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Review of the GAS3 Family of Proteins and their Relevance to Cancer

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Abstract

The GAS3 family of tetraspan proteins has recently been implicated in the progression of cancer. Currently, six members of the GAS3 family have been identified in humans and mice, and while their expressions in disease vary, data suggest that they play a role in epithelial cell structure and function. In this review, we highlight the studies implicating four of the members in disease pathogenesis as well as probe the structural similarities between the family members. Finally, the impact of targeting select members of the family such as PMP22 and EMP2 is discussed.

Keywords

epithelial membrane proteins; peripheral membrane protein-22; tetraspan; GAS3; four-transmembrane; cancer

I. INTRODUCTION

The tetraspan superfamily, also called transmembrane 4 superfamily (TM4SF) of membrane proteins, [1] is comprised of three subfamilies, namely, the connexins, tetraspanins, and growth arrest specific 3/PMP22 (GAS3) family. The current review focuses on the GAS3/PMP22 subfamily of TM4SF. This review will identify the known members of the GAS3/PMP22 family, describe their structure and function, and identify the different cancer types associated with each of the known members. Despite their association with many disease states, the GAS3 family of proteins largely remains enigmatic. Expressed in all metazoans, four members of the GAS3 family have been identified in humans and mice. This includes the prototype for this family peripheral myelin protein (PMP22), which has been linked to human demyelinating heredity neuropathies, [2,3] and all other members were identified based on their homology to PMP22[4,5] (Table 1). The GAS3 family can be distinguished from other four transmembrane proteins by their relatively small size, ranging from 157 (EMP1) to 160 amino acids (PMP22). They are characterized by two large extracellular domains of unequal sizes, which contain a number of N-linked glycosylation sites, and images are provided for each family member in Fig. 1.[6] Members have been shown to be expressed on the plasma membrane or in intercellular vesicles, and unique tissue and/or cell specific functions have

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been implicated for each.[7–10] In this review, we focus on identifying the structure, function, and the association of GAS3 family members PMP22, and the epithelial membrane proteins (EMPs) in cancer biology.

II. PMP22

A. Structure and Function

The *PMP22* gene (previously designated PASII, SR13, and gas3 for growth arrest-specific gene-3) codes for a 22 kDA glycoprotein consisting of 160 amino acids.[11–16] It has been proposed that PMP22 has four distinct functional roles. These include peripheral myelin formation, cell-cell interactions, cell proliferation, and peroxisomal biogenesis.[14–16] PMP22 was originally identified in two different systems. First, as a peripheral nervous system (PNS) myelin protein that is downregulated after sciatic nerve injury in the distal nerve stump,[12,13] and second as a mRNA that is strongly upregulated in growth-arrested NIH3T3 fibroblasts.[3,8] Induction of PMP22 expression occurs during cell cycle arrest and apoptosis in fibroblasts,[8,17] and hence, the family was named growth arrest specific or GAS3.

The highest levels of PMP22 expression are observed in myelinating Schwann cells in the PNS where it accounts for 2–5% of all myelin proteins.[18] Altered gene expression of PMP22 has been documented in peripheral neuropathies where amplification, deletions, or mutations in PMP22 result in Charcot-Marie-Tooth disease type 1A (CMT1A), an autosomal dominant demyelinating peripheral neuropathy, and Dejerine-Sottas disease, an inherited neurological disorder that progressively affects mobility.[19–22] Studies in mice have shown that PMP22 is required for the correct development of peripheral axons as mice devoid of PMP22 or carrying mutations in the gene display retarded myelination and develop hypermyelinated structures resulting in a Trembler phenotype.[23,24]

Outside of the peripheral nervous system, PMP22 transcripts have also been detected in the intestines, lungs, uterus, and heart, and it has been shown that PMP22 is regulated by two promoters that produce alternative gene transcripts in a tissue specific pattern.[25] It is known that both progesterone and glucocorticosteroids can upregulate the expression of PMP22 in sciatic nerve, and anti-progesterone therapy has been shown to reduce PMP22 levels, reducing the CMT1A phenotype. [22,26,27] However, outside of the PNS, estrogen appears to regulate its expression as within the uterus, high PMP22 mRNA and protein levels have been reported in proliferative stroma and endometrium. [28,29] Additional studies will be required to determine if the differences in expression are the result of the alternate promoters as well as the function of PMP22 in cells outside the PNS.

B. PMP22 and Cancer

The history of PMP22 in cancer has been varied as a number of studies have reported either up- or downregulation of the protein in different models. For example, decreased expression of the *PMP22* gene was observed during development of lung cancer in animal models.[30] In contrast to lung cancer, several studies have shown amplification of the PMP22 transcript in cancer. Amplification of the chromosome 17p11.2 region and PMP22 expression have been associated with human osteosarcoma and glioblastoma in tissue and cell lines.[31–36]

Moreover, *in vitro* and *in vivo* data suggest PMP22 may play a role in the neoplastic transformation process of the normal pancreas to premalignant lesions to pancreatic cancer.[37] This varied expression in different tumor types suggests that the role of PMP22 in growth arrest and differentiation may be cell and tissue specific.[37]

Similarly, the story in breast cancer is full of inconsistencies. Initial studies documented upregulation of PMP22 mRNA levels in a panel of invasive and noninvasive human mammary cancer cell lines,[38] and these studies were expanded on to investigate its prognostic potential in 249 primary breast cancer patients.[39] The authors concluded that patients who had higher than average median PMP22 gene expression were at higher risk of death from cancer when compared to patients with equal clinical covariables but lower PMP22 gene expression.[39] However, complicating these conclusions, other studies in breast cancer showed reduced PMP22 mRNA levels. Mimori and colleagues compared differential gene expression of PMP22 between normal, primary carcinoma cells, and metastatic carcinoma cells, and identified PMP22 as typically having diminished expression when comparing both primary tumor cells to normal cells and metastatic cells with primary tumor cells.[40]

The contradiction in mRNA levels highlights the need for better protein analysis of PMP22 in cancer, and it is likely that posttranscriptional regulation for PMP22 exists. For example, Lauer et al. reported that PMP22 may exist within peroxisomes. His group showed that while PMP22 mRNA levels of PMP22 in colon cancer are not altered compared to normal tissue, its protein levels, as measured by immunohistochemistry and Western blot analysis, were significantly reduced in colon carcinoma samples compared to normal tissue.[7] The authors conclude that the reduction of PMP22 along with other peroxisomal membrane proteins (for example, PMP70) in colon cancer may not be attributed to reduced transcription of their genes, as the mRNA levels were not altered in colon cancer, but possibly due to diminished rate of translation for these mRNAs in carcinoma cells.[7]

III. EMPS (EMP1, EMP2, AND EMP3)

A. EMP1: Structure and Function

The 25 kDa epithelial membrane protein 1 (EMP1), is a hydrophobic polypeptide protein composed of 160 amino acid residues that shares 40% of its sequence identity with PMP22.[4] As the hydrophobicity profiles of PMP22 and EMP1 are comparable, particularly within the first two transmembrane domains, Taylor and colleagues proposed that EMP1 and PMP22 were members of the same family and may share similar molecular functions.[41] Similarly, the N-linked glycosylation found in the extracellular domain of EMP1 is comparable to that of PMP22. The glycosylation domain in PMP22 carries an epitope known as L2/HNK-1, which is noted to mediate cell-cell recognition and adhesion.[42] EMP1 carries the N-linked glycosylation in the identical position to PMP22, implicating EMP1 as having functional roles in cell proliferation and differentiation.

EMP1 has been isolated from human, rat, rabbit, and mouse tissues and has been labeled with several compellations: EMP1, Tmp, PAP, CL-20, and B4B.[10,43–46] Despite being coexpressed on the transcriptional level in many tissues, especially PNS nerves, EMP1 and

PMP22 show significant differences in their relative tissue-specific expression levels. For example, although PMP22 mRNA is present at low to absent levels in the gastrointestinal tract, lung, brain, and skin, EMP1 levels are more abundantly expressed in these tissues.[10]

Although EMP1 and PMP22 are coexpressed in the PNS, they appear to be differentially regulated. [10] In animal models of sciatic nerve injury, mRNA levels of EMP1 and PMP22 were inversely regulated with significantly lower EMP1 mRNA than that observed for PMP22. Instead, EMP1 mRNA was upregulated four days post rat sciatic nerve injury in proliferating cells, whereas PMP22 mRNA was significantly reduced.[10] These results could be recapitulated *in vitro* using mitogen-expanded primary rat Schwann cells (pSc) and D6P2T Schwann cells grown in the presence or absence of forskolin, which induces PMP22 expression via axon-Schwann cell interactions during myelination. However, EMP1 mRNA levels decreased under similar experimental parameters. In other cell types such as NIH3T3 cells, similar results were observed. In a consecutive *in vitro* model, whereby NIH 3T3 fibroblasts were grown under serum deprivation, PMP22 mRNA expression was significantly increased but EMP1 mRNA decreased. This not only confirmed that PMP22 is regulated by growth arrest,[47] but again illustrated the inverse regulation of EMP1 mRNA.[4] The inverse regulation of EMP1 and PMP22 mRNAs during different stages of cell cycle provides indirect support for the role of these GAS3 family proteins in the regulation of cell proliferation and dormancy.[4] Currently, EMP1 has been identified as having many biological functions in cancer, including cell proliferation, apoptosis, invasion, and metastasis.[48–52]

1. EMP1 and Cancer—Similar to PMP22, the expression profile of EMP1 in cancer is mixed, with conflicting reports between mRNA and protein studies. On the mRNA side, reports have suggested both an upregulation and downregulation of EMP1 in cancer. For example, gene expression profiling of 50 human gliomas was analyzed using a cDNA microarray platform. EMP1 mRNA was upregulated and correlated with an increase in the *myc* oncogene.[53] Similar changes have been observed in other cancers. In breast cancer, immortalized human mammary luminal epithelial cells expressing moderate and high levels of the ERBB2 receptor, which is designated a proto-oncogene and belonging to the tyrosine kinase family (HER2/neu), were examined for changes in gene expression using cDNA microarrays corresponding to approximately 6000 genes.[54] EMP1 gene expression was significantly elevated in cells overexpressing ERBB2.[54] Finally, EMP1 overexpression was recently shown as a novel poor prognostic factor in pediatric leukemia that may regulate prednisolone resistance, cell proliferation, migration, and adhesion.[55] BCP-ALL patients with high levels of EMP1 showed a significantly poorer five-year event-free survival compared to patients with low expression with pathway analysis suggesting that EMP1 signals through the Src family kinases.[55]

In contrast, in other cancer systems, EMP1 has been linked with tumor suppression. EMP1 mRNA is reduced in oral squamous cell carcinoma (OSCC), nasopharyngeal carcinoma, and prostate cancer.[51,56,57] RT-PCR and immunohistochemistry were used to measure EMP1 mRNA levels and protein expression in OSCC and corresponding adjacent normal tissues. EMP1 mRNA levels and protein expression were significantly reduced in OSCC compared to control samples. In addition, decreased EMP1 expression was significantly correlated with clinical stage ($p = 0.002$) and lymph node metastasis ($p = 0.044$).[52] The following

findings suggest that EMP1 may function as a tumor suppressor. However, further studies are required to comprehend the functional mechanisms through which EMP1 may exert these effects in OSCC.

Similar to reports in OSCC, decreased EMP1 mRNA levels and protein expression have been reported in 75 cases of nasopharyngeal cancer relative to 31 cases of healthy controls. Decreased EMP1 expression significantly correlated with T stages (TNM staging system), lymph node metastasis, clinical stage, and histopathological grade. Furthermore, poor overall survival was significantly correlated with loss of EMP1 expression. In *in vitro* studies, transfection of the nasopharyngeal cancer cell line (CNE2) with overexpression of EMP1 resulted in significant decrease in cell proliferation but an increase in apoptosis. EMP1 overexpressing CNE2 cells exhibited increased caspase-9 ($p < 0.05$) but decreased VEGF-C protein levels.[57] Similarly, Sun and colleagues[56] again investigated the expression of EMP1 in colorectal carcinoma and prostate cancer. In both cases, decreased expression of EMP1 was significantly correlated with T stages, lymph node metastasis, clinical stage, and histopathological grade in patients with colorectal carcinoma as well as prostate cancer. In culture, transfection of human colorectal (SW-480) or human prostate PC-3 cancer cell lines with an EMP1 overexpression vector again resulted in increased apoptosis ($p < 0.05$) and decreased migration and invasion ($p < 0.05$). Both cell lines overexpressing EMP1 also showed significantly elevated caspase-9 protein levels but decreased VEGF-C levels. These findings suggest that EMP1 may induce apoptosis through the mitochondria-dependent pathway and suppress tumor metastasis through VEGF-C-mediated angiogenesis in OSCC, colorectal carcinoma, and prostate cancer.[56,57]

In cancers in women, a similar story exists in uterine leiomyomas and in cervical cancers. EMP1 gene expression was significantly downregulated (3.9-fold change) in leiomyoma samples compared to matched normal myometrium. These findings suggest that EMP1 may potentially alter smooth muscle cell differentiation in leiomyoma.[58] In an *in silico* study, microarray samples of 24 normal and 102 cervical cancer biopsies from four independent, publicly accessible databases were analyzed for gene expression profiling. Once more, EMP1 gene expression was significantly downregulated in cervical cancer biopsies compared to matched healthy controls.[59]

Finally, studies investigating the role of EMP1 in breast cancer are limited but suggest similarity to observations in other cancer types. Sun and colleagues examined EMP1 protein levels in breast tissue. Immunohistochemistry and Western blot were used to analyze EMP1 protein levels in 67 cases of breast cancer and 35 normal tissues. EMP1 protein levels were significantly lower ($p < 0.05$) in breast cancer samples relative to normal controls.[51] In addition, EMP1 protein levels were significantly correlated with T stages, lymph node metastasis, clinical stage, and histopathological grade. Poor overall survival was correlated ($p < 0.05$) with low EMP1 protein levels. Transfection of MCF-7 breast cancer cell lines in order to increase expression levels of EMP1 resulted in elevated apoptosis and a significant decline in migration and invasion. EMP1 significantly upregulated caspase-9 and decreased VEGF-C protein levels ($p < 0.05$). These findings suggest that EMP1 may negatively regulate breast cancer (*in vitro*) via modulation of caspase-9.[51]

B. EMP2

1. Structure and Function—Epithelial membrane protein-2 (EMP2) is believed to have resulted from a gene duplication of PMP22 as EMP2 and PMP22 are found on paralogous chromosomal regions.[60] Analogous to EMP1, EMP2 shares ~40% of its amino acid sequence with PMP22. Structurally, EMP2 is 160 amino acids translating into an 18 kDa polypeptide core with three N-linked glycosylation sites on its first extracellular loop.[5] Similar to the other tetraspan proteins, EMP2 mRNA is widely distributed with high expression in the lungs and moderate expression in the eyes, heart, thyroid, intestine, and uterus.[4,5,61] Similar to PMP22, mutations in EMP2 expression have been associated with disease. Mutations in EMP2 have been linked to childhood onset nephrotic syndrome with patients carrying a 21C > G (p.Phe7Leu) mutation.[62] Nonetheless, the protein expression of EMP2 is more discrete than its mRNA profile with limited expression observed in type 1 pneumocytes within the lung, endometrium, keratinocytes within the skin, and select sites including within retinal pigment epithelium and corneal epithelium of the eye.[63–66]

EMP2 expression is hormonally regulated, and *in vivo* studies have also delineated that upregulation of EMP2 expression within the endometrium occurs during the secretory phase.[67] It has been proposed that its expression is critical for successful implantation as knockdown of EMP2 within the uterus resulted in a significant decrease in the number of implantation sites within the uterine horns.[68]

Functionally, under physiological conditions, EMP2 appears to regulate the expression of select integrins, GPI proteins, and class 1 major histocompatibility complex proteins where it helps to traffic these proteins into lipid raft domains.[68–72] In this way, EMP2 appears to help stabilize select integrins, modulating adhesion onto various extracellular matrices.[70,73]

2. EMP2 and Cancer—Analogous to other tetraspan proteins, the history of EMP2 in cancer has been variable. Initially, EMP2 was also reported to have an apoptotic effect in a B-cell lymphoma cell line, and similar roles have been proposed in nasopharyngeal and urinary tract urothelial carcinoma.[61,74,75] However, multiple other studies suggest that EMP2 functions as an oncoprotein. Using microarray technology, gene profiling suggests that EMP2 mRNA is upregulated in a number of cancers including breast, ovarian, endometrial, and primary CNS malignancies. [76–79] Moreover, in many of these models, EMP2 mRNA was further upregulated during disease progression and metastasis. In a study examining gene expression from circulating tumor cells, EMP2 was one of six genes identified to be significantly overexpressed in blood samples obtained from patients with advanced and patients with primary breast cancer.[80] In addition, EMP2 was identified with twist expression in breast metastatic lesions in the bone, and its mRNA levels were upregulated in endocrine, dasatinib, and chemotherapy resistant tumors in multiple female tumors, suggesting that EMP2 promotes more aggressive disease.[81–84] These findings suggest that EMP2 mRNA may serve to be a potential diagnostic marker for female cancer pathologies.

To understand how the differences in mRNA expression correlate with protein levels, several studies have assessed EMP2 protein expression. The first of these studies examined endometrial neoplasm samples for EMP2 expression and correlated these findings to patient

clinical outcomes.[85] Endometrial tumors with high EMP2 expression were more likely to be myometrium invasive, high stage, recurrent, or fatal. In addition, the medial survival for patients with high EMP2 tumors was 23 months, but patients with low-to-negative EMP2 expression in their tumors had significantly better prognosis. Wadehra and colleagues concluded that endometrial carcinomas ($n = 99$) with an assortment of histologic subtypes, grades, and stages rendered EMP2 an independent negative predictor of disease-free survival linked to high disease stage.[85] These findings suggest that EMP2 may have significant prognostic implications in endometrial adenocarcinomas. To expand on the role of EMP2 in cancers in women, EMP2 protein expression was measured in patients with ovarian and breast cancers. EMP2 levels were upregulated in 63% of invasive breast cancers, and 73% in triple- negative breast tumors, and found to be normal in healthy controls. Similarly, EMP2 expression was observed in 68% of women with advanced ovarian cancers.[86]

Several studies have examined the mechanism through which EMP2 contributes to cancer progression. Using xenograft models in breast, endometrial, and primary CNS malignancies, alterations in EMP2 expression in turn modulated integrin-associated signaling cascades, specifically activating focal adhesion kinase (FAK)/Src.[69,87] In fact, even in tumors where EMP2 did not promote tumor growth, alternations in integrin expression were observed.[5] Subsequently, it has been shown that EMP2 also promotes induction of vascular endothelial growth factor (VEGF) and subsequently this leads to neoangiogenesis in endometrial tumors. This potentially occurs through a HIF-1a dependent mechanism mediated via hypoxia and FAK-Src activation.[88] The high expression and function associated with EMP2 in cancers suggested that it may function as a novel diagnostic and therapeutic target.

Since previous studies have established that EMP2 protein is significantly upregulated in endometrial and ovarian cancers, the significance of the prognostic and foretelling diagnostic knowledge that can be gained by imaging EMP2 expression in malignant tumors may be of value. Therefore, Fu and colleagues generated anti-EMP2 antibody conjugated to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and radiolabeled with Cu, which was then evaluated in mouse model of endometrial cancer as a positron emission tomography (PET) imaging molecule.[65] Cu-DOTA anti-EMP2 minibody successfully established high uptake in xenograft models of endometrial cancer overexpressing EMP2. On the contrary, EMP2-negative tumors exhibited low uptake with anti-EMP2 minibodies.[65] These findings suggest that patients with positive EMP2 malignancies may benefit from antibody specific imaging to localize EMP2-expressing tumors and eventually determining therapeutic efficacy.

To the best of our knowledge, EMP2 is the only protein in the GAS3 family that has been extensively investigated for its therapeutic potential in cancers in women.[86–88] *In vitro* studies investigated the efficacy of anti-EMP2 diabodies for the treatment of human endometrial adenocarcinoma (HEC-1A). Significant cell death and elevated caspase- 3 levels were evident following treatment in HEC-1A cell line, suggesting that anti-EMP2 diabody may exert its effects via caspase-dependent apoptotic cell death. *In vivo*, subcutaneous human xenografts of HEC-1A cells were generated and subsequently treated with anti-EMP2 diabodies. Significant tumor growth suppression and cell death ensued following treatment with anti-EMP2 antibody.[89] For the first time, these findings suggest that a GAS3

protein may have a pharmacological potential for the treatment of endometrial cancer. Given the successful treatment of endometrial tumors with the anti-EMP2 diabodies, fully human IgG1 antibodies were engineered. Anti-EMP2 IgG1 therapy significantly suppressed tumor growth devoid of any systemic toxicity.[87] Collectively, these results suggest anti-EMP2 antibody may have a therapeutic potential for the treatment of aggressive endometrial cancers.[65]

Outside of cancers in women, the therapeutic potential of EMP2 was investigated in glioblastoma (GBM). A GBM array from 110 patients was examined for EMP2 protein by immunohotochemisty and scored in a masked fashion. Approximately 95% of GBM patients were positive for EMP2 expression but normal brain tissues had low-to-undetectable levels. *In vivo*, GBM cells expressing EMP2 significantly heightened tumor growth, partially through $\alpha_v\beta_3$ integrin upregulation, FAK/Src activation, and increased cell migration and invasion. Anti-EMP2 antibody therapy successfully decreased tumor load in subcutaneous mouse models and enhanced GBM cell death *in vitro*.[90] These results suggest anti-EMP2 therapy may be of value in the pathogenesis of GBM.

C. EMP3

1. Structure and Function—Epithelial membrane protein-3 (EMP3) projects onto chromosome 19q13.3 band and consists of a165-amino acid sequence, four transmembrane domains, and two N-linked glycosylation sites within the first extracellular loop. The homology of EMP3 amino acid with PMP22, EMP1, and EMP2 is 41%, 33%, and 38%, respectively. The transmembrane domain is the site where homology is most significant among the GAS3 family proteins.[5,41,91] EMP3 mRNA levels are heightened in peripheral leukocytes, ovary, intestine, and embryonic tissues. In contrast to PMP22 and EMP1, EMP3 (and EMP2) transcripts are present in the liver.[5] Although the functional role of EMP3 has not been thoroughly investigated, speculations have been made that it may be involved in cell proliferation, cell-cell interactions, and apoptosis based on validated data from PMP22 and EMP1 studies.[5] EMP3 gene overexpression has been reported in oligodendroglial tumors, breast cancer cell lines, glioblastomas, and prostate cancer.[92–95] However, hypermethylation of EMP3 has extensively been studied in glioma and neuroblastoma, and has been reported to function as a tumor suppressor gene in these cancers.[96,97] Subsequent studies have linked the tumor suppressor properties of EMP3 to solid tumors, such as esophageal squamous cell carcinoma and phenochromocytomas.[94,98]

2. EMP3 and Cancer—Using microarray analysis, 89 neuroblastoma tumors were analyzed for EMP3 gene expression. EMP3 expression was significantly downregulated in neuroblastoma tumors compared to benign ganglioneuroma samples. Hypermethylation-mediated silencing of EMP3 gene was reported to be one known mechanism by which EMP3 gene is silenced in neuroblastoma tumors, and decreased EMP3 expression correlated with poor survival after two years following the diagnosis ($p = 0.03$). *In vitro*, use of a demethylating agent (5-aza-2-deoxycytidine) restored EMP3 gene expression in neuroblastoma cells, and resulted in significant decline in colony formation. *In vivo*, re-methylation of EMP3 significantly reduced tumor volume in mouse xenograft models of neuroblastoma.[96] These findings indicate that in neuroblastoma, EMP3 may function as a

tumor suppressor gene, and hypermeythylation of EMP3 is an indication of poor prognosis in patients diagnosed with neuroblastoma. Similarly, transcript levels of EMP3 were evaluated in 57 samples of oliodendrioglial tumors (OTs) by quantitative real-time RT-PCR. Reduced EMP3 expression was reported in 18% of OTs compared to healthy brains. However, EMP3 was overexpressed in 19% of OTs as well, with eight samples having greater than tenfold increase in EMP3 gene compared to healthy controls. And almost all OTs were positive for EMP3 methylation, but there was no significant correlation between methylation status and EMP3 gene expression.[99] These results suggest that in OTs, methylation alone does not regulate transcriptional silencing of EMP3, and the tumor suppressor properties of EMP3 that were reported in neuroblastoma do not apply to OTs.[96] Further research is required to elucidate the function of EMP3 in neuroblastoma, OTs, and normal cells. EMP3 expression has been characterized in non-small cell lung cancer (NSCLC), during early and advanced stages and the prognostic validity of EMP3 in NSCLC patients has been evaluated. EMP3 levels in NSCLC were significantly lower than healthy control tissues and correlated with TMN stage ($p < 0.05$). No significant correlation was found between EMP3 expression and other clinical parameters.[100] These findings provide further validation for the tumor suppressor properties of EMP3; however, further research is required to understand the molecular mechanisms of EMP3-mediated pathogenesis of NSCLC.

In upper urinary tract urothelial carcinoma (UUT-UC), EMP3 is associated with enhanced oncogenesis. *In vitro* and *in vivo* studies demonstrated that overexpression of EMP3 heightens cancer cell proliferation and migration, however suppresses cell adhesion (*in vitro*). In addition, the progression of urothelial carcinoma (UC) was positively correlated with EMP3 mRNA expression, providing evidence for the oncogenic property of EMP3 in UUT-UC. EMP3 mRNA and protein levels were evaluated in a cohort of UUT-UC patients ($n = 77$), and the most important critical indicator of poor prognosis was based on co-expression of EMP3 and ErbB2 integrin. *In vitro* studies validated the upregulation of ErbB2 in human UC cells and linked activation of ErbB2-PI3K-AKT pathway to increased expression of EMP3.[101] These findings suggest EMP3 possess cell proliferative properties in UUT-UC. Further research is needed to validate the potential of EMP3-mediated ErbB2 targeted therapy in UUT-UC.

IV. CONCLUSIONS

The GAS3 family of tetraspan proteins was expanded 20 years ago in a search for genes homologous to PMP22. For the first time, the structure, function, and association of the four major members of the GAS3 family of proteins to cancer has been reviewed. Although there is no consensus on the level of expression, dysregulation of each epithelial membrane protein member has been implicated in multiple cancers, perhaps highlighting the differential tissue and cell type specific expression of each family member. In addition, there is no consensus as to the etiology in disease as in some cases select members function as tumor suppressors and in others as oncoproteins. Some of the discrepancies may simply be the result of posttranscriptional regulation. Many of the studies performed to date analyze mRNA expression, and given the robust mRNA but discrete protein expression for both PMP22 and EMP2, it is likely that extensive posttranscriptional regulation exists to

determine the final protein levels of each of these family members. As such, targeting these proteins in specific cancers may yield new markers for diagnosis, prognosis, and therapy.

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ABBREVIATIONS

4-TM	four-transmembrane, tetraspan proteins
EMP2	epithelial membrane protein-2
GAS3	growth arrest stop 3
PMP22	peripheral membrane protein-22

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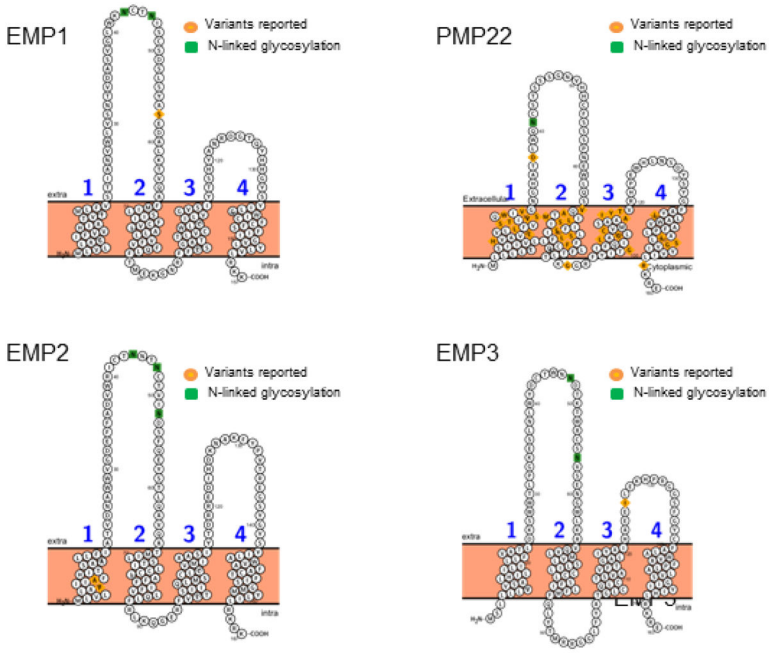

FIG. 1.

Putative structure of the GAS3 family of proteins. The topographical structure was determined using Protter software (adapted from Ref. 6). Reported variants as well as the sites for posttranslational modifications have been indicated.

TABLE 1

Amino acid homology between EMP2 family members

	EMP1	EMP3	PMP22	EMP2
EMP1	100	31.41	40.65	41.56
EMP3	31.41	100	43.12	39.75
PMP22	40.65	43.12	100	45.28
EMP2	41.56	39.75	45.28	100