finding that LGI1 controls the expression of MMPs is consistent with the downregulation of LGI1 expression during the malignant progression of brain tumors. The downregulation of LGI1 might result in the increase of MPP expression supporting tumor growth and metastasis formation. These findings raised the possibility that the tumor suppressor function of LGI1 might impede malignant development, thus it was proposed to function as a type-II tumor suppressor gene (Besleaga et al., 2003).

9.4 Cell specific inhibition of signaling pathways

The re-expression of LGI1 in T98G glioblastoma cells produced a specific inhibition of the ERK1/2 pathway without hindering the PI3/AKT pathway, whereas the PI3K/AKT pathway was inhibited by the expression of LGI1 in neuroblastoma cells (Fig. 11). In neuroblastoma cells the effects of LGI1 on the MAPK/ERK pathway were determined in the same neuroblastoma cell samples employed for the analysis of AKT. Western blot with antibodies directed to phopho-ERK1/2 and to total ERK1/2 was performed to determine the phosphorylation status of ERK1/2. Addition of serum on starved cells induced ERK1/2 phosphorylation about 2-fold in all cell clones independently on the expression of LGI1. This observation confirmed the specificity of the PI3K/AKT pathway inhibition by LGI1 in neuroblastoma cells.

This divergence might depend on the specific activation of signaling pathway linked to growth factor receptors (RTK) in different cancer cells (Fig.11). Further investigations are needed to clarify the mechanism of PI3K/AKT pathway and ERK1/2 pathway inhibition produced by LGI1; this might provide new strategies to control cell survival, proliferation and metastasis formation.

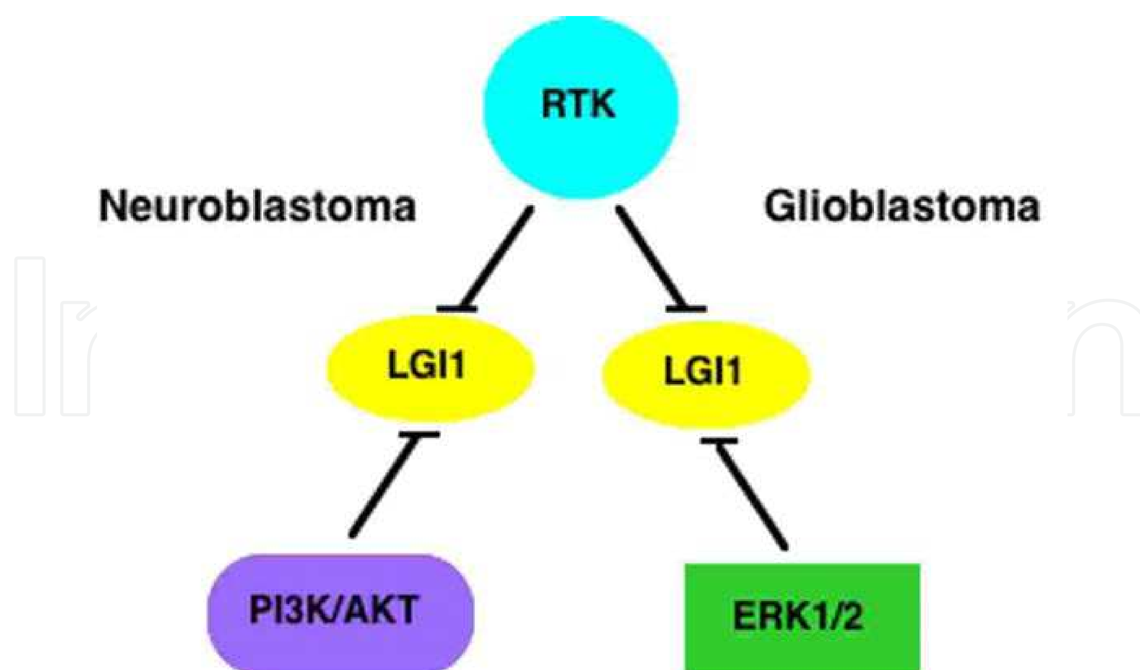

Fig. 11. Inhibition of signaling pathway downstream of RTK by LGI1 in neuroblastoma and glioblastoma cells.

10. Interacting proteins

LGI1 protein includes domains predicted to be involved protein-protein interaction (Section 3). The search for proteins interacting with LGI1 provided important indications on its function. First it was shown to interact with voltage gated potassium channel (Kv1.1) in axonal terminal of CNS neurons (Schulte et al., 2006), then it was described as a ligand for the epilepsy related neuronal receptor ADAM metallopeptidase 22 (Fukata et al., 2006). In contrast with the limited information on the function and signaling of ADAM22, Kv channels are well characterized and their activity is supposed to control a variety of cell functions. The voltage sensitive activity of Kv channels is particularly important in neurons where it controls membrane polarization, axon potential formation, firing properties and neurotransmitters release. However, it is also important in non-excitable cells, because it controls intracellular Ca^{2+} concentration, cell volume and cell survival (O'Grady & Lee, 2005).

10.1 Preclusion of Kv1 channels inactivation by Kvβ1

The membrane protein complex including Kv channels was affinity purified using antibodies to Kv1.1 subunit from rat brain and the protein complex was characterized using a proteomic approach (Schulte et al., 2006). Beside Kv1.1 also the regulatory subunit Kvβ1 and other subunits constituting the channels, plus several other proteins including Lgi1 were co-purified. The interaction of Lgi1 with Kv1.1 was unambiguous demonstrated by reverse co-purification with antibodies to Lgi1. In particular it was shown that Lgi1 co-purified with the Kv1.4 and Kvβ1 subunits. This was further confirmed by the results of immunohistochemitry on mouse brain showing co-localization of Lgi1 and Kv1.1 in the specific brain areas (described in Section 4), with prevalent pre-synaptic localization. To explore the functional role of Lgi1 on Kv channels activity, Kv1.1, Kv1.4, and Kvβ1 subunits,

which compose a type of channel capable of inactivation, were expressed in *Xenopus* oocytes and patch-clamp recordings were performed in the presence or absence of Lgi1 co-expression. These experiments demonstrated that Lgi1 clearly reduced channel inactivation, the decay of the recorded currents corresponded to that of channels composed of Kv1.1-Kv1.4 suggesting that Lgi1 prevents channel closure by the Kvβ1 subunit. The expression of LGI1 carrying ADLTE mutations failed to antagonize the inactivation by Kvβ1, suggesting that loss of LGI1 function may enhances channel inactivation by Kvβ1. This study suggested that modifications of the inactivation properties of Kv channels in ADLTE patients carrying LGI1 mutations support epileptic activity. However, the immunohystochemistry analysis revealed that expression of Lgi1 also occurs in neurons that do not express Kv1.1, suggesting that LGI1 might have different functions.

10.2 Increased K^+ permeability and induction of apoptosis

The regulation of Kv channel activity by LGI1 was discussed in the context of epilepsy; however, it might be related also to the tumor suppressor function of LGI1 because these channels are important regulator of cell survival. Indeed the upregulation of Kv1.1 activity was associated with neuronal apoptosis, while the inhibition or downregulation Kv channels with increased cell survival (Hu et al., 2008; Yu et al., 1997). Since it was shown that binding of LGI1 to Kv1β prevents channel inactivation, a deficit of LGI1 would results in a decreased of K^+ permeability and inhibition of apoptosis.

11. LGI1 as a therapeutic target

The discoveries that LGI1 inhibits central signaling pathways that regulate cell proliferation, survival, motility and angiogenesis render LGI1 an attractive therapeutic target. The decline of LGI1 expression in the malignant progression of gliomas and the downregulation of MMP production caused by the re-expression of LGI1 in glioblatoma cells, point out LGI1 as a target for the treatment of malignant brain tumors. However, a better understanding of the mechanisms of apoptosis and of MMP expression regulation is necessary to develop efficacious strategies of intervention.

11.1 Future directions

Gene therapy to achieve the re-expression of LGI1 might be a useful approach to treat malignant brain tumors with downregulated expression of LGI1, however, because gliomas are genetically heterogeneous, the determination of the genetic alterations that characterize each tumor is required to create effective strategies. An important mechanism of carcinogenesis tumor invasion, and metastasis operative in cancer cells is gene silencing by DNA hypermethylation of tumor suppressor genes. Epigenetic therapy to reduce promoter methylation might become suitable to re-express LGI1 in malignant brain tumors when an appropriate technology will be developed.

12. Conclusion

Several evidences supporting the tumor suppressor role of LGI1 have been presented here. The discovery of chromosomal rearrangements leading to loss of LGI1 expression in

glioblastoma cells, mutations in LGI1 gene specifically associated with gliobastoma and the downregulation of LGI1 expression in several tumors all point out a role of LGI1 in tumor suppression. Furthermore the findings that re-expression of LGI1 in glioblastoma cells impaired cell growth and migration ability through inhibition of the ERK1/2 pathway, with consequent downregulation of MMPs expression, support a role in the suppression of metastasis formation and tumor vascularization. This is in line with the downregulation of LGI1 expression observed in the malignant progression of gliomas. The findings that increased expression of LGI1 impaired growth and survival of neuroblastoma cells further strengthen the tumor suppressor role of LGI1. The involvement of LGI1 in the negative regulation of the PI3K/AKT pathway supporting cell proliferation and survival explains the mechanism of spontaneous cell death triggered by the elevation of LGI1 levels in neuroblatoma cells. The activation of intrinsic apoptosis triggered by LGI1 is consistent with a blockage of AKT activity, which regulates Bcl-2 family members involved in the control of mitochondrial membrane permeability. Furthermore, the interaction of LGI1 protein with voltage gated potassium channels (Kv1.1) shown to prevent channel inactivation by Kv1β subunit, provides an additional link with apoptosis since these channels are important regulators of cell survival. Because suppression of apoptosis in cancer cells is one of the main strategies to achieve the survival advantage required for malignant progression, it is feasible that alterations of LGI1 gene or downregulation of expression often observed in cancer cells might be required to suppress apoptosis through the inhibition of survival pathways linked to growth factor receptors and of Kv channels activity.

13. Chapter summary

- Alterations of LGI1 gene occur in some glioblastomas.
- Downregulation of LGI1 gene is associated with the malignant progression of gliomas.
- Downregulation of LGI1 gene occurs in several other tumors.
- Re-expression of LGI1 gene in glioblastoma cells, lacking endogenous LGI1 expression, decreases cell proliferation and invasiveness through the inhibition of the MAPK/ERK1-2 pathway and downregulation of MMP production.
- Overexpression of LGI1 in neuroblastoma cells, in which endogenous LGI1 expression is downregulated, impairs proliferation and induces apoptosis through the inhibition of the PI3K/AKT pathway.
- The pro-apoptotic function of LGI1 is also linked to the upregulation of Kv channels activity through the blockage of the negative regulator Kv beta subunit.
- The downregulation or inactivation of LGI1 gene often observed in tumor cells might be related to the suppression of apoptosis, beside enhancement of cell proliferation and invasion.

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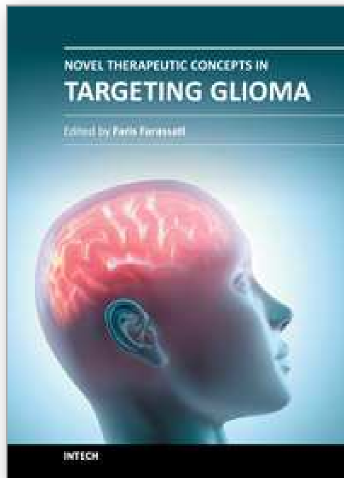

Novel Therapeutic Concepts in Targeting Glioma
Edited by Prof. Faris Farassati

ISBN 978-953-51-0491-9
Hard cover, 306 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Novel Therapeutic Concepts for Targeting Glioma offers a comprehensive collection of current information and the upcoming possibilities for designing new therapies for Glioma by an array of experts ranging from Cell Biologists to Oncologists and Neurosurgeons. A variety of topics cover therapeutic strategies based on Cell Signaling, Gene Therapy, Drug Therapy and Surgical methods providing the reader with a unique opportunity to expand and advance his knowledge of the field.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Nadia Gabellini (2012). The Tumor Suppressor Function of LGI1, Novel Therapeutic Concepts in Targeting Glioma, Prof. Faris Farassati (Ed.), ISBN: 978-953-51-0491-9, InTech, Available from: http://www.intechopen.com/books/novel-therapeutic-concepts-in-targeting-glioma/the-tumor-suppressor-function-of-lgi1

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