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Regulation of FAK Activity by Tetraspan Proteins: Potential Clinical Implications in Cancer

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Abstract

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that regulates multiple cell signaling pathways in both physiological and pathological conditions. Overexpression and activation of FAK is associated with many advanced stage cancers through promoting cancer cell tumorigenicity and progression as well as by regulating the tumor microenvironment. FAK has multiple binding partners through which FAK exerts its functions including RhoGEF, Src family, talin, cortactin, and paxilin. Over the last few years, it has been proposed that a novel group of four transmembrane proteins can interact with FAK and regulate its activity. These include select tetraspanins such as CD151 and CD9 as well as the GAS3 family members epithelial membrane protein-2 (EMP2) and peripheral myelin protein-22 (PMP22). In this review, we discuss the current knowledge of the interaction between FAK and tetraspan proteins in physiological and pathological conditions, with an emphasis on the potential of tetraspan family members as therapeutic targets in cancer.

Keywords

FAK; TM4SF; tetraspans; tetraspanins; connexins; claudins; proteins

I. INTRODUCTION

Focal adhesions are molecular complexes through which cells interact with the extracellular matrix (ECM). Discovered in the early 1990s, the FAK gene is positioned on human chromosomal region 8q24.3 and mouse chromosome 15,¹ and it consists of three major domains, i.e., an N-terminal FERM (band 4.1-ezrin-radixin-moesin) domain, a central

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kinase domain, and a C-terminal focal adhesion targeting (FAT) domain.² FAK is a non-receptor tyrosine kinase, ~120 kDa in size, but which functions in both kinase-dependent and independent manners. Much of the functional information about FAK comes from its association with integrin signaling, where it is essential in transmitting the physical communications between a cell and its extracellular environment. On activation via ECM binding, integrins cluster on the plasma membrane and recruit and activate adaptor proteins, non-receptor tyrosine kinases, small GTPase and cytoskeletal proteins.³ FAK interacts with integrins via its C-terminal domain within focal adhesions. The integrin-FAK linkage leads to the initial auto-phosphorylation of FAK at Y397, which results in a conformational change in the binding site for Src homology (SH2) domain-containing proteins such as Src family kinases, phosphoinositide 3-kinase, phospholipase C, and growth factor receptor-bound protein 7 (e.g., Grb7).^{4,5} FAK/Src complex formation further activates FAK at other tyrosine sites, including Y407, Y576, Y577, and Y925.⁶ Activation of FAK modulates cell adhesion, migration, proliferation, survival, apoptosis, and differentiation as well as angiogenesis and the immune response in the extracellular microenvironment.^{7,8}

FAK has multiple binding partners, through which it transmits signaling from ECM and growth factors to downstream molecules such as RhoGEF, Src family, talin, cortactin, and paxilin.⁷ Emerging evidence has also suggested that tetraspan proteins are novel binding partners and/or regulators of FAK activity. Tetraspan proteins are a family of widely expressed proteins implicated in a number of physiological and pathological functions.⁹ Consisting of the GAS3, tetraspanins, claudins, and connexins, each family has been shown to differentially regulate invasion and metastasis in multiple types of cancer.^{10–14} In this review, we highlight the research of select tetraspan family members implicated in FAK regulation with a focus on therapeutic intervention.

II. TETRASPANINS

Over the last two decades, tetraspanins have been implicated in diverse biological and pathological roles including proliferation, T- cell activation, differentiation, and metastasis.^{15–18} Tetraspanins are small integral proteins that contain two extracellular regions of unequal size and three short intra-cellular regions.¹⁹ Figure 1(a) depicts a typical tetraspanin, CD151.²⁰ All tetraspanins contain a CCG motif within the second extracellular loop as well as two conserved cysteines that form two intramolecular disulphide bonds.²¹ Nearly all tetraspanins also contain membrane-proximal cysteines that undergo palmitoylation.²² Currently, the family consists of 33 members, most of which reside in membrane or intracellular vesicular compartments.¹⁵ Many tetraspanins are widely expressed while others have a more restricted expression pattern. For example, CD81 is present on most cell types while CD53 is only present on lymphoid cells. Functionally, the majority of members are associated with integrins and regulate integrin-dependent cell migration, and in this regard tetraspanins have been shown to influence all facets of integrin function including their localization, ability to signal, and the rate at which they internalize (recently reviewed^{10,23})

With regard to FAK signaling in cells attached to an extracellular matrix, the integrin-tetraspanin complexes are localized into distinct clusters within cholesterol-rich lipid

microdomains. To date, at least seven members have been shown to regulate FAK activation. For example, tetraspanin 1 and tetraspanin 8 both promote FAK activation in cervical cancer and malignant glioma, respectively.^{18,23,24} In contrast, tetraspanin KAI1/CD82 reduces FAK activation in prostate cancer.²⁵ To highlight the role of tetraspanins in the control of FAK, two prototype tetraspanins, CD151 and CD9, are further discussed.

A. CD151 Tetraspanin Role and Function in FAK Signaling

The tetraspanin CD151 was originally identified as a resident of the tetraspanin-enriched microdomains found in many cell types. These microdomains have been proposed to cluster laminin binding integrins ($\alpha 3\beta 1$, $\alpha 6\beta 1$, and $\alpha 6\beta 4$), receptors for growth factors (HGFR, EGFR, and TGF- $\beta 1$ R), and matrix metalloproteinases (MMP-7, MMP-2, and MMP-9), implicating the role of CD151 in a number of physiological and pathological events.^{26,27} A 253 amino acid protein, approximately 28 kDa in size, expression of CD151 has been detected in many cell types. Located on chromosome 11p15.5,²⁹ CD151 was first cloned as PETA-3 and identified as a platelet cell surface antigen; it is also expressed in the majority of epithelia and mesenchymal cells.^{28,29} Functionally, the majority of biochemical and functional data generated suggest that CD151 regulates the function of laminin-5 binding integrins $\alpha 3$ and $\alpha 6$.³⁰ Laminin-5 is a component of the basement membrane that mediates either stable epithelial cell attachment or rapid cell motility, depending on its state of proteolytic processing.³¹ In human skin, CD151 is co-distributed with $\alpha 3\beta 1$ and $\alpha 6\beta 4$ at the basolateral surface of basal keratinocytes where it has been proposed to play a role in the formation and stability of hemidesmosomes.²⁹ In other cell types, CD151 colocalizes with $\alpha 3\beta 1$ integrin within microdomains at the most distal parts of lamellipodia and filopodia extension. Formation of integrin-tetraspanin CD151 microdomains trigger FAK phosphorylation, ultimately activating downstream binding partners of FAK such as Src, Cas, and Paxilin.^{22,32–35}

Data suggest that CD151 helps modulate epithelial integrity as well as migration, suggesting a role in tumor invasion and metastasis. CD151 expression is upregulated in a number of cancers including breast and prostate, and its expression is often associated with increased metastasis and/or a poor clinical outcome.^{27,36–38} Functionally, FAK expression is required for the CD151 mediated changes in cell motility, invasion, and metastasis, as it has been shown that FAK^{-/-} fibroblasts fail to migrate even in the presence of increased CD151.³⁹ Although CD151 mediated FAK activation can be dependent or independent of integrin, ErbB2 function in mammary tumor cells is promoted by CD151-integrin mediated adhesion to laminin-5 that results in FAK activation.^{36,40} Thus, inhibition of CD151 significantly sensitizes cells to anti-ErbB2 agents; and, interestingly, disrupting any of the three molecules, CD151, laminin-5, or integrins, renders the cells more susceptible to anti-ErbB2 therapy.⁴⁰

B. CD9 Tetraspanin and FAK Interaction

Located on chromosome 12p13.3, the CD9 gene encodes a cell surface tetraspan glycoprotein expressed at a molecular mass between 25 and 28 kDa. Originally identified on lymphohematopoietic cells, it is widely expressed on the plasma membrane of various normal cells and tumor cells.¹⁷ Physiologically, CD9 has been shown to be involved in a

number of functions including sperm-oocyte fusion as well as at the T-cell immunological synapse, and both responses occur in part through integrin mediated FAK activation.^{41–43} For example, CD9^{-/-} females display reduced fertility due to diminished sperm-egg fusion.^{42,44,45} It has been proposed that CD9 functions in concert with integrin $\alpha 6 \beta 1$ to mediate sperm fusion, which results in widespread activation of FAK as well as other tyrosine kinases. With regard to the immunological synapse, downregulation of CD9 diminishes IL-2 secretion by T lymphocytes linked to antigen presenting cells. This occurs through the reduced accumulation of high-affinity $\beta 1$ integrins within the immunological synapse, thereby reducing the activation of its downstream targets, namely, FAK and ERK1/2.⁴³

In contrast to its role in normal physiology, the ascribed role for CD9 in cancer is less clearly defined. In some cancers, including some lymphomas, nonsmall cell lung cancer, and myeloma, evidence supports a role for CD9 as a suppressor of tumor growth and metastasis.^{17,46} However, in other neoplasms such as in gastric and breast carcinomas, CD9 expression promotes cancer progression and/or metastasis.^{47,48} Thus, it is thought that CD9 may facilitate or suppress tumor growth in a cell type specific manner and/or its association with other plasma membrane proteins. For example, in human fibrosarcoma cell lines, CD9 can promote MMP-9 production in an integrin-independent, EGFR-dependent manner.⁴⁹

III. GAS-3 FAMILY OF PROTEINS

In contrast to the indirect association of tetraspanins with FAK, a novel direct binding partner of FAK is EMP2, a member of the growth arrest-specific-3 (GAS3)/peripheral myelin protein-22 (PMP22) tetraspan protein superfamily.⁵⁰ The GAS3 family consists of four members, i.e., PMP22, EMP1, EMP2, and EMP3, with each containing two extracellular loops of unequal size. Figure 1(b) depicts the representative structure of EMP2.²⁰ The first loop contains between two and three N-linked glycosylation sites, and all four proteins contain very small intracellular N and C-terminus tails.⁵¹ Although little information is known about the downstream signaling pathways associated with EMP1 and EMP3, under both physiological and pathological overexpression, EMP2 directly binds and activates FAK and Src kinases.^{52–54} EMP2 plays an important role in regulating cell migration, invasion, angiogenesis, and other cellular functions. One major function of EMP2 is to serve as an important scaffolding molecule that connects ECM-integrin signaling with downstream FAK/Src-mediated pathways. In this section, we discuss the interaction between FAK and EMP2 in some physiological and pathological conditions, with an emphasis on blastocyst implantation, epithelium from the retina, and specific cancers.

While EMP2 mRNA is prominently expressed in the adult ovary, heart, lung, and intestine, and fetal lung, its protein expression is more discrete.^{55,56} In NIH3T3 fibroblasts, EMP2 binds the $\beta 1$ integrin subunit and directly influences adhesion. Specifically, in these cells, EMP2 colocalizes with $\alpha 6 \beta 1$ integrin but not $\alpha 5 \beta 1$ integrin, regulating binding to laminin.⁵⁰ In other cell types, EMP2 binds and regulates a diverse set of integrins. In the uterus, EMP2 increases the surface expression of $\alpha v \beta 3$ integrin in the glandular and luminal uterine epithelium, and acute knockdown experiments suggest that EMP2 is required for an efficient endometrialblastocyst interaction.^{57,58} In the retinal pigment epithelium, EMP2 was shown

to directly bind to FAK as well, leading to FAK activation through increasing FAK phosphorylation at Y397, Y407, Y861, and Y925.^{54,59} While the binding sites between EMP2 and FAK have not yet been determined, the FAK N-terminal FERM domain binds to another membrane-associated tetraspan protein, TM4SF5,⁶⁰ suggesting that the FERM domain plays a prominent role in regulation of FAK activity with other membrane-associated proteins. We predict that integrins/FAK/EMP2 are able to form a triprotein complex and orchestrate a coordinated downstream signaling network in specific cell types.

A. FAK-EMP2 IN BLASTOCYST IMPLANTATION

FAK-EMP2 signaling plays a crucial role in women's physiology, contraception, and fertility. EMP2 is required for endometrial blastocyst implantation through upregulation of $\alpha v \beta 3$ integrin surface expression in glandular and luminal uterine epithelium. Both *in vitro* and *in vivo* studies suggest that EMP2 translocates from an intracellular location to the apical surface of the endometrial epithelium during the window of implantation in the mouse.⁵⁷ In line with EMP2 involvement of blastocyst implantation, FAK undergoes dynamic distributional changes during the same time frame. FAK is localized at the site of cell-to-cell contact on day one of pregnancy and expression of FAK is increased in the apical region of the rat uterine luminal epithelium and rat blastocysts, suggesting that FAK facilitates implantation.⁶¹ Since EMP2 and FAK directly bind each other and coexist during the same time frame of blastocyst implantation, it will be interesting to further investigate if these two proteins function in a synchronized and coordinated fashion. In addition, both EMP2 and FAK are regulated by steroid hormones. EMP2 expression is increased in the secretory phase compared to the proliferative phase within normal human endometrium, and progesterone induces EMP2 mRNA and protein expression, whereas estradiol only increases EMP2 mRNA levels in humans. Concordantly, in mice, progesterone upregulates EMP2 expression and translocation to the plasma membrane; however, estradiol only moderately upregulates EMP2 levels without promoting EMP2 translocation to the plasma membrane.⁶² FAK expression is higher in secretory endometrial tissues in women and regulated by steroid hormones.⁶³ FAK expression is sensitive to estrogen regulation and increases in endometrial stromal cells after estrogen treatment, which contributes to pathogenesis and progression of endometriosis.⁶⁴ Furthermore, estradiol and tamoxifen (anti-estrogen) activate FAK through the non-genomic transmembrane estrogen receptor GPR30.⁶⁵ Expression of FAK is not changed by progesterone;⁶⁴ however, progesterone upregulates CD82, which activates FAK during human endometrial cycles.⁶⁶ Blastocyst implantation is a complex process that requires the spatial and temporal synchronization of both uterine endometrium and blastocyst during the window of implantation. As EMP2 and FAK are both expressed at higher levels during blastocyst implantation and their expressions or activity are tightly regulated by estrogen and progesterone, it suggests that a better understanding of how FAK/EMP2 signaling promotes blastocyst invasion into uterine endometrium is needed.

B. FAK-EMP2 in Ocular Functions

Both FAK and EMP2 are expressed in the eye. In both the mouse and human, EMP2 is expressed at high levels in the eye and is localized to epithelial layers of the cornea, ciliary body, and retinal pigment epithelium; it also distributes at the stromal layer of the sclera, the nerve fiber layer, and optic nerve.⁶⁷ FAK is expressed in multiple layers of eye and involved

in multiple cellular functions both in the normal eye and in specific diseases. FAK is widely expressed and activated during lens development through regulating cell cycle, migration, and differentiation.⁶⁸ FAK is activated and forms a complex with ERK and paxillin, which modulates human corneal epithelial cell migration during wound healing.⁶⁹ Corneal epithelial cell adhesion, migration, and proliferation are enhanced through $\beta 1$ integrin-FAK-PI3K/Akt signaling pathway.⁷⁰ FAK and EMP2 form a complex and play an important role in regulating retinal pigment epithelium (RPE) functions. Collagen gel contraction by RPE is mediated by the FAK/Src pathway⁷¹ and EMP2 positively regulates integrin ligation and FAK/Src complex activation during collagen gel contraction.⁵⁹ An anti-EMP2 diabody decreases EMP2 protein levels, FAK activation, and collagen gel contraction by ARPE-19 cells without an adverse effect on cell survival.⁷¹ EMP2 and FAK also regulate neovascularization in the eye. FAK regulates proliferation and migration of choroidal microvascular endothelial cells via HIF-1 and VEGF expression in RPE cells.⁷² EMP2 also controls VEGF expression in RPE,⁷³ although there is no direct evidence that EMP2 regulation of VEGF expression is dependent on FAK. In addition, $\beta 1$ integrin-FAK signaling maintains retinal ganglion cell homeostasis.⁷⁴ Since FAK and EMP2 are both expressed in similar locations of the eye and directly bind each other, it is interesting to further investigate if integrin-EMP2-FAK form a complex in different cell types in the eye and how they function together to maintain retinal homeostasis.

IV. CLAUDINS

Claudins comprise another family of tetraspan proteins and function as integral components of tight junctions (TJs). Claudins, via their extracellular loops interacting with one another on adjacent cells, help maintain epithelial cell polarity as well as regulate paracellular permeability (barrier function) between the luminal and basolateral spaces.⁷⁵ In vertebrates, there are currently 27 known claudin members, and most are within the 20–34 kDa size range. All are reported to have four transmembrane domains with varying sizes of amino- and carboxyl-terminal tails extending into the cytoplasm. As a representative example, the structure of claudin-3 is provided [Fig. 1(c)].²⁰ Structurally, the first extracellular loop contains charged amino acids that regulate paracellular anion and cation selectivity. The first extracellular loop also contains a highly conserved signature motif [Gly-Leu-Trp-xx-Cys-(8–10aa)-Cys].^{12,76} Interestingly, this signature motif is also present on the GAS3/PMP22 family of proteins, resulting in speculation that the GAS3/PMP22 and claudins may actually be part of the same family.^{77,78} The second extracellular loop of a claudin is shorter than the first (16–33 residues), and although it is not as well characterized, it is believed to help regulate claudin-claudin interactions.⁷⁶ Finally, claudins possess a C-terminal PDZ binding motif which enable them to associate with cytoplasmic proteins such as ZO-1, -2, and -3, multi-PDZ domain protein (MUPP)-1 and PALS-1 associated TJ protein (PATJ), and this allows claudins to be indirectly linked to the actin cytoskeleton.⁷⁹

Although they have been described primarily in epithelia and endothelia, claudins have been described in almost every cell type.⁸⁰ Claudins interact with each other in two different ways. First, they can bind laterally to each other in the plane of the membrane (heteromeric interactions) or by head-to-head binding between adjacent cells (heterotypic interactions). Thus, while multiple claudin isoforms are expressed simultaneously within tight junctions,

others are distributed throughout facets where cell-cell contact occurs. Similar to the connexins (discussed below), it has been proposed that claudins form hexameric units, and, consistent with this idea, homo- and hetero-multimer of up to six claudins have been observed biochemically.^{81–83} Under physiological conditions, FAK can colocalize within tight junctions and directly influence its function.^{84,85} For example, in colonic epithelial cell lines, reduction of FAK using the inhibitor PF-228 decreases transepithelial resistance while increasing paracellular permeability.⁸⁶

A. Claudins, FAK, and Cancer

Altered expression of several claudin proteins has been linked to the development of various cancers, and it has been shown that they can play a cancer-promoting or tumor-suppressor role in a tissue-dependent manner.^{13,87} The downregulation of several claudins in cancer is consistent with the disruption of TJs during tumorigenesis. While it intuitively seems that all claudins should be downregulated in carcinogenesis, specific claudin family members have rather been documented to be upregulated in multiple cancers.^{12,13} Overexpression of multiple claudins such as claudin-1, -3, -4, and -7 have been reported. As an example, overexpression of claudin-3 and -4 have both been observed in ovarian cancer and implicated in metastasis, and this has been shown in part mediated through FAK activation.^{88,89} Functionally, several claudins have been implicated in regulating epithelial-to-mesenchymal transition (EMT), the formation of cancer stem cells or tumor-initiating cells (CSCs/TICs), and chemoresistance, suggesting that they may be promising targets for the treatment of chemoresistant and recurrent tumors.^{87,90,91} We highlight the role of claudin-1 below as an example of claudin regulation in cancer.

B. Claudin-1 and Cancer

Metastasis of cancer cells from the primary site to other tissues is the principal cause of cancer-associated deaths in malignancy. One of the key events for invasion of tumor cells is EMT where epithelial cells downregulate contacts with neighboring cells and lose polarity, reorganize their cytoskeleton, to ultimately become isolated and motile. One such example is hepatitis C co-receptor claudin-1.^{92,93} Claudin-1 overexpression has been reported in multiple cancers including colon, cervical, basal, and invasive breast cancers, but interestingly, its expression has been shown to be downregulated in prostate and liver carcinomas.^{91,94–96}

Part of the discrepancies in claudin expression during carcinogenesis may occur due to its multiple modes of regulation. Claudin proteins can be regulated by a number of transcription factors including Snail, a repressor that plays a critical role in regulating EMT, as well as caudal homeobox proteins (Cdx1 and Cdx2) and GATA4.^{94,97,98} Outside of transcription, claudin-1 mRNA levels are controlled through epigenetic silencing as well as by micro-RNA expression.^{99–102} Finally, it has been shown that claudin-1 protein expression is commonly mislocalized in neoplastic tissue. In colorectal cancer, for example, claudin-1 expression is localized to the cytoplasm as well as within the nucleus.¹⁰³

While the mechanism of claudin-1 in promoting tumorigenesis is poorly understood, recent work suggest claudin-1 physically associates with Src/p-Src in a multiprotein complex that

also includes ZO-1, a PDZ-binding tight junction protein.¹⁰⁴ In this way, it has been speculated that it may modulate the susceptibility to anoikis in colon cancer in an Src-dependent manner.

V. CONNEXINS

In humans, connexins constitute a large family of 21 tetraspan proteins that form the membrane channels known as gap junctions. Typically, connexin nomenclature is based on a number system representing the predicted molecular weight based on the cDNA sequence of an individual connexin.¹⁰⁵ As an example, Cx32 refers to a connexin with a molecular weight of 32 kDa. Structurally, each connexin has four transmembrane domains that create the wall of the channel while the two extracellular loops play roles in the cell-cell recognition as well as docking processes.¹⁰⁶ While the N-terminus, trans-membrane domains, and the extracellular loops are highly conserved among family members, the cytoplasmic loop and C-terminus are variable with regard to both sequence and length [Fig. 1(d)].²⁰ Connexins assemble into hexameric structures called connexons that can be comprised of homomeric or heteromeric subunits that organize within the Golgi apparatus. These connexons or hemichannels are transported into the lateral side of a cell plasma membrane where it will dock head-to-head with a hemichannel on the adjacent cell to form an intercellular channel. In this way, connexins create gap junctions to allow for the communication and transfer of ions and small signaling molecules such as K⁺, Ca²⁺, cAMP, and glucose between cells.¹⁰⁷

A. Connexin Complexes

Outside of their interactions with each other, connexins associate with a diverse set of partners. Under normal physiological conditions, many of these partners associate with connexins within the gap junctional complex and include cytoskeletal elements, a number of protein phosphatases and kinases, and enzymes.^{108,109} However, connexins have also been shown to have roles outside of gap junctions, namely, in the regulation of motility and migration. For example, within neural cells, reduction in Cx43 protein levels reduced migration of neurons during cortical development in mice, and in other cell types, Cx43 expression correlated with alterations in cell morphology, adhesion, motility and migration.^{110–112}

Recent studies have shown, similar to many other tetraspan families, that connexins display complex translational and posttranslational mechanisms that regulate their synthesis, maturation, membrane transport, and degradation.^{109,113,114} This occurs in part to ensure that connexons can rapidly respond to environment cues, and as such, they are dynamically regulated and have a half-life of only 1.5–5 h.¹¹⁴ Select connexins have been shown to be amendable to phosphorylation, hydroxylation, acetylation, disulfide binding, nitrosylation, and palmitoylation.^{110,115–118} The best characterized of these posttranslational modifications is phosphorylation, and this appears to be essential for the proper function of gap junction channels. Several connexins have been shown to undergo phosphorylation including Cx31, Cx32, and Cx56, while others such as Cx25 are not modified in this way.¹¹⁰ Connexin phosphorylation can occur by a variety of kinases such as Src, PKC, and MAPKs, which

affects their trafficking to the plasma membrane, stability, and ultimately the gating of gap junction channels.^{119–122} Phosphorylation events may also alter other cellular functions independent of gap junctions such as growth and proliferation, and these are discussed below in the context of cancer.

B. Connexins, FAK, and Cancer

Outside of their role as channels between neighboring cells, connexins have independent roles in maintaining cell morphology, establishing polarity, and cytoskeleton rearrangements.¹⁰⁹ Via their cytoplasmic carboxyl domain, connexins have been shown to interact with a myriad of proteins including cytoskeletal elements, other junctional proteins, and enzymes.¹⁰⁸ In this way, gap junctions also function as signaling complexes that can affect cellular function and oncogenic transformation. While original observations suggested that connexins function as tumor suppressor proteins, it is now known that modulation of connexin expression in specific cancers produces characteristic tissue and progression changes.¹²³ For example, connexin 32 (Cx32) expression is upregulated in select breast cancers but its expression is lost in hepatocellular carcinoma.^{124,125} Similarly, whereas Cx43 expression does not affect squamous cell carcinomas, it functions as a tumor suppressor in lung, cervical, and bladder carcinoma.¹²⁶ Disruption of Cx43 further increases the susceptibility to develop tumors in response to administration of chemical carcinogens, and it can be upregulated as a cancer preventive agent in the skin by retinoic acid.^{127,128} In some of these tumor models, restoration of connexin expression partially reversed the transformed phenotype.¹²⁹

With regard to binding partners, in some tumors, a link between another gap junction protein and integrins has been described. For example, Cx26 expression correlated with migration and invasion by interacting with FAK in prostate cancer cells. In these cases, adhesion was not affected by inhibition of the gap junction, but rather, invasion and migration were dramatically reduced. Moreover, in these studies, Cx26 directly interacted with FAK and appeared to regulate the activity of it and other integrin binding proteins.¹³⁰ In contrast, in breast cancer cell lines, Cx26 overexpression correlates with a reduction in migration and invasion, and the authors conclude that this reduction is linked to diminished expression of integrin $\beta 1$.¹³¹ These data tend to demonstrate that interactions between the integrin system and connexins may act differently on the migration capacity of cancer cells.

VI. FAK INHIBITORS

FAK inhibitors are being evaluated in several clinical trials with an underlying rationale that they would alter both tumor and stromal cell functions. FAK inhibitory effects on tumor cells involve the prevention of cell motility and invasion *in vitro* as well as tumor growth and metastasis in mouse models. FAK inhibitor administration can slow tumor growth and trigger increased tumor cell apoptosis *in vivo*. While pharmacologic targeting of FAK scaffold function is still at an early stage of development, a number of small molecule-based FAK tyrosine kinase inhibitors are currently undergoing preclinical and clinical testing. In particular, PF-00562271, VS-4718, and VS-6063 show promising clinical activities in patients with selected solid cancers (Table 1). Clinical testing of rationally designed FAK-

targeting agents with implementation of predictive response biomarkers, such as merlin deficiency for VS-4718 in mesothelioma, may help improve clinical outcome for cancer patients.¹³²

Given the ubiquitous expression of FAK, more specific targeting of the kinase would be expected to be beneficial. In a number of cancers, a number of studies suggest that targeting of tetraspan proteins in selected tumors may downregulate FAK activity. We predict that this may be a more focused way to target FAK activation and, as such, highlight targeting EMP2 and CD151 as representative examples.

A. Targeting EMP2

In a number of cancer models, upregulation of EMP2 promotes FAK activation. This has been shown in both epithelial tumors of the uterus and breast, as well as in primary CNS malignancies.^{11,52,53} As such, targeting EMP2 would be predicted to reduce FAK activation. Our laboratory has been instrumental in creating a panel of antibodies and antibody fragments to target EMP2, and we have shown that these reagents specifically localize to EMP2 positive tumors with minimal targeting to normal tissue, suggesting that EMP2 is normally sequestered.⁵⁶ While targeting EMP2 positive tumors with anti-EMP2 agents reduces tumor load, the mechanism by which this occurs is still unclear. However, this appears to occur, at least in part, through the targeting of FAK and Src. Using either an anti-EMP2 diabody or fully human IgG1 antibody, targeting of EMP2 reduces FAK and Src phosphorylation, ultimately reducing invasion.^{9,48,49}

B. Targeting CD151

Similarly, despite the fact that tetraspanins have relatively small extracellular domains, antibodies to select tetraspanins have been shown to have a number of antitumor effects.¹³³ Anti-CD151 antibody targeting CD151 protein has been created and utilized in several cancer models including those utilizing fibrosarcomas, prostate cancer, and colon cancer cell lines.^{134,135} Treatment with an anti-CD151 antibody blocked metastasis, indicating its potential for cancer therapy.¹³⁴ The specific mAb developed inhibited migration, in part through its ability to bind and inactivate FAK activity, as well as preventing tumor cells from entering the vasculature to colonize secondary organs.^{39,136} This effect appears to be two pronged as anti-CD151 treatment can inhibit FAK activation in both integrin -dependent and -independent ways.

VII. CONCLUSIONS

The non-receptor tyrosine kinase FAK regulates multiple cell signaling pathways in both physiological and pathological conditions, and activation of FAK has been shown to be regulated by a number of tetraspan proteins. Comprised of the GAS3, claudins, tetraspanins, and connexins families, each has been shown to regulate FAK activity. In many cases, activation of tetraspans promotes FAK activation, often leading to an increase in invasion and metastasis. While several FAK inhibitors are currently in clinical testing, we hypothesize that the targeting of tetraspans may be a useful and more specific way to inhibit FAK in cancer.

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ABBREVIATIONS

FAK	focal adhesion kinase
4-TM	four-transmembrane
TJ	tight junction
EMP2	epithelial membrane protein-2
PMP22	peripheral myelin protein-22
ECM	extracellular matrix

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FIG. 1.

Representative structure analysis of tetraspanin, GAS3, claudin, and the connexin family of proteins. Predicted structure of (a) CD151, (b) EMP2, (c) claudin-3, and (d) Cx32 using Protter interactive software(adapted from Ref. 20).

TABLE 1

List of FAK inhibitors available in the market

Name	Target proteins(s)	Company	Patients	Trial phase	Status/ conclusion	NIH numbers
PF-00562271	FAK, PyK2	Pfizer/Verastem	Pancreatic Head and neck prostatic neoplasms	Phase 1	Completed ^{**}	NCT00666926
PF-04554878/VS-6063	FAK, PyK2	Pfizer/Verastem	Mesothelioma	Phase 2	Ceased [*]	NCTT0187060
			NSCLC	Phase 2	Recruiting	NCT01951690
VS-4718	FAK	Verastem	Ovarian	Phase 1B/2	Recruiting	NCT01778803
GSK2256098	FAK	GlaxoSmithKline	Hematologic cancers	Phase 1	Recruiting	NCT01849744
			Healthy subjects	Phase 1	Completed ^{**}	NCT00996671
			Solid tumors	Phase 1	Recruiting	NCT01138033
			Solid tumors	Phase 1	Recruiting	NCT01938443
BI 853520	FAK	Boehringer Ingelheim	Advanced solid cancers (Canada, Netherlands)	Phase 1	Recruiting	NCT01335269
			Advanced solid cancers (Japan, Taiwan)	Phase 1	Completed ^{**}	NCT01905111

^{*} Ceased, due to lack of response^{**} maximum tolerated dose favorable for advancement to phase II