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Transgelin-2: Biochemical and Clinical Implications in Cancer and Asthma

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Abstract

Transgelin-2 has been regarded as an actin-binding protein that induces actin gelation and regulates actin cytoskeleton. However, transgelin-2 has recently been shown to relax the myosin cytoskeleton of the airway smooth muscle cells by acting as a receptor for extracellular metallothionein-2. From a clinical perspective, these results support transgelin-2 as a promising therapeutic target for diseases such as cancer and asthma. The inhibition of transgelin-2 prevents actin gelation and thereby cancer cell proliferation, invasion, and metastasis. Conversely, the activation of transgelin-2 with specific agonists relaxes airway smooth muscles and reduces pulmonary resistance in asthma. Here, we review new studies on the biochemical properties of transgelin-2 and discuss their clinical implications for the treatment of immune, oncogenic, and respiratory disorders.

Transgelin-2 Is a Unique Actin-Binding Protein Involved in Cancer and Asthma

Actin is a critical component of the cellular cytoskeleton that controls most cellular functions, from cellular differentiation and migration to receptor location and intracellular signaling [1]. **Actin-binding proteins** (see Glossary) account for approximately 20% of the total cellular proteins and control cellular functions by regulating **actin polymerization** and remodeling [2,3]. The transgelins are a family of actin-binding proteins that were named for their potential to induce **actin gelation** [4]. Transgelin proteins were first isolated from chicken gizzards, in which they were the most abundant proteins in **smooth** but not **skeletal muscles** [5]. These experiments revealed three proteins with the same molecular weight of 22 kDa but different isoelectric points (pI) in two-dimensional gel electrophoresis [6]. These proteins were named transgelin-1, the most basic polypeptide with a pI of 9.0; transgelin-2, with a pI of 8.4; and transgelin-3, the most acidic polypeptide with a pI of 7.0 [7–9].

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Of these, transgelin-2 is among the most abundant proteins expressed in smooth muscles, and it was first named smooth muscle 22 α (known as transgelin-1)-homolog or smooth muscle 22 β [10]. Transgelin-2 is expressed shortly after the other actin-binding proteins, such as tropomyosin and desmin, and at the same time as the **myosin light chains (MLC)** during the development of the chicken gizzard [5,11]. Transgelin-2 has distinct biochemical and biological properties, different from the other transgelin proteins, making it a promising pharmacological target that may provide therapeutic advantages for treating different diseases [12–14]. In this review, we highlight new studies on the functions of transgelin-2 and discuss the clinical implications of this actin-binding protein in immune diseases, cancer, and asthma.

Transgelin-2 Gene, Structure, and Biochemical Features

Transgelin-2 has a unique genetic organization and transcriptional regulation compared with the other transgelins [4]. Transgelin-1 and -3 are encoded in the *TAGLN1* and *TAGLN3* genes located on the human chromosomes 11q23.3 and 3q13.2, respectively, while transgelin-2 is encoded in the *TAGLN2* gene located on human chromosome 1q23.2 [4,15] (Figure 1). This special location is near other important genes, such as the Fc fragment of IgE receptor Ia (closely related to asthma and eczema) and IGSF8 immunoglobulin superfamily member 8 (functioning as a tumor suppressor) (<https://www.ncbi.nlm.nih.gov/gene/8407>).

Transgelins are first regulated by alternative splicing, producing multiple transcripts. *TAGLN1* and *TAGLN3* have five exons that can produce eight and seven transcripts, respectively (Ensembl IDs of human *TAGLN1* and *TAGLN3* are ENSG00000149591 and ENSG00000144834, respectively) [16,17]. However, the *TAGLN2* gene has seven exons that can produce five transcripts (201–205; Ensembl ID: ENSG00000158710). Of these, transcript 205 does not contain an open reading frame, thus does not encode a protein, and transcript 204 has an incomplete 3' coding sequence. Transcripts 201 and 203 encode the same polypeptide with 199 amino acids, while transcript 202 encodes a polypeptide with 220 amino acids due to an extra sequence after the initial methionine in the N terminal, produced in exon 3 (Figure 1).

From a structural perspective, the transgelin proteins contain an N terminal single calponin-homolog (CH)-domain, an actin-binding motif, and a C terminal calponin-like repeated (CLR) region [4,14] (Figure 2). The CH-domain refers to a region of approximately 100 residues that was first identified at the N terminus of calponin, an actin- and calcium-binding protein that inhibits the ATPase activity of myosin in smooth muscles [18]. The CH-domain is preserved across the three transgelin isoforms with approximately 60% homology [19]. Although the actin-binding domain is critical for the transgelin proteins [4], it has less sequence identity among the three transgelins (approximately 20%–40%) [20]. By contrast, the C terminal CLR region that binds actin to stabilize the cytoskeleton represents the section with the highest grade of homology between the three transgelin proteins, with approximately 88% identity [20,21].

Unlike the other transgelins, transgelin-2 localizes at the cellular membrane; this is supported by confocal microscopy and proteomic analyses of plasma membranes isolated from either human hepatoma HepaRG cells or murine dendritic cells [22,23]. Transgelin-2 also appears in the membrane radial lamellipodium of T lymphocytes, accumulates in the immunological synapse of B lymphocytes, and localizes at the membrane ruffles of lipopolysaccharide (LPS)-stimulated macrophages [24–26]. Similar results have been found at the membranes of the **airway smooth muscle cells** (ASMCs) [12]. Confocal analyses of live rat ASMCs incubated with anti-transgelin-2 antibody revealed a strong signal at the cellular membrane [12]. These results suggest that transgelin-2 may be a membrane protein or interacts with transmembrane proteins such as **cadherins** [27].

The three transgelin proteins are expressed in different cell types (Table 1). Transgelin-1 is mainly expressed in visceral and vascular smooth muscle cells, and transgelin-3 is only expressed in neurons [18,28,29]. By contrast, transgelin-2 is widely expressed in most cell types, from smooth muscle cells to immune cells, but it is mainly expressed in the bronchial epithelium, lung mesenchyme, gastrointestinal epithelium, and the cartilaginous and periosteal layers of bones, although it is not expressed in the smooth muscle cells of the muscularis mucosa of the gastrointestinal tract [28]. In immune tissues, transgelin-2 is expressed in the thymus, spleen, lymph nodes, and immune cells (mostly in T and B lymphocytes and activated macrophages) [24]. These data suggest that transgelin-2 encodes a novel cell lineage-restricted cytoskeletal protein with a unique pattern of expression during development [28].

Regulation of Transgelin-2 Expression

Gene Expression Regulation

Transgelin-2 expression is regulated at the transcriptional level by three major signaling pathways, including **NF- κ B**, **transforming growth factor β (TGF β)**, and **extracellular signal-regulated kinase (KRAS-ERK)** pathways [10,30,31].

Transgelin-2 expression is regulated by the NF- κ B pathway in response to stimuli such as bacterial endotoxin [10]. Though transgelin-2 has a constitutively high expression in all subsets of T lymphocytes, regardless of their activation, transgelin-2 expression in macrophages is induced by LPS via Toll-like receptor-4 (TLR4) [10]. Consistently, the induction of transgelin-2 expression by LPS is completely suppressed after treatment with NF- κ B inhibitors [10]. Transgelin-2 is the only transgelin containing the NF- κ B consensus motif (–174 to –179) upstream of its coding sequence, and point mutation of this region was shown to significantly reduce the transgelin-2 promoter activity by luciferase reporter assay [10]. Given that LPS activates the NF- κ B pathway via TLR4, other agonists for TLR4 are expected to induce transgelin-2 expression to modulate macrophage activity [32].

In addition to the NF- κ B pathway, transgelin expression is also regulated by TGF β [30,33]. Specifically, low concentrations of TGF β (2 ng/ml) were shown to induce the rapid expression of transgelin-1, within 30 minutes after treatment, without affecting the transgelin-2 expression in human lung epithelial A549 cells [34]. TGF β also induced transgelin-2 expression in human lung epithelial A549 cells, but at higher TGF β

concentrations (5 ng/ml) and with longer treatment times (72 hours) [30]. Similar results were found in other cell lines; for example, high concentrations of TGF β (5 ng/ml) induced transgelin-2 expression in SW480 colon carcinoma cells after 48 hours of treatment [33]. As TGF β is an important cytokine in cell growth, cell differentiation, and immune system regulation, its role in regulating transgelin-2 may be an important mechanism linking transgelin-2 with both immune and oncogenic alterations.

The third pathway modulating transgelin-2 expression is the epidermal growth factor receptor (EGFR) and KRAS-ERK signaling pathway [31]. The most relevant results of these studies are directly related to high transgelin-2 expression in pancreatic ductal adenocarcinoma (PDAC), representing the fourth leading cause of cancer-related death in the United States [35,36]. The most common KRAS mutation, KRAS-G12D, is a typical hallmark in more than 90% of PDACs and induces both tumor cell proliferation and transgelin-2 expression via ERK2 [31]. These results are clinically relevant because the direct inhibition of KRAS is clinically challenging and KRAS targeted therapy has not been successful [31]. Furthermore, the KRAS signal network is different in PDACs, non-small cell lung cancers (NSCLC), and colon cancers [37]. Thus, the total inhibition of the KRAS pathway may produce unspecific deleterious effects that can be avoided by specifically targeting transgelin-2 [31].

Protein Expression Regulation

Similarly, recent studies indicate that transgelin-2 expression can be inhibited by microRNAs [4], such as miR-1 [38,39], miR-133 [40], miR133b [41], and miR-145-5p [42]. miR-1 binds to bases 71–77, 185–191, and 348–354 of the 3'-untranslated regions (UTRs) of messenger RNA to inhibit transgelin-2 transcription in human head and neck squamous cell carcinomas [38]. miR-1 has also been shown to suppress cell proliferation, invasion, and metastasis of esophageal squamous cell carcinoma by targeting transgelin-2 [39]. Likewise, miR-133 reduces the proliferation and migration of vascular smooth muscle cells by specifically inhibiting transgelin-2 expression without affecting transgelin-1 expression [40]. miR-133b inhibits invasion, angiogenesis, and colony formation in human bladder cancer cell lines 5637 and T24 by targeting transgelin-2 via the direct binding of the 215–221 of the 3'-UTR [41]. miR-145-5p also suppresses transgelin-2 expression in human bladder cancer by targeting the 211–217 of the 3'-UTR [42].

Post-Translational Regulation of Transgelin-2

The biological activity of transgelin-2 is mainly regulated by phosphorylation (Figure 2). A phosphorylation analysis identified at least six serine or threonine phosphorylation sites in the transgelin-2 of Jurkat T cells [43]. More specifically, a large-scale mass spectrometry analysis showed that the transgelin-2 in HeLa and K562 cells can be phosphorylated at Ser-11, Ser-163, Ser-185, Thr-180, Thr-190, and Tyr-192 [44].

Although the actin-binding domain is not phosphorylated, it is flanked by two phosphorylation sites at Ser-145 and Ser-163, which are phosphorylated by ERK2 and PKA, respectively [43] (Figure 2), and appear to have major clinical implications in tumor malignancy by contributing to cellular migration and invasion [31]. The transgelin-2

phosphorylation at Ser-145 stabilizes transgelin-2, as shown by the phospho-deficient mutant of transgelin-2, S145A, which has a lower protein stability in a proteasome-mediated protein degradation assay [31]. This phosphorylation has also been associated with the prognosis of PDAC; during PDAC, the KRAS-G12D mutation enhances the ERK2 interaction with transgelin-2 and induces Ser-145 phosphorylation [31]. On the other side of the actin-binding domain, PKA phosphorylates Ser-163 located between the actin-binding domain and the CLR-region [43]. This phosphorylation also contributes to oncogenesis because the phospho-deficient mutants of transgelin-2 S83D and S163D both enhance the cell motility of hepatocellular carcinoma cells by preventing actin polymerization [45]. This phosphorylation also plays an important role in metabolism by modulating insulin production in insulinomas [46]. High glucose levels decrease the transgelin-2 phosphorylation at Ser-163 within 5 minutes in rat insulinoma-derived INS-1E cells [46]. Phospho-deficient mutants of transgelin-2 S163A or S163D decrease insulin production in INS-1E cells at high concentrations of glucose (16 mM) [46].

In addition to ERK2 and PKA, transgelin-2 is regulated by PKC, which can phosphorylate Thr-180 and Ser-185 located at the CLR-region [43]. Given that transgelin-1 Ser-181 phosphorylation at the CLR-region reduces the transgelin-1 binding to actin *in vitro* [47], it is suggested that phosphorylation of transgelin-2 Thr-180 and Ser-185 in the CLR-region can contribute to actin depolymerization [43,47]. These studies also reveal that transgelin-2 appears to have only one tyrosine phosphorylation site [44]. Tyr-192 is located at the distal part of the CLR-region and is phosphorylated in HeLa and K562 cells [44]. The phosphorylation of transgelin-2 at Tyr-192 was found in different kinds of sarcoma cell lines, suggesting its importance in the malignant process of sarcoma [48]. Future studies are required to determine the specific extracellular signals and intracellular pathways modulating these phosphorylation events, as well as their biological and clinical implications.

Transgelin-2 Protein Interaction Networks

Transgelin proteins are defined by their potential to induce actin gelation [4]. In contrast to the strong, rope-like structures formed by most actin-binding proteins, the bundles of transgelin-2 and actin are loose, gel-like structures that allow a higher flexibility [24]. The principle role of transgelin-2 is to modulate actin polymerization [24]. From a biochemical perspective, transgelin-2 binds to actin with a substantially higher binding ratio of 2–8:1 compared with that of common actin-binding proteins with a bundling activity of 0.05–0.5:1 [24,49]. Further, the dimerization of transgelin-2 may help combine actin filaments into bundles [19,24]. Structurally, the CH-domain and actin-binding motif of transgelin-2 are essential to mechanically connect two adjacent actin filaments and thereby mediate multimeric interactions [50]. Transgelin-2 can hinder the binding sites of actin for other accessory proteins and thus prevent their binding to actin [24,50]. For example, transgelin-2 competes with cofilin to bind to actin and thus blocks cofilin-mediated actin depolymerization [24]. Transgelin-2 also prevents actin-related protein 2/3 (Arp2/3)-mediated **actin nucleation** by overlapping and competing with its binding site to actin [50].

Transgelin-2 can interact with **ezrin** and regulate critical signal transduction pathways (Figure 2) [12]. Ezrin has emerged as a key regulator of the actin cytoskeleton by orchestrating signals from the membrane surface to the actin, and contains an N terminal **four-point-one, ezrin, radixin, moesin (FERM)** domain, a central linker region, and a **C-terminal ERM-associated domain (C-ERMAD)** [51]. Of these domains, transgelin-2 interacts with the N terminal FERM domain of ezrin through its CLR region (K_D of 73.3 nM) to stabilize the activated membrane receptor complex [12]. RhoA regulates this interaction [51]; activation of RhoA increases the intracellular levels of phosphatidylinositol 4,5-bisphosphate (PIP₂), causing the binding of PIP₂ to the FERM domain of ezrin [52], and induces the phosphorylation of ezrin Thr-567 by Akt [53], or calcineurin homologous protein-1 (CHP1) [54], etc. [51]. Together, this causes the release of the ezrin C-ERMAD domain from the FERM domain, allowing its binding to F-actin [55]. Sustained activation of ezrin by RhoA allows transgelin-2 to bind to the ezrin N terminal FERM domain [12]. Conversely, ezrin also interacts with the RhoA-GDP dissociation inhibitor (GDI) complex through its FERM domain to sustain RhoA activation [56], thereby, it in turn continues to activate ezrin and promote the interaction of ezrin with transgelin-2 [12,51]. Unlike reversible biochemical reactions, the regulatory mode of transgelin-2 and ezrin contains a positive-feedback loop that creates a switch-like, irreversible bistable response [57]. Once the transgelin-2 and ezrin complex is formed, it can be reinforced by this positive-feedback loop, even long after the initial stimulus is removed or until a new stimulus occurs.

Clinical Implications of Transgelin-2

Transgelin-2 in Immune Responses

The specific expression of transgelin-2 in the immune system suggests its role in immunological disorders [24]. Transgelin-2 can regulate the actin cytoskeleton of multiple immune cells, thus modulating the activation, differentiation, and phagocytosis of lymphocytes and macrophages [4]. It regulates the activation, cytokine production, and interaction of T lymphocytes with antigen-presenting cells [10,58] by stabilizing cortical interactions with F-actin at the immunological synapse and acquiring the leukocyte function-associated antigen-1 (LFA-1) activation after T cell receptor (TCR) stimulation [24,49] (Figure 3). The inhibition of transgelin-2 expression reduces the F-actin content and destabilizes the F-actin ring formation, resulting in decreased T cell adhesion and spreading [24]. Transgelin-2-deficient T cells also exhibit lower cytokine production and cytotoxic effector function, and deletion of the actin-binding motif of transgelin-2 dramatically reduces the production of interleukin-2 (IL-2) in T lymphocytes [24]. Transgelin-2 is also expressed in B lymphocytes, although at lower levels than in T lymphocytes and with a more specific cellular pattern [59,60]. B lymphocytes expressing transgelin-2 are mainly located at **germinal centers** of secondary lymphoid tissues, including the lymph nodes and the spleen [59]. B1 lymphocytes have 60-fold higher levels of transgelin-2 protein than B2 cells, suggesting that transgelin-2 could be a potential biomarker to identify B2 lymphocytes and study their differentiation [60]. The knockout of transgelin-2 in B lymphocytes prevents T lymphocyte activation by reducing cytokine production, such as IL-2 and interferon- γ [25]. Finally, while transgelin-2 expression in inactive macrophages is very low, its expression is highly upregulated by bacterial endotoxins to enhance cytokine production and phagocytosis

[10]. The inhibition of transgelin-2 expression in macrophages decreases their phagocytic potential, and transgelin-2 knockout mice are more susceptible to bacterial infection and have higher mortality rates during bacteremia [10,26]. These results support the role of transgelin-2 in the formation of phagocytic synapses in macrophages that facilitate the clearance of infectious bacteria [10].

From a clinical perspective, recent studies indicate that transgelin-2 may serve as a biomarker of immune disorders such as lupus erythematosus [59]. Clinically, transgelin-2 expression is significantly increased in B lymphocytes in the lymph nodes and kidneys of patients with systemic lupus erythematosus [59]. Furthermore, transgelin-2 may be a suitable target to balance immune responses because it compensates for the lack of co-stimulatory signals. For example, transgelin-2 is involved in LFA-1 activation in TCR-activated T lymphocytes [24,49]. Transgelin-2 binds LFA-1 and the small GTPase Rap1 at the immunological synapse to enhance the interaction with the intercellular adhesion molecule, thereby providing co-stimulation for T lymphocyte activation [4,61] (Figure 3).

Transgelin-2 as a Biomarker and Target for Cancer

The disorganization and rearrangement of actin filaments represent a fundamental event in cancer cell invasion, metastasis, and drug resistance [62,63], and transgelin-2 directly regulates the actin filaments that are closely associated with these activities [13,64] (Figure 4, Key Figure). Transgelin-2 may facilitate the migration of cancer cells because the stability of the transgelin-2/actin complex is stronger than that of F-actin [50]. Transgelin-2 overexpression increases cell invasion, migration capacity, and γ -radiation resistance in human NSCLC cells, thus promoting tumor relapse and recurrence [65]. The overexpression of transgelin-2 causes a significant increase in cell invasion and proliferation in both U87 MG glioma cells and primary GBM30 glioma neurospheres [66]. Consistently, the decrease of transgelin-2 expression by promoter hypermethylation in isocitrate dehydrogenase mutant gliomas is associated with favorable outcomes compared with gliomas without the mutation [66]. The downregulation of the transgelin-2 expression significantly decreases cell invasion, proliferation, and colony formation in the human malignant meningioma cell line CH157, while significantly increasing the apoptosis rate of the cells [67].

Additionally, transgelin-2 expression is strongly upregulated in pancreatic cancer [68,69], colorectal cancer [70,71], lung adenocarcinomas [72], as well as hepatocellular carcinomas [73], uterine cervical squamous cell carcinomas [74], papillary thyroid carcinoma [75], and bladder cancers [76,77], suggesting that transgelin-2 may serve as a new biomarker to predict the progression and prognosis of cancers [13] (Table 2). More specifically, tissue microarray analyses show a positive staining rate for transgelin-2 of 85% in colorectal cancer, 50% in adenomas, and 75% in hepatic metastasis [70]. A study indicates that transgelin-2 expression is upregulated by 70% in 11 of 16 patients with hepatocellular carcinoma [73]. The mRNA and protein levels of transgelin-2 are increased in human pulmonary adenocarcinoma tissues by 14.3- and 7.0-fold, respectively [78]. Further, the expression of transgelin-2 is correlated with the prognosis of colorectal cancer [70,71]. The mean survival rates of colorectal cancer patients with low, moderate, and high expression levels of transgelin-2 are 49.8 ± 13.6 , 31.6 ± 19.0 , and 11.4 ± 11.0 months, respectively [70].

The phosphorylation of transgelin-2 also plays an important role in the prognosis of cancer [31, 44,45]. Higher levels of transgelin-2 phosphorylation at Ser-145 are associated with poor prognosis in cancer patients with PDAC [31]; among 114 patients, 61 patients (54%) with high levels of transgelin-2 Ser-145 phosphorylation had a median survival of 9 months compared with the 33-month survival rate in the other 53 patients with lower levels of transgelin-2 phosphorylation [31]. Together, these studies reveal that transgelin-2 expression and phosphorylation are associated with a poor prognosis and survival expectancy in cancer.

The predictive significance of transgelin-2 appears to be different from that of transgelin-1, a potentially similar biomarker candidate for cancer [13]. Unlike transgelin-1, transgelin-2 expression is abundant in the neoplastic glands but poor in the tumor stroma in human pulmonary adenocarcinoma tissues [78]. Consistently, transgelin-1 is stained mainly in the tumor stroma of human breast cancer and is observed in b 20% of tumor cells, while transgelin-2 is stained with higher intensities in tumor cells [79]. Furthermore, the expression of transgelin-2, but not transgelin-1, is also increased in alveolar pneumocytes affected by atypical adenomatous hyperplasia [78]. The main difference between transgelin-1 and transgelin-2 is that their levels change conversely during tumor development [13]. Transgelin-1 expression is mainly in the tumor stroma and its level increases with differentiation of the tumors [13]. On the contrary, transgelin-2 expression is restricted to the tumor cells and its level decreases with differentiation of the tumors [13]. The difference from transgelin-1 may be important to highlight the specificity of transgelin-2 as a potential therapeutic target in cancer.

Targeting transgelin-2 may provide therapeutic advantages in cancer treatment [13,14]. The inhibition of transgelin-2 expression prevents drug resistance in cancer chemotherapy. For example, the inhibition of transgelin-2 expression with small interfering RNA sensitizes a paclitaxel-resistant human breast cancer cell line (MCF-7/PTX) to paclitaxel [80,81]. Phenolic compounds such as paeonol and salvianolic acid A can reverse the paclitaxel resistance in MCF-7/PTX cells as these compounds can both decrease transgelin-2 expression [80,81]. Consistently, the incubation of MCF-7/PTX cells with the taxoid compound SB-T-121205 (20 nM for 48 hours) significantly suppresses transgelin-2 expression and induces apoptosis [82]. Given that the transgelin-2 phosphorylation is closely related to the cellular motility, the inhibition of transgelin-2 phosphorylation may also be a potential therapeutic strategy in cancer treatment to prevent cell migration and proliferation in metastasis [31,45]. The phospho-deficient transgelin-2 mutants S83A and S163A significantly decrease cell migration in hepatocellular carcinoma cells, whereas the phospho-mimetic mutants S83D and S163D increase cell migration [45]. Transgelin-2 Ser-145 phosphorylation can be blocked with the ERK inhibitor U0126, and the phospho-deficient transgelin-2 mutant S145A reduces cell proliferation and induces smaller tumors [31]. Together, these results suggest that transgelin-2 can be used as a potential biomarker for prognosis and a clinical target for cancer treatment.

Receptor-like Relaxation of ASMCs in Asthma

Recent studies indicate that, in addition to being an actin-binding protein, transgelin-2 can relax the myosin cytoskeleton of the ASMCs and thus reduce pulmonary resistance in

asthma [12]. These studies also suggest that transgelin-2 may act as a receptor to trigger an intracellular signaling pathway leading to the dephosphorylation of MLC and relaxation of the myosin cytoskeleton (Figure 4); a protein signaling study showed that transgelin-2 activation by metallothionein-2 induced the phosphorylation of RhoA Ser-188 and the dephosphorylation of ROCK Tyr-722, **myosin phosphatase target subunit 1 (MYPT1)** Thr-853, and MLC Ser-20 in ASMCs [12]. Therefore, the active **MLC phosphatase (MLCP)** complex dephosphorylates MLC, inducing ASMC relaxation to reduce asthmatic pulmonary resistance [12] (Figure 4). This potential of metallothionein-2 to relax ASMCs is specific for transgelin-2 and is not shared with the other transgelins [12]. Although transgelin-1 expression is increased in patients with asthma, the overexpression of transgelin-1 affects neither actin filament propulsion nor the asthmatic airway [83]. These results suggest that transgelin-1 may contribute to slow actin filament remodeling, while transgelin-2 influences actin and myosin in the rapid contraction–relaxation cycle of ASMCs.

From a pharmacological perspective, selective transgelin-2 agonists may provide therapeutic advantages to reduce pulmonary resistance in asthma [84]. Current treatments for asthma are based on β_2 -agonists, activating β_2 -adrenoceptors to relax ASMCs [85]. However, the β_2 -adrenoceptors are susceptible to desensitization [86]. TSG12, a transgelin-2 agonist, is more effective than β_2 -agonists in relaxing human ASMCs and TSG12 can provide clinical advantages for asthma treatment by preventing receptor desensitization [12]. Additional studies to elucidate more transgelin-2 agonists may enable alternative treatments for asthma.

Concluding Remarks

Herein, we have discussed recent studies that show transgelin-2 has unique biochemical properties for the coordination of both the actin and myosin cytoskeletons and may provide therapeutic advantages in treating immune diseases, cancer, and asthma. However, because transgelin-2 is a structural protein and it is widely expressed, the potential side effects should be noted [87] and specific targeting of transgelin-2 should be considered (see Outstanding Questions). We note that, as compared with transgelin-1, transgelin-2 expression is mainly restricted to the tumor cells [13]. We believe this restricted expression of transgelin-2 in tumor cells increases its clinical implications and diminishes the potential toxic side effects if transgelin-2 can be selectively targeted using appropriate drug delivery systems [88]. The possible selective inhibition of transgelin-2 in cancer cells may be crucial for further development and the lowest effective dose should be identified [89]. Specific transgelin-2 targeting has been observed; the inhalation of transgelin-2 agonist TSG12 significantly abrogated pulmonary resistance in asthma without disturbing the functions of the other organs, such as the blood pressure [12]. In the future, new compounds and new drug delivery methods that specifically regulate the expression and phosphorylation of transgelin-2 can be designed and used to confirm its biological functions and provide new treatments for immune, oncogenic, and respiratory disorders.

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Glossary

Actin-binding proteins

proteins bind actin and regulate the functions of actin filaments

Actin gelation

actin filaments crosslinked with other proteins to form a gel-like network

Actin nucleation

the initiation of actin polymerization from free monomers

Actin polymerization

the rapid conversion of monomeric actin (G-actin or globular-actin) to the polymeric form of actin (F-actin or filamentous-actin)

Airway smooth muscle cells

a major structural component of the airway; these play an important role in both normal breathing and pathophysiologic conditions such as asthma

Cadherins

a type of transmembrane protein that mediates in cell–cell adhesion

C-terminal ERM-associated domain (C-ERMAD)

domain of ezrin that is important for regulating F-actin and intramolecular interaction

Extracellular signal-regulated kinase (KRAS-ERK)

member of the mitogen-activated protein kinase family that transmits signals from extracellular agents to regulate extensive cellular processes

Ezrin

a protein that has the ability to interact with both the plasma membrane and actin

Four-point-one, ezrin, radixin, moesin (FERM)

domain of ezrin that is required for membrane binding

Germinal centers

specific areas that maintain the mature stage of naive lymphocytes and initiate the adaptive immune responses

MLC phosphatase (MLCP)

a heterotrimer consisting of a phosphatase catalytic subunit (PP1C δ), a myosin phosphatase targeting subunit (MYPT1), and a 20-kDa subunit (M20) that dephosphorylates MLC

Myosin light chains (MLC)

the low molecular weight component of myosin; the myosin light chain phosphorylation plays an important role in muscle contraction

Myosin phosphatase target subunit 1 (MYPT1)

the 110-kDa subunit of MLCP that can enhance the catalytic activity and specificity

NF- κ B

a family of highly conserved transcription factors that plays a key role in regulating immune response to infection

Skeletal muscles

a striated muscle that is under the voluntary control of the somatic nervous system

Smooth muscles

an involuntary nonstriated muscle that causes a hollow organ, such as airway smooth muscle, to contract or relax

Transforming growth factor β (TGF β)

a multifunctional cytokine that plays a pivotal role in diverse cellular processes

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Highlights

Actin is a critical component of the cellular cytoskeleton and interacts with a plethora of actin-binding proteins

Transgelin-2 is an important actin-binding protein and misregulation of transgelin-2 may contribute to diseases such as cancer and asthma

Ezrin has emerged as an essential regulator of transgelin-2 in biological functions other than those involving actin.

Specific targeting of transgelin-2 may result in a high potential to selectively and effectively regulate various diseases.

Outstanding Questions

How does transgelin-2 phosphorylation regulate its binding to either actin or ezrin?

If transgelin-2 indeed interacts with the membrane, what is the exact transmembrane model?

What other necessary proteins are required for transgelin-2 to orchestrate cellular signals?

Because transgelin-2 is widely expressed, can specific agonists be designed and applied in the clinic selectively and effectively?

Can specific targeting of transgelin-2 be a way to treat diseases with reduced side effects and more benefits?

If targeting transgelin-2 is toxic for healthy cells, are there alternative targets with which it interacts that may yield high specificity and less toxicity?

Is targeting the specific phosphorylations of transgelin-2 a possible way to reduce side effects while treating diseases?

Figure 1. Human Transgelin-2 Gene and Protein Structures.

Transgelin-2 gene (*TAGLN2*) structure showing exonic (numbered boxes) and intronic (line) regions and sizes. The transcripts 201–203 are shown with the translated regions and the gray regions are the coding sequence of the transcript. The protein structure (bottom) shows the extra (EX) sequence, calponin-homolog (CH)-domain, actin-binding motif (ABM), and C terminal calponin-like repeated (CLR)-region.

Figure 2. Structural Characteristics of Transgelin-2.

Human transgelin-2 contains an N terminal calponin-homolog (CH)-domain, an actin-binding motif (ABM), and a C terminal calponin-like repeated (CLR)-region. Transcript 202 encodes a polypeptide with 220 amino acids due to an extra (EX) N terminal sequence (SAFSLALALVSSQPPPPIGM) after the initial methionine. The CH domain binds to metallothionein-2 (MT-2), ERK2, and TSG12. The ABM domain binds to actin. The C terminal CLR domain binds to ezrin. Transgelin-2 is regulated by phosphorylation at Ser-11, Ser-83, Thr-84, Ser-145, Ser-163, Thr-180, Ser-185, Thr-190, and Tyr-192. This phosphorylation is regulated by multiple extracellular factors (EGF and TGFβ) and intracellular kinases (ERK2, PKA, and PKC).

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Figure 3. Schematic of Transgelin-2 at the Immunological Synapse.

Stimulation of T cell receptor (TCR) of T lymphocyte in response to antigen (Ag) is firstly triggered by major histocompatibility complex (MHC) molecules on the antigen-presenting cell. Transgelin-2 (TG2) stabilizes F-actin and binds the leukocyte function-associated antigen-1 (LFA-1) at the immunological synapse after TCR stimulation, thus enhancing the activity of small GTPase Rap1 to increase LFA-1 affinity and clustering. The subsequent interaction between LFA-1 and the intercellular adhesion molecule (ICAM) is an essential co-stimulatory signal for the prolonged adhesion of T lymphocyte to the antigen-presenting cell. The stabilization of F-actin by TG2 at the immunological synapse may help overcome the insufficient co-stimulatory signals under immunosuppressive conditions.

Key Figure

Schematic of Transgelin-2 in the Intracellular Interaction and Signaling Mechanism

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Figure 4. Key Figure Schematic of Transgelin-2 in the Intracellular Interaction and Signaling Mechanism

Transgelin-2 (TG2) induces actin gelation by binding in a high ratio to actin (high concentrations of TG2 represented by green ‘gel’ on bundled actin filaments) (left). Misregulation of intracellular interactions of TG2 with actin can lead to malignant tumor invasion, metastasis, and drug resistance. Additionally, TG2 may provide a signaling pathway that relaxes the myosin cytoskeleton of the airway smooth muscle cells (right). In this pathway, we propose that TG2 may act as a receptor for extracellular ligands or agonists, such as metallothionein-2 (MT-2) or TSG12. The activation of TG2 binds ezrin to stabilize the membrane receptor complex and induces RhoA phosphorylation, thereby inactivating the RhoA-ROCK-MYPT1 pathway. Myosin light chain phosphatase (MLCP), a heterotrimer consisting of MYPT1, PP1Cδ, and M20, can be activated by the dephosphorylation of MYPT1. The catalytic activity of PP1Cδ is enhanced to dephosphorylate myosin light chains (MLC), causing the relaxation of airway smooth muscle cells. Abbreviations: COPD, Chronic obstructive pulmonary disease; M20, the 20-kDa subunit of MLCP with unknown function; MYPT1, myosin phosphatase target subunit 1; PP1Cδ, phosphatase catalytic subunit of MLCP; ROCK, Rho-kinase.

Table 1.

Physical and Biochemical Characteristics of Human Transgelin Isoforms

Isoforms	Transgelin-1	Transgelin-2	Transgelin-3	Refs
Chromosome location	11q23.3	1q23.2	3q13.2	[4]
Exons	5	7	5	[4]
Transcripts	8	5	7	[16]
Length (aa)	201	199/220	199	[13]
Identity to transgelin-2 (%)	64.7%	100.0%	69.8%	[13]
Isoelectric points	9.0	8.4	7.0	[7–9]
Tissue expression	Visceral and vascular smooth muscle cells	Smooth muscle cells and the immune system	Nervous system	[18,28,29]

Abbreviations: aa, Amino acids.

Table 2.

The Transgelin-2 Expression in Cancers

Cancer types	Patient numbers	Tissue analyzed	Methods	Transgelin-2 expression in tumor	Validations	Refs
Bladder cancer	23	Whole tissue	miRNA screening using a low-density array	>2-fold higher	RT-PCR, IHC	[90]
Colorectal cancer	20	Microdissected tumor cells	Acetylation stable isotopic labeling method and MS	4-fold higher	WB, IHC	[70]
Hepatocellular cancer	16	Whole tissue	SEREX	>2-fold higher	RT-PCR	[73]
Hepatocellular cancer	27	Microdissected tumor cells	2-DE and MS	>2-fold higher	IHC	[91]
Lung cancer	17	Whole tissue	2-DE and MS	7-fold higher	RT-PCR, WB, IHC	[78]
Maxillary sinus squamous cell carcinoma	20	Whole tissue	Oligo-microarray	2.7-fold higher	RT-PCR, WB	[92]
Uterine cervical squamous cell carcinoma	18	Whole tissue	2-DE and MS	>2-fold higher	WB	[7]

Abbreviations: 2-DE, Two-dimensional electrophoresis; IHC, immunohistochemistry; MS, mass spectrometry; RT-PCR, real-time quantitative polymerase chain reaction; SEREX, serological analysis of recombinant cDNA expression libraries; WB, Western blot.