Published in final edited form as:

Exp Mol Pathol. 2010 February ; 88(1): 77. doi:10.1016/j.yexmp.2009.09.008.

Inactivation of LGI1 expression accompanies early stage hyperplasia of prostate epithelium in the TRAMP murine model of prostate cancer

John K Cowell, Karen Head¹, Padmaja Kunapuli², Mary Vaughan³, Ellen Karasik⁴, and Barbara Foster⁴

MCG Cancer Center, Medical College of Georgia, School of Medicine, 1120 Fifteenth Street, Augusta, GA 30912

¹ Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263

² Department of Cell Stress Biology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263

³ Department of Pathology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263

⁴ Department of Pharmacology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263

Abstract

The LGI1 gene has been implicated in tumor cell invasion through regulation of the ERK pathway. To determine whether human prostate cancer cells (PC3, 22RV, Du145) are similarly affected by exposure to LGI1, we conducted scratch wound assays and demonstrated that the secreted LGI1 protein can reduce cell motility, an essential component of invasion and metastasis. These studies have now been extended to an in vivo mouse model of prostate cancer. Using a BAC transgenic mouse expressing a GFP reporter gene under the control of cis regulatory elements, we demonstrated that LGI1 is highly expressed in the normal prostate epithelium. To determine whether loss of LGI1 expression is associated with development and progression of murine prostate cancer, we bred the GFP reporter BAC transgenic mice with TRAMP mice which undergo early hyperplasia and progressive stages of prostate cancer. In the F1 animals, although the surrounding normal prostate epithelium expressed high levels of LGI1 in the double transgenic mice, the LGI1 gene had been inactivated even at the earliest stages of hyperplasia. This observation supports the suggestion that inactivation of LGI1 in certain cell types is related to tumor progression. Taken together these results suggest that LGI1 may be an important molecule for the arrest of prostate cancer cell invasion and possibly as a biomarker for early detection of prostate hyperplasia.

Keywords

LGI1; Prostate cancer; TRAMP mouse; Tumor development; Gene inactivation

Address Correspondence to: John K Cowell, MCG Cancer Center, Medical College of Georgia, School of Medicine, 1120 Fifteenth Street, Augusta, GA 30912., Tel: 706 721 4381, Fax: 706 721 1670 jcowell@mcg.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The LGI1 gene, located in 10q24, was isolated through its association with a chromosome translocation breakpoint in the T98G glioma cell line (Chernova et al. 1998) and has since been shown to regulate cell movement, invasion, and growth in soft agar phenotypes in glioma cells (Kunapuli et al. 2003, 2004), and is frequently inactivated in head and neck cancer (Fialka et al. 2008). Loss of chromosome region 10q24 has been associated with the development of advanced stages of glioma development (von Deimling et al 1992) and is also a frequent event in other tumor types such as prostate cancer (Gray et al. 1995, Ittman et al 1998). Early studies using RT-PCR analysis suggested that LGI1 expression was lost, or significantly down regulated in advanced stage gliomas (Chernova et al. 1998, Krex et al 2002, Basleaga et al. 2003), although the subsequent demonstration of high levels of LGI1 expression in neurons (Morante-Redolat et al 2002) made interpretation of these results more difficult because of the co-mingling of this cell type in gliomas. Although reports of LGI1-specific antibodies support the loss of expression in high grade gliomas, the suggestion that these antibodies may cross react with other members of the LGI family of proteins cautions over interpretation of these observations (Head et al 2007). To specifically characterize the cellular distribution of LGI1 gene expression, therefore, we created BAC transgenic mice (Head et al. 2007) carrying recombiner substitution of the LGI1 gene with the open reading frame of the green fluorescent protein (GFP). The GFP gene was introduced at the ATG start codon of LGI1 using homologous recombination within the context of the LGI1 cis regulatory elements present on the BAC (Head et al. 2007). In this way, any cell with the transcription capability of activating the endogenous LGI1 gene, also demonstrates transcription of the GFP gene, and these cells can then be identified using standard immunohistochemistry.

Through this BAC recombineering approach we have demonstrated that the pattern of LGI1 expression was highly restricted to specific organs (Head et al. 2007). In addition to widespread expression in different parts of the brain, high levels of LGI1 expression was also identified in the epithelial lining of the prostate ducts, the islets of Langerhans, endometrium, sympathetic ganglia and sebaceous glands (Head et al. 2007). The demonstration of expression in the prostate epithelium, in particular, raised the possibility of specifically investigating LGI1 expression in these cells during in vivo development and progression of prostate cancer in transgenic mouse models.

The transgenic adenocarcinoma of mouse prostate (TRAMP) model (Greenberg et al. 1995) has been used extensively as a model for early stages of prostate cancer. In this model, expression of the SV40 large and small T oncoproteins is directed to the secretory cells of the prostate epithelium, under the control of the minimal rat probasin promoter. Male TRAMP mice develop evidence of hyperplasia of the prostate within the first four weeks of life and these histological changes closely parallel events during development of human prostate hyperplasia.

We have now generated homozygous mice expressing the GFP reporter for LGI1 expression and crossed them with TRAMP mice to investigate LGI1 expression during the early stages of hyperplasia in the prostate epithelium in F1 animals. Using immunohistochemistry we have shown that while LGI1 is expressed extensively throughout the normal prostate epithelium, cells showing early stages of hyperplasia lose LGI1 expression. These observations indicate that, as suggested for gliomas, inactivation of LGI1 may be an important event for tumor development in prostate cancer cells.

Materials and methods

Homozygous TRAMP mice in the C57Bl/6 background were crossed with homozygous LGI1 mice in the FVB/N background. The F1 male offspring were all heterozygous for the TRAMP and LGI1 transgenes and used for the following experiments.

Immunohistochemistry

Organs and tissues were fixed overnight in 10% Neutral Buffered Formalin and then embedded in paraffin. 5 µm sections were deposited onto charged slides and dried at 60°C for 1 hour. Slides were deparaffinized at room temperature in three changes of Xylene and rehydrated using graded alcohols. Endogenous peroxidase was quenched with aqueous 3% H₂O₂ for 15 minutes and washed with PBS/T. Antigen retrieval was then performed using citrate buffer, pH 6, in the microwave for 10 minutes, and allowed to cool for 15 minutes followed by a PBS/T wash. The slides were then placed on a DAKO autostainer and further processed with a PBS/T wash followed by Casein (0.03%) treatment (in PBS/T) for 30 minutes. The primary anti-GFP antibody (Abcam #ab6556 used at 1.25 µg/ml) was then applied to slides and left for one hour at room temperature. An isotype-matched control (1.25 µg/ml rabbit whole molecule IgG) was used on a duplicate slide in place of the primary antibody as a negative control. A PBS/T wash was followed by application of the biotinylated secondary antibody (goat anti-rabbit) for 30 minutes. A PBS/T wash was followed by ABC Elite reagent (Vector Lab) for 30 minutes. PBS/T was used as a wash and the DAB chromogen (DAKO, Carpinteria CA) was applied for 5 minutes (color reaction product - brown). The slides were then counterstained with Hematoxylin, and dehydrated, cleared, and a cover slip applied. Hematoxylin and eosin stained sections were generated for orientation, and these sections were counterstained with trypan blue. All images were visualized using conventional microscopy.

Wound Healing Assay

Prostate cancer cell lines 22Rv, Du145 and PC3 were maintained in DMEM supplemented with 10% fetal calf serum. The wound-healing assay is a simple, inexpensive method to study directional cell migration in vitro. In brief, cells were cultured in six well plates to confluency. Cultures were then washed once with PBS and a linear scratch was created in the cell monolayer with a pipette tip. After marking the position of the 'wound', the cells were again washed twice with PBS and fresh culture medium was added. Using an inverted microscope (4x magnification) each wound was photographed at time = 0 and then again 24 hours later. The relative efficiency of wound closure was calculated using the Image J software and then plotted using the Graphpad prism software. The wound closure was defined as the ratio of the extent of the remaining wounded area relative to that of the initial area. To study the effect of secreted LGI1 on wound closure, supernatant from LGI1 secreting cells was collected and concentrated using Amicon Millipore columns and then added to the T98G cells immediately after the scratch wound was made. After 24 hours, these cell monolayers were again photographed and the extent of wound closure was measured and compared to control cells treated in the same way. Experiments were repeated at least three times in 6 replicates.

RESULTS

Analysis of cell motility in human prostate cancer cell lines

The observation that LGI1 was expressed in the prostate epithelium (Head et al. 2007) raised the issue whether it was inactivated in prostate cancer cells and also whether it could affect cell motility of prostate cancer cells as suggested previously for gliomas (Cowell 2002, Kunapuli et al. 2004). We have recently demonstrated that reexpression of LGI1 in glioma cells that are null for its activity lose their cell invasion and motility phenotypes (Kunapuli et al. 2004). This phenotypic change is correlated with loss of cell movement in scratch wound

assays (unpublished observations), which measures the ability of cells to close a 'wound' created in a monolayer of cells in culture. In an extension of these studies, we recently showed that the secreted LGI1 protein recovered from cells expressing it, when applied to glioma cells in a scratch wound assay, also suppressed their motility (Manuscript submitted). Analysis of the Gene Expression Omnibus (GEO) database for normal human prostate tissue demonstrates that LGI1 is expressed in this tissue (e.g. entry GDS2545). In one report, where cellular subcomponents of the prostate were isolated, the luminal secretory epithelial cells were specifically shown to express LGI1 at high levels (entry GDS1973). To determine whether prostate cancer cell motility is also affected by the LGI1 protein, we analyzed LGI1 expression in several well-characterized human prostate cancer cells lines; PC3, 22RV and DU145. RT-PCR analysis showed that while 22RV cells expressed detectable levels of LGI1, PC3 and DU145 did not (Figure 1). These latter two cell lines in particular provided the opportunity to determine whether the addition of exogenous LGI1 protein would affect motility in scratch wound assays. To evaluate the influence of the LGI1 protein on cell motility, we harvested supernatant from glioma cells expressing and secreting LGI1 protein (Kunapuli et al. 2004) as well as from a parallel culture of the parental glioma cells which did not. Prostate cancer cells were then treated in parallel with the two supernatants which differed only by the presence of LGI1. In this assay, 22RV ($p = 0.000713$), PC3 ($p = 0.0000241$) and DU145 ($p = 0.000732$) cells showed a significant inhibition of wound closure, compared with the control supernatant. These observations support the idea that the LGI1 protein can inhibit cell motility of prostate cancer cells. To determine whether this inactivation can be detected in vivo, we analyzed LGI1 expression during early stage hyperplasia of the prostate in the TRAMP mouse model.

Analysis of LGI1 expression in developing prostate cancer in a mouse model

One of the LGI1-BAC transgenic mouse strains described by Head et al. (2007) carries a tandem integration of multiple copies of the RP23-213g7 BAC into chromosome 16. This BAC was recombineered to insert the GFP open reading frame into the ATG start site of LGI1 by homologous recombination and in context with the cis regulatory elements. Cells with the transcriptional ability to promote LGI1 expression, therefore, also express GFP as demonstrated by Heintz and colleagues (Gong et al. 1998). To generate 'homozygous' mice, heterozygous BAC transgenic mice were mated and the individual progeny assayed for the presence of the GFP gene using PCR as described previously (Head et al. 2007). Those F1 mice shown to carry GFP were then crossed with wild type mice and the progeny again assayed for the GFP gene. Homozygous LGI1-GFP mice were identified where 100% of offspring in this cross were shown to express GFP. The homozygous LGI1-GFP mice showed normal development and, in particular, the prostate glands were structurally and histologically normal. The TRAMP mice used in these experiments were the kind gift of Dr. N. Greenberg (Fred Hutchinson Cancer Research Center) and carry multiple copies of the transgene. All male offspring carrying the transgene develop prostate hyperplasia and eventually develop prostate cancer. Crosses between the homozygous LGI1 and homozygous TRAMP line result in all F1 animals carrying both transgenes.

The prostate glands from the F1 LGI1/TRAMP males were collected at 4, 8 and 12 weeks of age, during which time early hyperplastic events have been consistently reported and various levels of progression are seen. Immunohistochemical (IHC) analysis of various organs using an anti-GFP antibody, demonstrated that the normal prostate epithelium of the LGI1 transgenic mice show strong expression (Head et al. 2007). As a control, IgG staining showed no immunoreactivity. On each slide, the pancreas from the same mouse was included, since we have shown previously (Head et al. 2007) that the Islets of Langerhans are strongly positive for LGI1 but the parenchyma was not (Figure 2). This control served to demonstrate the efficiency of the anti-GFP staining. In the F1 double transgenic mice, normal epithelium, as evidenced by large cytoplasm/nuclear ratios and evenly spaced cells, showed expression of

LGI1. In contrast, adjacent regions of the prostate showing hyperplasia, which is characterized by increased cell density, abnormal nuclear shape, a reduced cytoplasmic to nuclear ratio and disruption of the glandular structure, did not show LGI1 expression as assessed by GFP immunoreactivity (Figure 2). Remarkably, in an extensive survey of the prostate tissue from six different double transgenic mice, there was a complete inverse correlation between LGI1 expression and atypical hyperplastic prostatic tissue. Although the more advanced hyperplastic regions showed closely packed cells, which might have obscured LGI1 staining, in the examples shown in Figure 2D and 2E, many of the hyperplastic regions show lower densities of nuclei where LGI1 staining is clearly absent. Higher magnification analysis of these areas clearly and consistently showed absence of LGI1 staining (data not shown).

To demonstrate whether the LGI1 null cells were derived from prostate epithelium, we analyzed atypical hyperplastic lesions in serial sections of the prostates of double transgenic mice using an anti-cytokeratin-18 antibody, which has been well characterized as an epithelial cell-specific marker. Analysis of the same 4, 8 and 12 week old prostate tissue clearly demonstrated that both normal and hyperplastic epithelial regions were positive for cytokeratin 18 (Figure 3), demonstrating that the individual cells lacking LGI1 expression are epithelial. E-cadherin has also been used as an epithelial cell marker (Huss et al. 2007) and when the prostates from the double transgenic (TRAMP/LGI1) mice were stained with this antibody, both normal and atypical hyperplastic areas stained strongly, especially on the lateral cell borders, further confirming the epithelial cell origin of the LGI1 null cells.

We also analyzed the prostate tissue using the neuroendocrine cell specific marker, synaptophysin (SYPH). This is an important control because it has been suggested that the aggressive prostate cancers and metastases that ultimately develop in, and kill, TRAMP mice frequently have a neuroendocrine phenotype (Huss et al. 2007, Chiaverotti et al. 2008). Using anti-synaptophysin antibodies to specifically identify neuroendocrine cells, we demonstrated that these hyperplastic prostate cells in the F1 animals were negative for synaptophysin (Figure 3) demonstrating that the hyperplastic regions did not have a neuroendocrine phenotype. These IHC analyses demonstrate that inactivation of LGI1 expression has already occurred, even in prostate epithelium cells in the earliest stages of tumor development in the TRAMP mouse, which supports our hypothesis that loss of LGI1 function in transformed cells may be associated with tumor progression.

Since late stage tumors in older TRAMP mice frequently demonstrate a neuroendocrine phenotype, we also analyzed the prostates from TRAMP/LGI1 mice at 20–24 weeks of age using anti-synaptophysin antibodies. Prostate tumors from these mice (Figure 2) were analyzed using anti-GFP which demonstrated that LGI1 was not expressed whereas synaptophysin is. These observations confirm that the aggressive cancer cells that eventually develop in these animals were not derived from an epithelial cell type, and do not express LGI1.

Discussion

The LGI1 gene has been implicated in cancer cell motility and invasion in glioma cells (Kunapuli et al. 2003, Kunapuli et al. 2004). Molecular studies have suggested that control of the in vitro invasion phenotype is exerted through down regulation of the MAPK/ERK pathway, where one of the consequences is suppression of MMP production (Kunapuli et al. 2004, Sirerol-Piquer et al 2006). Since the LGI1 protein is secreted (Senechal et al. 2005, Head et al. 2007), it presumably exerts its effect through specific binding to components on the outer cell membrane, which is supported by IHC analysis (Sirerol-Piquer et al 2006) and proteomics studies demonstrating that LGI1 binds to members of the ADAM family of extracellular receptors (Fukata et al 2006, Sagane et al 2008, Kunapuli et al 2009). The emerging view of LGI1, therefore, is that it can affect signaling pathways in different cell types that result in cell

movement, possibly through modification of the events that lead to actin reorganization. As a secreted protein, however, LGI1 is a potential drug to prevent invasion and metastasis.

Analysis of metastasis in the brain has challenging complications not seen in other cell types, and one rationale for studying the role of LGI1 in prostate cancer development was to determine whether the protein can affect cell motility in vitro and whether it is inactivated as part of in vivo prostate cancer development for which there are more tractable models. The lack of a critically evaluated antibody for LGI1 (Head et al. 2007) led us to generate a BAC transgenic line which serves as a reporter for LGI1 expression at the single cell level. Using this approach we have clearly shown that even in the earliest stages of prostate hyperplasia in the TRAMP mouse, LGI1 expression has been eliminated. Thus, when the analysis was performed in mice at 4–12 weeks of age there was a clear loss of expression of LGI1 that was specific to the low grade hyperplastic areas of the prostate and not the normal prostatic tissue. These results support the idea that in prostate cells, early inactivation of LGI1 is important for the development of abnormal foci as also suggested for glioma development (Cowell 2002). Remarkably, wherever hyperplasia was detected, LGI1 expression was absent, making this a potentially very valuable marker for early stage cancer if a robust antibody assay can be developed. There is also evidence that some advanced stage human prostate cancer cells, as shown from the cell line analyses, have also inactivated the LGI1 gene, although as we noted in gliomas, not all tumors show this inactivation event. Nonetheless, exposure to high levels of exogenous LGI1 to all prostate cancer cells tested, significantly reduced cell motility in a scratch wound assay, which suggests a potential use of this protein in the treatment of prostate cancer.

Acknowledgments

Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263

This work was supported in part by grant NS46706 from the National Institutes of Health and from the Georgia Cancer Coalition.

References

- Besleaga R, Montesinos-Rongen M, Perez-Tur J, Siebert R, Deckert M. Expression of the LGI1 gene product in astrocytic gliomas: downregulation with malignant progression. *Virchows Arch* 2003;443:561–564. [PubMed: 12942323]
- Chernova OB, Somerville RPT, Cowell JK. A novel gene LGI1 from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene* 1998;17:2873–2881. [PubMed: 9879993]
- Chiaverotti T, Couto SS, Donjacour A, Mao JH, Nagase H, Cardiff RD, Cunha GR, Balmain A. Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. *Am J Pathol* 2008;172:236–246. [PubMed: 18156212]
- Cowell JK. Epilepsy research gets new guidance. *Nature Medicine* 2002;8:219–220.
- Fialka F, Gruber RM, Hitt R, Opitz L, Brunner E, Schliephake H, Kramer FJ. CPA6, FMO2, LGI1, SIAT1 and TNC are differentially expressed in early- and late-stage oral squamous cell carcinoma—a pilot study. *Oral Oncol* 2008;44:941–948. [PubMed: 18234543]
- Fukata Y, Adesnik H, Iwanaga T, Brecht DS, Nicoll RA, Fukata M. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science* 2006;313:1792–1795. [PubMed: 16990550]
- Gray IC, Nobile C, Muresu R, Ford S, Spurr NK. A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Cancer Res* 1995;28:328–332.
- Gong S, Yang XW, Li C, Heintz N. Highly efficient modification of bacterial artificial chromosomes (BACs) using novel shuttle vectors containing the R6gamma origin of replication. *Genome Res* 1998;12:1992–1998. [PubMed: 12466304]

- Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci* 1995;92:3439–3443. [PubMed: 7724580]
- Head K, Gong S, Joseph S, Wang C, Burkhardt T, Rossi MR, LaDuca J, Matsui SI, Vaughan M, Hicks DG, Cowell JK. The expression pattern of the LGI1 gene in tissues and organs from BAC transgenic mice demonstrate a neuronal and glial expression pattern as well as other distinct cell types in the adult animal. *Mamm Genome* 2007;18:328–337. [PubMed: 17565425]
- Huss WJ, Gray DR, Tavakoli K, Marmillion ME, Durham LE, Johnson MA, Greenberg NM, Smith GJ. Origin of androgen-insensitive poorly differentiated tumors in the transgenic adenocarcinoma of mouse prostate model. *Neoplasia* 2007;9:938–950. [PubMed: 18030362]
- Ittmann MM. Chromosome 10 alterations in prostate adenocarcinoma. *Oncol Rep* 1998;5:1329–1335. [PubMed: 9769364]
- Krex D, Hausess M, Appelt H, Mohr B, Ehninger G, Schackert HK, Schackert G. Physical and functional characterization of the human LGI1 gene and its possible role in glioma development. *Acta Neuropathol* 103:255–266. [PubMed: 11907806]
- Kunapuli P, Kasyapa C, Hawthorn L, Cowell JK. LGI1, a putative tumor metastasis suppressor gene, controls in vitro invasiveness and expression of matrix metalloproteinases in glioma cells through the Erk1/2 pathway. *J Biol Chem* 2004;279:23151–23157. [PubMed: 15047712]
- Kunapuli P, Chitta K, Cowell JK. Suppression of the cell proliferation and invasion phenotypes in glioma cells by the LGI1 gene. *Oncogene* 2003;22:3985–3991. [PubMed: 12821932]
- Kunapuli P, Jang G, Kazim L, Cowell JK. Mass Spectrometry identifies LGI1-interacting proteins that are involved in synaptic vesicle function in the human brain. *J Molec Neurosci*. 2009 In Press.
- Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, Saenz A, Poza JJ, Galan J, Gesk S, Sarafidou T, Mautner VF, Binelli S, Staub E, Hinzmann B, French L, Prud'homme JF, Passarelli D, Scannapieco P, Tassinari CA, Avanzini G, Marti-Masso JF, Kluwe L, Deloukas P, Moschonas NK, Michelucci R, Siebert R, Nobile C, Perez-Tur J, Lopez de Munain A. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet* 2002;11:1119–1128. [PubMed: 11978770]
- Sagane K, Ishihama Y, Sugimoto H. LGI1 and LGI4 bind to ADAM22, ADAM23 and ADAM11. *Int J Biol Sci* 2008;4:387–96. [PubMed: 18974846]
- Senechal KR, Thaller C, Noebels JL. ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. *Hum Mol Genet* 14:1613–1620. [PubMed: 15857855]
- Sirerol-Piquer MS, Ayerdi-Izquierdo A, Morante-Redolat JM, Herranz-Pérez V, Favell K, Barker PA, Pérez-Tur J. The epilepsy gene LGI1 encodes a secreted glycoprotein that binds to the cell surface. *Hum Mol Genet* 2006;15:3436–3434. [PubMed: 17067999]
- von Deimling A, Louis DN, von Ammon K, Petersen I, Hoell T, Chung RY, Martuza RL, Schoenfeld DA, Yaşargil MG, Wiestler OD, et al. Association of epidermal growth factor receptor gene amplification with loss of chromosome 10 in human glioblastoma multiforme. *J Neurosurg* 1992;77:295–301. [PubMed: 1320666]

Figure 1.

RT PCR analysis of LGI1 expression in prostate cell lines shows that 22RV cells expressed abundant LGI1, but cell lines PC3, Du145, LNCaP and LAPC4 did not. Glioma cells lines T98G (no expression) and A172 and U87 (weak expression were included as known cell line controls) as were normal human brain and breast samples.

Figure 2.

Histopathological analysis of prostate samples from TRAMP/LGI1 double transgenic mice. Normal prostate ducts show expression in epithelial cells (A). During early stages of hyperplasia, LGI1 expression is lost (B). Each paraffin block analysis included pancreatic tissue from the same mouse where staining of the Islets of Langerhans acted as a positive control for LGI1 (C). In ducts from older mice the hyperplasia was more pronounced (D–E) but the surrounding normal prostate epithelium was still positive for LGI1 but the hyperplastic cells were not. The prostate cancer that develops in older mice was positive for synaptophysin (F) but negative for LGI1 (G).

Figure 3.

Serial sections through prostate ducts in TRAMP/LGI1 double transgenic mice show the loss of staining for LGI1 in hyperplastic region which were positive for the epithelial markers cytokeratin-18 (CTYO18) and E-cadherin (ECAD), but negative for the neuroendocrine marker synaptophysin (SYPH).