Review

Targeting Tropomyosin-receptor Kinase Fused Gene in Cancer

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Abstract. *Tropomyosin-receptor kinase fused gene (TRK-fused gene, TFG) encodes a protein which is a conserved regulator of protein secretion that localizes in the endoplasmic reticulum exit sites and controls the export of materials from the endoplasmic reticulum. It is important for intracellular trafficking of protein secretion. TFG belongs to the systems which control cell size, and is involved in regulatory mechanisms of apoptosis and cell proliferation. The TFG fusion proteins have been found to play a role in oncogenesis, with the activity of TFG fusion proteins promoting tumor development. In addition, TFG alone has been demonstrated to function like an oncoprotein; however, there are contradictory data suggesting TFG might act as a tumor suppressor. In this article, we will review the functions and regulation of TFG, the TFG fusion proteins, and the role of TFG in tumorigenesis. Finally, the potential of targeting TFG in cancer treatment is discussed.*

Intracellular Trafficking and Tropomyosin-receptor Kinase Fused Gene (TRK-fused gene, *TFG*)

Intracellular trafficking of proteins is an essential biological process that controls the correct distribution of proteins within eukaryotic cells (1). The proteins are exported from the endoplasmic reticulum (ER), and then packaged into vesicles

that emerge at defined sites on the ER and finally fuse with the ER-Golgi intermediate compartment (ERGIC) (2). Then the proteins are exported from the ER in COPII-coated vesicles (3). To complete the transport, the tropomyosin-receptor kinase-fused gene (TRK-fused gene, TFG) protein is required (4). TFG protein is a conserved regulator of protein secretion that localizes in the ER exit sites and controls the export of proteins from the ER (4). *TFG* is located on the q arm of human chromosome 3 and encodes a ubiquitously expressed cytoplasmic protein (5). The total length of *TFG* cDNA is 1677 bp, encoding a 400-amino-acid protein containing putative functional domains, such as Phox and Bem 1p (PBI), a coiled-coil (CC) domain and a serine, proline, tyrosine, glycine and glutamate (SPYGQ)-rich region (6). TFG forms hexamers that facilitate the co-assembly of SEC-16 with COPII subunits (7). TFG levels at ER exit sites are correlated with levels of SEC-16 and COPII (3). Extra TFG could increase the COPII recruitment and stimulate the secretion from ER, while TFG depletion leads to a decline in both SEC-16 and COPII levels at ER exit sites (3). In addition, the membrane flux mediated by TFG is also important for maintaining proper Golgi organization (3). TFG depletion leads to a defect in normal ER function and Golgi assembly, causes ERGIC and Golgi membranes to become smaller and more poorly-stacked, the ER becomes fragmented and Golgi networks become fewer (3). Therefore, TFG is important for intracellular trafficking of protein secretion.

TFG Functions and Regulation

In addition to its important role in intracellular trafficking, the functions of TFG are complex. In *Caenorhabditis elegans*, TFG acts both as apoptotic suppressor and activator of cells and nuclei to grow to normal size (4). Loss of TFG in *C. elegans*, results in supernumerary apoptotic corpses, whereas its overexpression inhibits programmed cell death

(4). TFG cooperates with another protein, apoptotic protease activating factor-1 (APAF-1), to control cell and body size (4, 8). APAF-1 is an adaptor protein and plays a pivotal role in activating apoptosis and restricting cell and nuclear size, thereby determining the appropriate overall size of an animal (8). TFG and APAF-1 act antagonistically to control cell and body size through modulation of the protein production and accumulation, and regulate apoptosis (4). In *Xenopus laevis*, TFG interacts with the SH3 domain of v-src avian sarcoma viral oncogene homolog (Src), phospholipase C, and p85 phosphoinositide 3-kinase (PI3K) subunit (9). Therefore, TFG belongs to the systems which control cell size, and is involved in the mechanisms regulating apoptosis and cell proliferation; all these functions determine the overall dimensions of organs and organisms, and their dysregulation can lead to tumor formation (4). In addition, TFG has been found to be mutated in hereditary motor and sensory neuropathy with proximal dominant involvement, which is an autosomal-dominant neurodegenerative disorder (10). Inhibition of TFG function is considered to cause hereditary axon degeneration by impairing ER structure (11).

The regulation of TFG is still not fully-understood. TFG can regulate phosphotyrosine-specific phosphatase-1 activity, which is important in proteolysis (12). Because proteolysis is a key regulatory event that controls intracellular and extracellular signaling, TFG might affect the functions of proteins through irreversible changes of their structure (13). TFG is up-regulated in response to tumor necrosis factor (TNF) and APOL-related leukocyte expressed ligand-1 (TALL1) (14), a member of the TNF family implicated in B-cell proliferation and autoimmunity (4). In addition, TFG has been noted to interact with phosphatase and tensin homolog (PTEN), a key tumor suppressor that modulates cell growth, division, and death (4, 15). TFG also interacts with cyclic AMP response element-binding protein (CREB), a transcription factor required for cell growth and survival (16). Inactivation of TFG or CREB by RNA interference results in a significant increase in the number of apoptotic corpses during embryogenesis; and embryos overexpressing TFG contain fewer cell corpses (4, 17). These data suggest the TFG seems to interact with factors related to cell growth, proliferation, and apoptosis.

Fusion Proteins

Structural genomic aberrations have been found to produce fusion oncogenes, which are one of the most common mechanisms of oncogenesis (18, 19). Among them, chromosomal translocations are well-known genetic aberration in cancer such as leukemia, lymphoma and sarcoma, and several types of epithelial carcinomas, such as thyroid carcinoma, salivary gland mucoepidermoid carcinoma, renal cell carcinoma, secretory breast carcinoma, and prostate carcinoma (19, 20). Translocation links two distinct chromosomes, and often generates chimeric proteins by fusing segments of two distinct genes, and can lead to cancer (21). Such chromosomal translocation is a high level of intrinsic structural disorder, enabling fusion proteins to evade cellular surveillance mechanisms that eliminate misfolded proteins (21). All fusion genes have common features such as constitutive expression due to house-keeping promoters, nuclear/cytoplasmic localization of the respective fusion proteins, harboring a di-merization domain within the NH2-terminus, and constitutive activation due to autophosphorylation of tyrosine residues of the kinase domain (20). Furthermore, fusion proteins contribute to generating an oncogenic signal mainly through three major mechanisms: a phosphorylation site and a tyrosine-kinase domain are fused, and structural disorder of the intervening region enables for intra-molecular phosphorylation; the fusion of a DNA-binding element to a transactivator domain results in an aberrant transcription factor that causes severe misregulation of transcription; and a dimerization domain fuses with a tyrosine kinase domain and disorder enables the two subunits within the homodimer to engage in permanent intermolecular phosphorylation (21). Therefore, receptor tyrosine kinase (RTK) appears to play an important role in the functions of the fusion proteins. RTKs act as regulators of normal cellular physiology, and genetic lesions of RTKs such as point-mutations, overexpression, and structural genomic re-arrangements, could induce oncogenic activations of RTKs, and lead to the development and progression of human cancer (18). In total, approximately 60 human RTKs have been identified and some are involved in fusion proteins, and display constitutive tyrosine kinase activity (18). Almost all RTK-derived fusion proteins lack the transmembrane domain and are assumed to be prevalently cytosolic (18). In addition, most effector proteins involved in the RTK-mediated signal transductions are re-distributed to the plasma membrane after activation of RTKs (18).

TFG Fusion Protein and Cancer

TFG has been identified as a fusion partner of neurotrophic tyrosine kinase receptor 1 (*NTRK1*) in generating the thyroid *TRK−T3* oncogene, and is also involved in oncogenic re-arrangement with anaplastic lymphoma receptor tyrosine kinase (*ALK*) in anaplastic lymphoma and neuron-derived orphan receptor 1 (*NOR1*) in extraskeletal myxoid chondrosarcoma (5, 22-25). Thus, TFG fusion proteins may act as oncoproteins in the tumorigenesis of human cancer. The *TFG* fusion genes occur mainly through fusion between the 5' end of *TFG* and the 3' end of the associated kinase gene (26). This is an important feature as not only is TFG ubiquitously and highly expressed across tissues, but the

presence of a coiled coil domain also allows for constitutive autophosphorylation and oncogenic activation of the fused RTK (27). In such an RTK fusion protein, the constitutive tyrosine kinase activity will activate signal transduction pathways and lead to cellular transformation (18). On the other hand, TFG may re-localize the activities of the TFG fusion proteins to ER exit sites, where they may prematurely phosphorylate substrates during ER export, and cause the premature stimulation of multiple effectors, including components of the extracellular signal-regulated kinase 1/2 (ERK1/2) kinase cascade, which leads to cell transformation (3). In such a case, stimulation of ERK1/2 at ER exit sites may further cause hyperphosphorylation of Sec-16, resulting in the formation of new exit sites that would recruit extra TFG fusion proteins (3). As a whole, the TFG fusion proteins act through the mechanism of dimerization or cytoplasmic relocalization which brings about activation of RTKs (21). Such a fusion between TFG and RTK induces a feed-forward mechanism that may activate tyrosine kinase activity and lead to oncogenesis (3). Furthermore, both the RTK portion and the TFG portion of the oncogenic fusion proteins are essential for transforming activity (4).

The possible fusion partners of TFG have been found to include NTRK1, ALK, nuclear factor-kB (NF-kB) essential modulator (NEMO), TNF receptor-associated factor-associated NF-kB activator (TRAF-associated NF-kB activator, TANK), NOR1, and translocated in extraskeletal chondrosarcoma (TEC), and peptidyl-prolyl cis/trans isomerase NIMA interacting 1 protein (PIN1) (28, 29). For example, NTRK1 is a high-affinity receptor for nerve growth factor (NGF) (30). Binding of NGF to NTRK1 leads to its dimerization and autophosphorylation, ultimately causing the activation of several downstream signaling cascades, including RAS-RAF-mitogen-activated protein kinase kinase (MAPKK, MEK)−ERK pathway, to promote cell survival and growth (31). The sequences encoding the *N*-terminus of TFG can fuse to the *C*-terminus of NTRK1 (25), and in addition, *N*-terminal domain of TFG localizes to ER exit sites and is also capable of redirecting the *C*-terminal domain of NTRK1 there (3). The fusion between TFG and NTRK1 in thyroid carcinomas may yield the chimeric protein product TRK−T3, which exhibits transforming activity in NIH3T3 cells (5, 27). The *ALK* gene encodes a tyrosine kinase receptor belonging to the insulin growth factor receptor superfamily (32, 33). ALK is expressed in the central and peripheral nervous systems and is important in the development of the mouse embryos, but not in adults (20). Genetic alterations involving *ALK*, including gene fusion, amplification, and mutations, have been identified in anaplastic large cell lymphoma, inflammatory myofibroblastic tumors, lung cancer and neuroblastoma (33). In addition, transfection of NIH3T3 cells with *TFG−ALK* fusion genes transforms the cells and the transforming capacity of these cells is related to the level of TFG−ALK fusion proteins (33). Furthermore, the inhibition of apoptosis and the promotion of cellular proliferation in the TFG−ALK-transfected NIH-3T3 cells occur through the activation of downstream PI3K/protein kinase B (PKB or AKT) and MAPK signaling pathways (33). TANK, also a TFG fusion partner, has been considered to play a role in oncogenesis as a constitutive expression of TRAF-1, TRAF-2 and TANK/TRAF-interacting protein (I-TRAF) has been noted in cancer (34). TNF has been noted to interact with its receptors and recruits these factors including TANK to activate NF-kB and then amplify the expression of anti-apoptotic genes in glioma tissues (25, 35). In human extraskeletal myxoid chondrosarcoma, t(3;9)(q11-q12;q22) translocation is associated with a chimeric molecule in which the N-terminal domain of *TFG* is fused to the *TEC* gene (29). The chimeric gene encodes a nuclear protein that binds DNA with the sequence specificity of the parental TEC protein (29). The N-terminal domain of *TFG* in *TFG−TEC* induces a 12-fold increase in the activation of luciferase, indicating that the N-terminal domain of TFG in the TFG−TEC protein has intrinsic transcriptional activation properties (29). This means the fusion gene encodes a transactivator that is more potent than TEC, and full integrity of the *N*-terminal domain of the TFG is necessary for full transactivation (29). In addition, ectopically-expressed TFG−TEC and TEC can up-regulate the expression of S-phase kinase-associated protein 2 (SKP2), L-Myc, suppressor of cytokine signaling 2 (SOCS2) and signal transducer and activator of transcription 3 (STAT3), which are more strongly up-regulated by TFG−TEC than by TEC; and these findings may explain how TFG−TEC plays an oncogenic role in the development of human extraskeletal myxoid chondrosarcoma (29).

TFG Mutation and Expression in Cancer

From the evidence above, it is probable that TFG−RTK fusion proteins are important in oncogenesis (18); however, the role of TFG-alone in oncogenesis is still unclear. In prostate cancers, PIN1, a peptidyl-prolyl isomerase, is involved in cell transformation and the maintenance of the malignant phenotype in prostate cancer (36). From PIN1-proteomic analysis, TFG has been found to be a PIN1-binding phosphorylated protein and is up-regulated in prostate cancer cell lines and tissues (36). The TFG expression levels in prostate cancer tissues are higher than in non-cancerous tissues in 63.9% of the cases (36). Whilst the sole expression of either PIN1 or TFG has only minor effects on NF-kB luciferase reporter activity, the co-expression of PIN1 and TFG produces significant reporter activity (36). Targeted inhibition of TFG by specific silencing RNA results in reduced cell proliferation and

induction of premature senescence in PC3 prostate cancer cells, with the cells being relatively larger and containing vacuolated nuclei and a granular cytoplasm (36). Furthermore, TFG expression is noted to be closely associated with both a higher probability and shorter period of tumor recurrence following surgery (36). Because TFG activates the cell signaling mediated by NF-kB and androgen receptor in prostate cancer cells, TFG is considered to engage in the hormone dependency of recurrent prostate cancers (36). As a whole, TFG is a PIN1-interacting oncogenic protein and plays a role in the cell growth and tumorigenesis of prostate cancer, and, thus, it can be considered a potential diagnostic and prognostic marker and therapeutic target in prostate cancer (36). From these data, TFG appears to function like an oncoprotein in its role in the TFG fusion proteins.

In contrast, there are several reports suggesting TFG is more likely to function as a tumor suppressor (9, 26). Ikaros, a Kruppel-type zinc finger protein, is essential for normal lymphocyte development and differentiation, and it is frequently inactivated in both human and mouse leukemia and lymphoma (9). Therefore, Ikaros is considered a tumor suppressor and Ikaros inactivation affects the cellular response to radiation (9). In Ikaros-transfected mouse 3T3-L1 fibroblasts, the expression of TFG is up-regulated (9). In contrast, the expression of TFG is consistently down-regulated in radiation-induced T-cell lymphoma in B6C3F1 mice that exhibit defective Ikaros expression (9). These data suggest that TFG may function downstream of Ikaros and may be involved in radiation-induced lymphomagenesis (9). In another study, single-nucleotide polymorphism arrays were used to interrogate DNA copy number changes in a panel of 39 metastatic melanoma cell lines (26). Several genes were demonstrated to have focal homozygous deletions and sequenced to reveal non-synonymous somatic mutations in *TFG* in approximately 5% of melanomas (26). The *TFG* mutations include a mini mutation hotspot at amino acid residue 380 (P380S and P380L) and the presence of multiple mutations in two melanomas (26). In addition, there is a trend for non-synonymous mutations to occur toward the carboxyl terminus of TFG and the overlap between the location of these mutations and the deletion in oncogenic fusion events suggests an important structural or functional role of the 3' end of TFG (26). Because TFG is involved in the NF-kB and MAPK pathways, and activation of MAPK pathway occurs in about 70% of melanomas and the NF-kB pathway is important in inhibition of apoptosis and treatment resistance in melanomas, *TFG* mutation is considered important in the tumorigenesis of melanomas (26). However, the number of melanomas with a *TFG* mutation is low; therefore, the mutation may represent an alternative mechanism of tumorigenesis (26).

Conclusion

Identification of chromosomal events that lead to fusion proteins were mainly restricted primarily to hematological malignancies. However, data in the literature suggest that the mechanisms for creating oncogenic fusion proteins may occur in solid tumors and the fusion proteins may contribute to oncogenesis in solid tumors. TFG fusion proteins have been found to play a role in the oncogenesis, with the activity of TFG fusion proteins promoting tumor development by inhibiting the normal cell-death program and activating the cell growth-regulatory machinery (4). Because there is heterogeneity between different tumor types and intra-tumor heterogeneity (37), technologies such as genome-wide comparative genomic hybridization arrays, single-nucleotide polymorphism arrays, *in situ* polymerase chain reaction (PCR), fluorescent *in situ* hybridization, rapid amplification of cDNA end-coupled PCR and sequencing might be helpful to identify the presence of TFG fusion proteins or other fusion proteins in cancer (19, 26, 33). In addition, more *in vitro* and *in vivo* studies are necessary to demonstrate the transforming properties of TFG fusion proteins. Furthermore, the expression of TFG may also cause changes of the expression of fusion partners and modify tumorigenesis. Experiments that blocking RTK or TFG, or both RTK and TFG should be performed to delineate their actual roles in the tumorigenesis. The role of TFG itself in tumorigenesis, functioning as an oncoprotein or tumor suppressor, is still unclear and deserves further studies. To clarify this, it is important to know the degree of TFG expression in more cancer cell lines and tumor tissues of different grades of malignancies. The *in vitro* and *in vivo* experiments should also include the effects of blockade and overexpression of TFG on the proliferation rate, colony formation, *in vitro* invasiveness, migration through the endothelial barrier, and tumorigenicity of cancer. Finally, it is important to define the molecular details of TFG, and determine which downstream target genes are critical for tumorigenesis and whether TFG fusion proteins collaborate with these genes to generate cancer. As a whole, since TFG fusion proteins and probably TFG-alone play a role in tumorigenesis, identification and characterization of fusion proteins or TFG-alone may provide a valuable approach to the development of novel and effective pharmacological strategies for therapeutic management of solid tumors (18).

Acknowledgements

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