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Review

IGSF4: a new intercellular adhesion molecule that is called by three names, TSLC1, SgIGSF and SynCAM, by virtue of its diverse function

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Summary. Members of the immunoglobulin superfamily often play key roles in intercellular adhesion. IGSF4 is a novel immunoglobulin (Ig)-like intercellular adhesion molecule. Three Ig-like domains are included in the extracellular domain of IGSF4 and mediate homophilic or heterophilic interactions independently of Ca^{2+}. The cytoplasmic domain of IGSF4 contains the binding motifs that connect to actin fibers. Since IGSF4 has been characterized by several independent research groups, this molecule is called by three names, TSLC1, SgIGSF and SynCAM. IGSF4 was first characterized as a tumor suppressor of non-small cell lung cancer and termed TSLC1, although how IGSF4 suppresses tumor growth remains unknown. Silencing of the IGSF4 gene was primarily achieved by allelic loss and promoter methylation in this type of cancers. Soon after this discovery, IGSF4 was found to have roles in adhesion of spermatogenic cells to Sertoli cells and mast cells to fibroblasts and termed SgIGSF. Other researchers revealed that IGSF4 drives synaptic formation of neural cells and termed it SynCAM.

Key words: TSLC1, NSCLC, SgIGSF, Spermatogenesis, Sertoli cell, Mast cell, SynCAM, Synapse formation

Introduction

Cell adhesion molecules comprise of four categories: the integrins, the selectins, the cadherins and the immunoglobulin superfamily (IGSF) (Hynes, 1999). IGSF is the largest and consists of cell surface receptors like major histocompatibility (MHC), neural cell adhesion molecule (NCAM), intercellular adhesion molecule (ICAMs) and nectins (Edelman, 1986; Williams and Barclay, 1988; Brummendorf and Rathjen, 1995; Takai and Nakanishi, 2003). IGSF is defined with their characteristic immunoglobulin-like domains. The highly conserved domains contain two cystein residues located at both ends and they play a major role in the loop formation of the molecule. The members of IGSF are Ca^{2+}-independent and have preferences for homophilic and/or heterophilic interactions (Benson et al., 2000). In small intestine epithelial cells, nectin plays roles in the organization of E-Cadherin-based adherence junctions and claudin-based tight junctions (Takai and Nakanishi, 2003). NCAM is involved in the induction of neurite growth by modulating astrocyte proliferation (Walsh and Doherty, 1996). NCAM is also expressed in Leydig cells and is postulated to be involved in the development and functions of Leydig cells and in the adhesion among Leydig cells and between Leydig cells and the extracellular matrix (Mayerhofer et al., 1992). Thus, IGSF serves not only as an active adhesion molecule but also as a cell surface recognition molecule involved in various cellular processes, including morphogenesis, proliferation, differentiation and migration (Takeichi, 1988).

In 1999, a new member of IGSF was isolated and named as IGSF4 (Gomyo et al., 1999) (GenBank accession number NM_014333). In 2001, this molecule was shown to be a tumor suppressor of lung cancer and was renamed as *Tumor Suppressor of Lung Cancer 1* (TSLC1) (Kuramochi et al., 2001). In the same year and the next, researchers of testis and brain found that this molecule played a significant role in spermatogenic-cell attachment and synapse formation (Wakayama et al., 2001; Biederer et al., 2002). Accordingly, IGSF4 was renamed as Spermatogenic Immunoglobulin Superfamily (SgIGSF) (GenBank accession number AB052293)and Synaptic Cell Adhesion Molecule (SynCAM) (GenBank accession number AF539424), respectively. During this period, we also found a role for

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the molecule in mast-cell adhesion in the course of mast cell research (Ito et al., 2003). We will desribe here the structure, expression, and functional roles of the molecule, an emerging intercellular adhesion molecule that is involved not only in the pathogenesis of cancer but also in the organization of normal tissues. In this article, the name of the molecule is uniformly termed as IGSF4.

Molecular structure of IGSF4

IGSF4 is composed of a number of recognizable functional domains (Fig. 1) (Gomyo et al., 1999; Wakayama et al., 2001; Biederer et al., 2002). First, a signal sequence resides at the N-terminal. There are three Immunoglobulin-like domains, suggesting an involvement of this molecule in cell adhesion. The interaction through the domains is not only by a homophilic manner but also a heterophilic one (Kuramochi et al., 2001; Wakayama et al., 2001; Masuda et al., 2002; Ito et al., 2003). However, the counterpart of heterophilic interaction remains to be identified. The protein also has a transmembrane domain, followed by a short cytoplasmic domain. The cytoplasmic domain contains the protein 4.1 binding motif homologous to that of human glycophorin C and Drosophila Neurexin IV (Marfatia et al., 1995; Ward et al., 1998) . Through the protein 4.1 binding motif, IGSF4 directly associates with DAL-1, a gene product of another lung tumor suppressor belonging to the protein 4.1 family (Yageta et al., 2002). IGSF4 interacts with the actin filament through an anchoring protein, DAL1. The cytoplasmic C-terminal sequence of IGSF4 harbors a PDZ-domain protein-interaction sequence that is homologous to that of the synaptic cell-surface proteins neurexin and syndecan, which bind to the PDZ-domain protein CASK and syntenin (Hata et al., 1996; Grootjans et al., 1997). Consistently, the cytoplasmic tail of IGSF4 binds to CASK and syntenin (Biederer et al., 2002). IGSF4 also contains six possible sites for the N-linked glycosylation motif (N-X-S/T) (Kornfeld and Kornfeld, 1985).

IGSF4 is preserved throughout vertebrate genomes, with homologous sequences identifiable in mouse, human, cows, chicken or puffer fish (Biederer et al., 2002). EST databank searches also revealed extensive alternative splicing of IGSF4. Recently, we have cloned a splicing variant of mouse IGSF4 that lacks transmembrane and cytoplasmic domains (Koma et al., unpublished data). We have demonstrated that this isoform produces a soluble protein and binds the extracellular domain of the membrane-bound isoform.

Rabbit polyclonal antibody raised against C-terminal 15 amino acids of mouse IGSF4 detected variable sizes of bands in mast cells, testis, lung, spleen and stomach of mouse (Ito et al., 2003). Enzymatic deglycosylation experiments revealed that the variability of the molecular weights seemed to reflect the presence of various forms of IGSF4 that have received cell and tissue type-specific N-glycosylation. In testis, the extent and the pattern of glycosylation are developmentally regulated (Wakayama et al., 2001). Rabbit polyclonal antibody raised against C-terminal 11 amino acids reacted with multiple protein in brain (Biederer et al., 2002). The multipe bands are also due to complex N-glycosylation and the level and pattern of N-glycosylation of IGSF4 varied during development.

IGSF4 as a tumor suppressor in lung cancer-TSLC1

Non-small cell lung cancer (NSCLC) shows loss of heterozygosity (LOH) on specific chromosomal regions, including 3p, 13q, 17p, and 11q at high frequency (Weston et al., 1989; Kawanishi et al., 1997; Kohno and Yokota 1999). When focused on chromosome 11, the presence of tumor suppressor gene(s) was initially demonstrated through the suppression of turmorigenecity of a human adenocarcinoma cell line, A549, by the introduction of chromosome 11 (functional complementation) (Satoh et al., 1993). An LOH study on 11q in primary NSCLC tumors restricted a common deleted region to 5 cM in 11q23 (Iizuka et al., 1995).

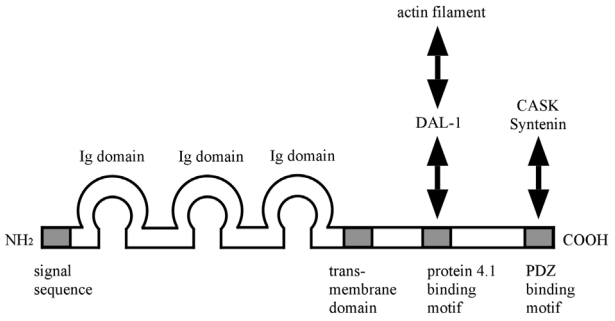

Fig. 1. Schematic representation of the structure of IGSF4. IGSF4 contains from the NH2-terminal to the COOH-terminal signal peptide, three Ig domains, a transmembrane domain, protein 4.1 binding motif and PDZ binding motif. IGSF4 binds to DAL-1 and indirectly anchors to actin filaments through the protein 4.1 binding motif. The PDZ binding motif mediates the association of IGSF4 with CASK and Syntenin.

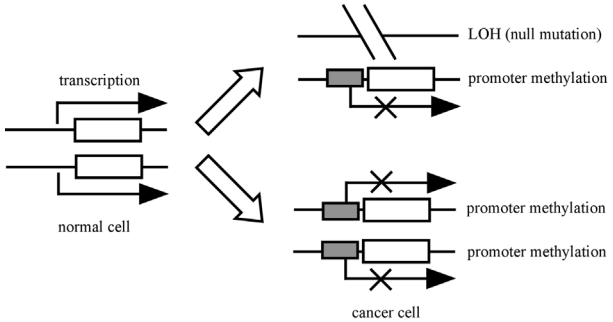

Fig. 2. Alteration of IGSF4 gene in human cancer. In tumors or cell lines with LOH, hypermethylation of the promoter represents the second hit to inactivate the IGSF4 gene (upper right). In contrast, promoter methylation is also important and well correlated with silencing of the IGSF4 gene in cancer without LOH (below right).

Kuramochi et al. (2001) prepared continuous YAC clones covering this region and confirmed by the functional complementation method that one clone had suppressor activity. This clone was fragmented and further examined for suppressor activity. Thus, the tumor suppressing segment was finally restricted to a single gene, TSLC1. This gene is a tumor suppressor in this experimental system.

Kuramochi et al. next examined the two-hit inactivation of IGSF4 in primary tumors to determine whether IGSF4 was really involved in human tumors. LOH at IGSF4 locus was detected in 42, 33 and 17% of the primary NSCLC, hepatocellular carcinoma (HCC) and pancreatic cancer (PaC) (Table 1). In these tumors exhibiting LOH at the IGSF4 locus, inactivating mutations, including a frameshift and a missense mutation, were rarely found, whereas loss of expression associated with promoter methylation was observed with high incidence. Thus, the silencing of IGSF4 by the promoter methylation is mainly involved in the primary tumors of NSCLC, HCC and PaC showing LOH on 11q23 (Fig. 2). On the other hand, a study on tumors that retained heterozygosity in this region revealed that 25% of them exhibited hypermethylation, indicating that in some tumors both IGSF4 alleles may be silenced by methylation, as occurs in other cancer-related genes. These results indicate that IGSF4 is a tumor suppressor in NSCLC.

IGSF4 mediates cell-to-cell or cell-to-substrate interaction and contributes to the pathogenesis of NSCLC. However, the precise mechanism underlying this contribution is not clear. IGSF4 was shown to interact with the actin filament through an anchoring protein, DAL-1, through the protein 4.1 binding motif (Fig. 1) (Yageta et al., 2002). This suggests that IGSF4 participates in the organization of cytoskeleton and thereby establishes stable adhesion. Loss of IGSF4 in a tumor may disturb cell adhesion and help the cell to invade into the surrounding environment. Furthermore, the complex of IGSF4 and DAL-1 was accumulated in TPA-induced membrane ruffling areas where the actin cytoskeleton was dynamically reorganized. In addition, IGSF4 restoration in IGSF4-deficient NSCLC cells suppressed the metastasis of the cells. Taken together, IGSF4 is involved not only in cell adhesion but also in cell motility.

The involvement of IGSF4 has been studied in prostatic cancer and gastric cancer (Fukuhara et al., 2002; Honda et al., 2002). IGSF4 promoter was methylated in 1 out of 4 prostatic cancer cell lines, 7 out of 22 primary prostatic cancers, 2 out of 10 gastric cancer cell lines and 15 out of 97 primary gastric cancers (Table 1). The two gastric cancer cell lines and one prostatic cancer cell line that exhibited hypermethylation in IGSF4 promoter retained two alleles of IGSF4 and completely lost the expression of IGSF4. In primary NSCLC tumors or cell lines with LOH, hypermethylation of the promoter represents the second hit to inactivate the IGSF4 gene. Likewise, promoter methylation is also important and well correlated with gene silencing of the IGSF4 gene in prostatic cancer cells and gastric cancer cells. Tumor suppressing activity

Table 1. Incidence of TSLC1 gene alteration in human cancer.

CANCER	REDUCED OR LOSS OF EXPRESSION	LOH	PROMOTER METHYLATION	MUTATION	REFERENCE
NSCLC					
Cell lines	6/12 (50)	5/12 (42)	4/12 (33)	0/12 (0)	Kuramochi et al., 2001
Primary tumors	ND	15/36 (42)	8/20 (40)	1/54 (1.9)	Kuramochi et al., 2001
	19/47 (40)	ND	ND	ND	Ito et al., in press
HCC					
Cell lines	3/8 (38)	3/8 (38)	ND	0/8 (0)	Kuramochi et al., 2001
Primary tumors	ND	7/21 (33)	4/14 (29)	1/36 (2.8)	Kuramochi et al., 2001
Pancreatic cancer					
Cell lines	9/11 (82)	ND	3/11 (27)	0/11 (0)	Kuramochi et al., 2001
Primary tumors	ND	3/18 (17)	3/12 (25)	0/40 (0)	Kuramochi et al., 2001
Prostatic cancer					
Cell lines	3/4 (75)	ND	3/4 (75)	ND	Fukuhara et al., 2002
Primary tumors	ND	4/18 (22)	7/22 (32)	ND	Fukuhara et al., 2002
Gastric cancer					
Cell lines	ND	0/2*	2/10 (20)	ND	Honda et al., 2002
Primary tumors	ND	ND	15/97 (15)	ND	Honda et al., 2002

*: LOH was examined in the two cell lines of which the promoter was methylated. ND: not determined.

was confirmed by restoration of IGSF4 in one prostatic cancer cell that lost the IGSF4 expression. These results suggest that alteration of IGSF4 is involved in prostatic cancer and gastric cancer through gene silencing by hypermethylation of the promoter (Fig. 2).

IGSF4 as an adhesion molecule of spermatogenic Cells-SgIGSF

Wakayama et al. (2001) cloned a novel mouse IGSF gene from the mouse testis. In brief, they scanned the database of mouse-expressed sequence tag (EST) clones and selected an EST clone homologous to NCAM. NCAM is expressed by Leydig cells and is postulated to be involved in the development and functions of Leydig cells (Mayerhofer et al., 1992). With this EST clone, they screened a cDNA library of mouse testis cDNA library and identified IGSF4. *In situ* hybridization revealed that expression of the gene was localized to spermatogonia and early premeiotic spermatocytes in the preleptotene to zygote stages, whereas other cell types including Sertoli cells lacked the expression. Hence, they renamed the gene Spermatogenic Immunoglobulin Superfamily (SgIGSF).

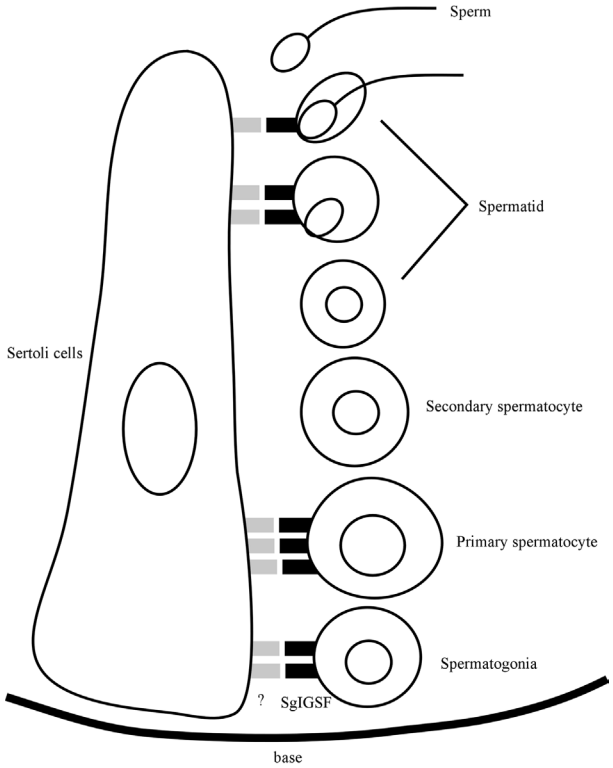

Fig. 3. IGSF4 expression in spermatogenesis. Immunohistochemistry revealed that IGSF4 was expressed in the eariler spermatogenesis cells ranging from spermatogonia to primary spermatocytes and in the elongating of spermatids. IGSF4 is supposed to mediate the binding of spermatogenesis cells to Sertoli cells via the specific receptor(s) on Sertoli cells other than IGSF4. This adhesion may help spermatonegesis cells to receive the structual and nutritional support by Sertoli cells in seminiferous tubules.

They produced a polyclonal antibody raised against C-terminal 15 amino acids of IGSF4 (Wakayama et al., in press). Western blot analysis of the testis from developing mice detected multiple immunoreactive bands in a range of 80-130kDa. The pattern of the bands varied during development. Enzymatic deglycosylation resulted in a shift of the multiple bands of 80-130kDa to a single band at 70kDa. Considering the predicted molecular mass of IGSF4, about 56kDa, and the lack of developmental changes in the pattern of IGSF4 mRNA expression in Northern blot analysis, IGSF4 is a glycoprotein and its glycosylation pattern and extent are developmentally regulated.

Immunohistochemisty with this antibody revealed that IGSF4 protein was localized in the seminiferous tubules and that the seminiferous epithelia were immunostained at two separate portions, near the base of the seminiferous tubules and close to the lumen (Fig. 3). The former represented the earlier spermatogenesis cells ranging from spermatogonia to primary spermatocytes. The latter represented elongating spermatids. The present localization of the IGSF4 protein in early spermatogenic cells is consistent with the result of in situ hybridization. In contrast, the localization of the IGSF4 protein in later spermatogenic cells is not accomapanied with detectable mRNA expression in these cells. The similar split of transcription and translation is known for some testis-specific proteins, including the transition proteins and the protamines (Heckt, 1993). In these proteins, the transcription ceases in the early spermatogenic stage, whereas the translation occurs in the later stage using the remaining mRNA. The same mechanism may explain the split of transcription and translation in IGSF4.

The immunoreactivity within the cells was localized primarily on the cell membranes. Developing spermatogenic cells receive structural and nutritional support by Sertoli cells in seminiferous tubules. Thus, the presence of specific receptor(s) for IGSF4 on Sertoli cells was suspected. Wakayama et al. then examined the binding activity of IGSF4 on these cells. Sertoli cells were cultured primarily and incubated with the immunoprecipitated endogeneous IGSF4 and the recombinant IGSF4 to allow the in situ binding of SgIGSF to putative receptor moecule(s) on the cell surface. Immunostaining of Sertoli cells with anti-IGSF4 antibody revealed that both endogeneous and recombinant IGSF4 bound to the surface of Sertoli cells. Expression of IGSF4 in Sertoli cells was not observed in either immunostaining or immunoblotting. These results suggested that IGSF4 on the suface of spermatogenic cells binds to some molecule(s) on Sertoli cells in a heterophilic manner and thereby may play diverse roles in spermatogenesis.

IGSF4 as a mast-cell adhesion molecule-sgIGSF

Microphthalmia transcription factor (MITF) is a

basic-helix-loop-helix-leucine zipper-type transcription factor (Hodgkinson et al., 1993; Hughes et al., 1993). The mutant *mi* allele encodes MITFs with deletion (*mi*-MITF) of a single amino acid, while the *tg* is a null mutation. The *mi*-MITF has a significant inhibitory effect on transcription of various genes. Although these mutant alleles have different structural and functional abnormalities, poor adhesion of cultured mast cells (CMCs) to fibroblasts was common among CMCs derived from all 3 MITF mutant mice (Ebi et al., 1992). We subtracted a cDNA library of Wild-type CMCs with mRNAs expressed by *mi*/*mi* CMCs and found a clone encoding IGSF4 (Ito et al., 2003). Northern and Western blot analyses revealed that IGSF4 was expressed in wild-type CMCs but not in CMCs derived from MITF mutants.

Immunocytochemistry using anti-IGSF4 antibody showed that the subcellular localization of IGSF4 was restricted to cell-to-cell contact areas among wild-type CMCs (Fig. 4a). In wild-type CMCs adhering to fibroblasts, IGSF4 was located primarily in the lamellipodial structure (Fig. 4b), where cytoskeletal components and regulators, such as vinculin and Rac-1, are known to accumulate in mast cells (Guillemot et al., 1997). These results suggested that IGSF4 may mediate adhesion either among CMCs or between CMCs and fibroblasts.

We overexpressed IGSF4 in *tg*/*tg* and *mi*/*mi* CMCs and examined the adhesion property of these cells. Consistent with the results of immunocytochemistry, these cells formed macroscopic aggregates in suspension culture, although intact *tg*/*tg* and *mi*/*mi* CMCs did not form such aggregates. IGSF4 appeared to function as a homophilic adhesion molecule. However, this homophilic interaction may take place only when IGSF4 is overexpressed. In fact, wild-type CMCs did not form such macroscopic aggregates.

We next examined the attachment of *tg*/*tg* and *mi*/*mi* CMCs overexpressing IGSF4 to fibroblasts. These cells adhered to fibroblasts as well as to wild-type CMCs, although original *tg*/*tg* and *mi*/*mi* CMCs did not (Table 2). This indicates that IGSF4 is necessary for adhesion of CMCs to fibroblasts. Because fibroblasts did not express IGSF4, IGSF4 appeared to serve as a heterophilic adhesion molecule in the interaction between CMCs and fibroblasts. The counterpart of IGSF4 that is expressed by fibroblasts remains to be identified. Probably the heterophilic interaction may be more physiological than the homophilic interaction, because the overexpression of IGSF4 was not necessary for the heterophilic interaction.

Defective expression of IGSF4 in CMCs derived from all MITF mutant mice suggested that the presence of wild-type MITF was essential for the expression of IGSF4 in mast cells. In fact, transfection with the wild-type MITF cDNA normalized the expression of IGSF4 in *tg*/*tg* CMCs. In addition, the transactivation effect of wild-type MITF on the IGSF4 gene promoter was confirmed in MST mastocytoma cells by Luciferase assay and Electrophoretic gel mobility shift assay (EGMSA). However, wild-type MITF did not appear necessary for the expression of IGSF4 in cells other than mast cells. The expression of IGSF4 was comparable between testes, spleens, lungs, and stomachs of wild-type and *tg*/*tg* mice. Probably, other transcription factors

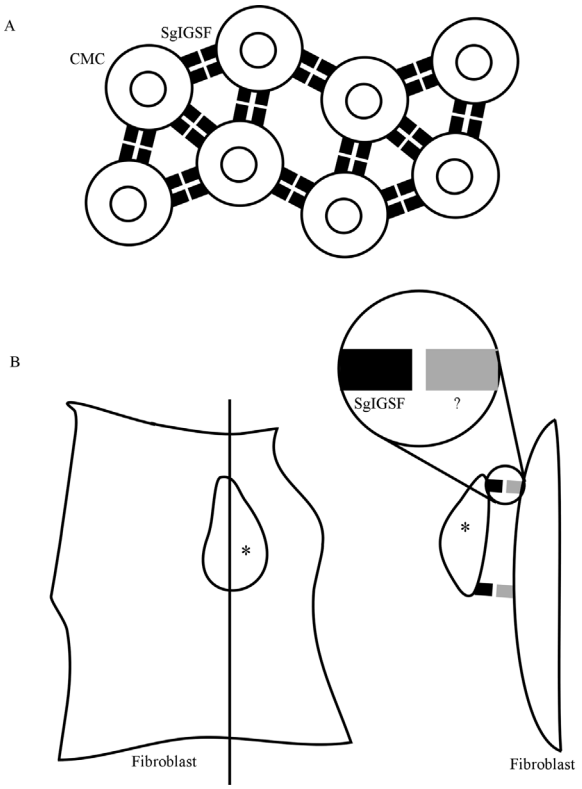

Fig. 4. Localization of IGSF4 in CMCs. **A.** In microscopic aggregates of wild-type CMCs, IGSF4 was localized in the area of cell-to-cell contact. **B.** Wild-type CMCs were cocultured on the monolayer of fibroblasts. CMCs adhere to fibroblasts (left) and the cross-sectional view (right) reveals that IGSF4 is localized on the lateral membrane of CMCs which exhibit the lamellipodial structure. The vertical line in the left is a plane for the cross-sectional view of the right. Asteisk indicates wild-type CMCs.

Table 2. Normalization of attachment of *tg*/*tg* and *mi*/*mi* CMCs to fibroblasts by transfection with IGSF4.

CMCs	No. OF ADHERING CMCs PER FIBROBLAST
wild-type CMCs	0.163±0.006
tg/tg CMCs	0.053±0.006*
tg/tg CMCs transfected with IGSF4	0.189±0.026
tg/tg CMCs transfected with empty vector	0.057±0.005*
mi/mi CMCs	0.045±0.005*
mi/mi CMCs transfected with IGSF4	0.156±0.029

*: $p<0.01$ by t-test when compared with the values of wild-type CMCs.

may compensate for wild-type MITF in these tissues.

IGSF4 as an adhesion molecule involved in synaptic formation and function-SynCAM

Synapses are specialized intercellular junctions that are assembled when an immature presynaptic terminal contacts a postsynaptic cell. Ig domain proteins play a fundamental role in synaptic cell adhesion. For example, Drosophila fasciclin II and Aplysia apCAM are essential for normal synapse formation, probably through homophilic extracellular interactions that are coupled to intracellular PDZ-domain proteins (Bailey et al., 1992; Mayford et al., 1992; Davis et al., 1997; Thomas et al., 1997; Zito et al., 1997). However, no corresponding Ig domain protein has been reported in the vertebrate brain. Bieder et al. (2002) searched GenBank databanks for proteins that contain extracellular Ig domains and intracellular PDZ-domain-interacting sequences and identified SynCAM (IGSF4).

The cytoplasmic C-terminal sequence of IGSF4 contains a PDZ-domain protein-interaction sequence that is homologous to synaptic cell-surface proteins Neurexins and syndecans. Since these PDZ-domain proteins bind to the PDZ-domain proteins CASK (Hata et al., 1996; Hsueh et al., 1998; Biederer and Sudhof, 2001) and syntenin (Grootjans et al., 1997), IGSF4 was also supposed to bind to CASK and/or syntenin. Coexpression of IGSF4 with CASK in HEK 293 cells recruited CASK from the cytosol to the membrane and resulted in the colocalization of both proteins. In addition, affinity chromatography showed the binding activity of the cytoplasmic tail of IGSF4 to CASK and syntenin. Thus, the interaction between IGSF4 and PDZ-domain proteins, CASK and syntenin, was confirmed.

The extracellular N-terminal sequence of IGSF4 harbors three Ig domains, suggesting the involvement of cell adhesion. IGSF4 was examined for cell adhesion property. Affinity chromatography experiments showed the binding of the recombinant extracellular sequence of IGSF4 to rat brain IGSF4. The binding was independent of Ca^{2+} and specific for IGSF4, because three other neuronal Ig domain proteins (L1, N-CAM and TAG-1) failed to bind to IGSF4. Then, *Drosophila* S2 cells were stably transfected with IGSF4. The transfectants aggregated into large clumps and immunocytochemistry confirmed the presence of IGSF4 in the plasma membrane. These data suggest that IGSF4 functions as a homophilic cell adhesion molecule and the homophilic binding by IGSF4 mediates cell adhesion.

The localization of IGSF4 in brain was examined. Subcellular fractionation of rat brains showed the enrichment of IGSF4 in synaptic plasma membranes. This distribution pattern was identical to that of synaptic proteins, CASK and neuroligin. Immunohistochemistry revelaed that IGSF4 was colocalized with synaptophysin, an abundant synaptic vesicle protein. Immunoelectron microscopy demonstrated the presence of IGSF4 in both pre- and postsynaptic compartments symmetrically. These suggest a possible role of IGSF4 as a homophillic cell adhesion molecue that spans the synaptic cleft.

To determine the involvement of IGSF4 in synaptic function, spontaneous miniature synaptic currents (minis) were examined. Hippocampal neurons of primary culture transfected with IGSF4 demonstrated the increased mini frequency. Since mini frequency depends primarily on the number of synapses and their release probability, IGSF4 was supposed to induce formation of new synapses or enhanced presynaptic neurotransmitter release. Then neurons were transfected with dominant the negative fragment of IGSF4, the cytoplasmic tail of IGSF4. Visualization of synapse formation showed that synapse density was decreased in the transfectants. Quantifications of synaptic transmission after nerve stimulation revealed the decreased transmission in the transfectants. These results indicate that IGSF4 affects synaptic function through the modulation of synaptic formation and neurotransmitter release.

An active role of IGSF4 in synaptic function was examined using non-neuronal cells. HEK 293 cells were transfected with IGSF4 and cocultured with hippocampal neurons. Synapse formation was induced on the transfectants. When stimulated, the presynaptic activity (exocytosis and recycling of vesicle pool) was not different between regular interneuronal synapses (between neurons) and heterologous synapses (between the transfectant and the neuron). Then HEK 293 cells were cotransfected with IGSF4 and glutamate receptor (GluR2) and electrical activity was recorded by whole-cell voltage-clamp. The transfectants exhibited postsynaptic glutamatergic responses resembling those of neurons (Fig. 5). These suggest that a single signal provided by IGSF4 is sufficient to instruct the presynaptic terminal for differentiation.

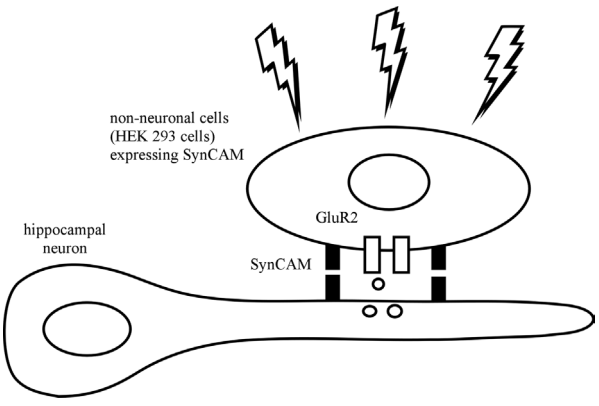

Fig. 5. Reconstitution of glutamatergic synaptic transmission onto transfected HEK 293 cells. HEK 293 cells were transfected with IGSF4 and GluR2 and seeded on cultured hippocampal neurons. Synapse formation was induced on the neurons with normal release property and the transfectants exhibited glutamatergic responses resembling those of neurons.

Conclusions and perspectives

IGSF4 is an emerging member of the immunoglobulin superfamily and serves as an intercellular adhesion system. IGSF4 was first found as a tumor supressor in NSCLC, followed by the prompt discovery of its physiological role in spermatogenic-cell adhesion, mast-cell adhesion to fibroblast and formation of functional synapses. In spermatogenic cells and mast cells, the intercellular adhesion property of IGSF4 was demonstrated. In contrast, in NSCLC and neurons, IGSF4 serves not solely as an intercellular adhesion molecule but also as a regulator of cellular function. This indicates a dual role of IGSF4 as an active adhesion moecule and a cell surface recognition molecule. We recently found that IGSF4 was expressed in the pulmonary epithelium and localized at cell-cell boundaries (Ito et al., in press). This may suggest a role of IGSF4 in cell-to-cell interactions of the epithelium and open up a new category in physiological roles of IGSF4.

IGSF4 was previously named as Necl2, nectin-like protein 2, in the direct submission to GenBank (GenBank accession number AAF69029). Nectin is involved in organization of the junctional complex in epithelial cells, formation of synapses in neurons and the organization of heterotypic junctions between Sertoli cells and spermatids in the testis (Takai and Nakanishi, 2003). These features resemble those of IGSF4, suggesting that IGSF4 may have similar and complementary roles to nectin in epithelium interaction, synapse formation and spermatogenesis.

Due to the diverse roles of the gene, IGSF4 was renamed differently according to its distinctive role, such as TSLC1, SgIGSF and SynCAM. Furthermore, in the GenBank search, we found other names for the gene, IGSFB12 and Necl2, which were registered before IGSF4, TSLC1, SgIGSF and SynCAM. To prevent the confusion about the gene name, we have termed the gene IGSF4 in this article. IGSF4 was deposited into the GenBank after mapping on chromosome, clarifying the exon-intron boundaries and indicating its potential role as a tumor suppressor. Here we would like to propose to uniformly name the gene as IGSF4.

In this article, the diversity of roles of IGSF4 has been well demonstrated. However, the detailed molecular mechanisms underlying the adhesion remain to be elucidated. 1) If the IGSF4 acts as simple glue, the presence of IGSF4, rather than its absence, might possibly assist tumor formation in nude mice. This question could be answered if IGSF4 were involved in the negative signals for cell growth, turned on by cell adhesion, are disturbed. 2) IGSF4 is supposed to function in a heterophilic manner in the binding of spermatogenic cells to Sertoli cells and mast cells to fibroblasts. The partner of heterophilic binding of IGSF4 should be identified. 3) IGSF4 on non-neuronal cells induces reconstitution of a functional synaptic glutamatergic response. Intestinal mucosal mast cells in intestines were in intimate contact with peptidergic nerves (Stead et al., 1987; Stead et al., 1989; Arizono et al., 1990). Pavlovian conditioning caused mast cell degranulation in gastrointestinal tract (MacQueen et al., 1989). Other evidence supports the functional coupling of nerve and mast cells (Santos et al.,1996; Santos et al., 1998). We have recently observed that both components, nerve and mast cells, express IGSF4 in gastrointestinal tract (Ito et al., unpublished data). These findings raise the possibility that IGSF4 has a role in funtional coupling of these components through the formation of synapses. Elucidation of these unresolved issues will provide us profound insights into the molecular linkage between intercellular junctions mediated by IGSF4 and various cell funtions, such as morphogenesis, differentiation, proliferation and migration, and also into the molecular mechanisms underlying cancer.

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