FAK and WNT Signaling: The Meeting of Two Pathways in Cancer and Development

Yuri Fonar and Dale Frank*

Department of Biochemistry, The Rappaport Family Institute for Research in the Medical Sciences, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa 31096, Israel

Abstract: Recent studies connect the FAK and Wnt/β-catenin signaling pathways, both which promote cancer when aberrantly activated in mammalian cells. Over-stimulation of either Wnt/β-catenin or FAK activities was independently shown to promote numerous types of human cancers, including colon, breast, prostate and ovary. Observations in different model systems suggest a complex and dynamic cross-talk between these two pathways. During early vertebrate development, FAK protein is required for the proper regulation of Wnt/β-catenin signaling that controls pattern formation in the developing nervous system. In Xenopus laevis embryos, FAK protein depletion eliminated Wnt3a gene expression in the neural plate. In mouse osteoclast cells, mechanical stimulation through FAK activation stabilized β-catenin protein to promote its nuclear translocation. In contrast, in the mouse intestine, FAK activity was induced downstream of Wnt to promote intestinal regeneration and was also essential for tumorigenesis in an APC deletion model of colorectal cancer. Adding to this complexity, in human cell lines, FAK induced a context-dependent modulation of Wnt signaling to activate target-gene expression. Other diseases are also associated with FAK and Wnt pathway over-activation. Increased FAK and Wnt pathway activities were independently implicated in idiopathic pulmonary fibrosis (IPF), a lung disease of unknown etiology. Revealing the FAK-Wnt connection in IPF could provide a better understanding of disease pathology. There appear to be multiple interactions between the Wnt/β-catenin and FAK signaling pathways in different cell types and organisms. Mutual FAK-Wnt pathway regulation could be a general phenomenon, having many still understated roles in either normal physiological or disease processes.

Keywords: Focal Adhesion Kinase (FAK), Wnt/β-catenin, cancer, development.

INTRODUCTION

New Roles for FAK Protein

In recent years, studies on FAK protein and its downstream signaling have expanded into many diverse directions, generating much interest, especially in the field of cancer research. New roles for FAK have been revealed, so the classic picture of FAK exclusively acting as cytoplasmic protein, recruited to focal adhesions is undergoing a major revision. A new and major role for FAK protein has emerged that links its function to the nucleus. FAK protein localizes in the nucleus [1, 2] and nuclear FAK protein was shown to interact with the chromatin modulating methyl CpG binding protein 2 (MBD2). FAK binding to MBD2 reduces its binding to HDAC protein, thus suggesting a role for FAK in modulating chromatin structure and gene expression [3]. FAK promotes cell cycle progression through transcriptional control of the CyclinD1 gene, by affecting two transcription factors: by increasing the DNA binding activity of EtsB protein and increasing KLF8 gene expression [4-6]. Additionally, FAK nuclear accumulation and not its kinase activity was also shown to affect the stability and activity of another transcription factor, the p53 tumor suppressor protein [1, 2, reviewed in 7, 8]. When over expressed in cancer and normal fibroblast cell lines, the N-terminal FERM-domain of FAK physically interacts with the N-terminal domain of p53; this binding inhibits transcription of p53-target genes. Both proteins also undergo a clear nuclear co-localization in cancer cell lines, one of the first observations suggesting a nuclear function for FAK protein in regulating gene expression [2]. The depletion of FAK protein in mouse and human breast cancer cells modulated expression of many genes [9, 10]. However, it still needs to be determined if these changes in gene expression are all dependent on nuclear FAK function or indirectly dependent on the “classic” cytoplasmic FAK protein activity. Nevertheless, it is clear that FAK’s role in regulating gene expression adds an additional dimension to the multi-functionality of this protein.

FAK and Wnt Signaling

Most recently, studies have emerged that link FAK and Wnt signaling. This interaction is the focus of this review. Wnt signaling serves crucial roles in many biological processes ranging from cell proliferation, tissue organization, organ development and body pattern formation in invertebrate and vertebrate embryos. It would be difficult to find any normal cell, in any metazoan organism, in which Wnt signaling does not play a role. In mammals, Wnt signal participates in such a diverse processes as hair growth, bone mass development and maintenance, central nervous system (CNS) patterning, intestinal regeneration after injury and lymphocyte functioning. Not surprisingly, Wnt pathway deregulation is implicated in numerous types of human cancers, including colorectal, breast, prostate and ovary.

The Wnt Signaling Cascade

There are a number of different Wnt signaling pathways. These pathways differ according to specific Wnt ligands, Wnt receptors, and downstream effectors. In this review, we will mainly describe the “canonical” or Wnt/β-catenin signaling pathway and its interactions with FAK protein. The basic flowchart of the Wnt/β-catenin pathway is well established. In the absence of Wnt ligand, β-catenin, the downstream mediator and transcriptional activator of the pathway, is found in a cytoplasmic complex with APC, Axin and GSK3β proteins. In this complex GSK3β phosphorylates β-catenin, which leads to β-catenin ubiquitination and targeted degradation. Secreted Wnt ligand protein on the cell surface binds and activates the Frizzled receptor and the LRP 5/6 co-receptor complex. The intracellular motifs of the Frizzled-LRP5/6 complex activate the dishevelled protein in the cell cytoplasm. Dishevelled protein triggers the subsequent release of β-catenin from the APC/Axin/GSK3β complex, leading to cytoplasmic accumulation of β-catenin and nuclear translocation. In the nucleus, β-catenin binds DNA along with the TCF/LEF protein to activate transcription of specific target genes. In the absence of β-catenin in the nucleus, TCF/LEF acts as a transcriptional repressor.

Implication in Cancer

FAK’s role in many different cancer types makes its potential interactions with other cancer promoting signaling cascades an attractive subject of investigation. The recent results in both normal
cells and cancer cells link FAK protein activity to the Wnt/β-catenin pathway, thus making this a relevant system to elucidate how FAK protein functions in normal and aberrant physiological processes. FAK, being a kinase, is an attractive target for small molecule kinase inhibitor drugs [11]. Identifying FAK’s interconnections to other cancer promoting signaling pathways could be clinically important, especially for pathways in which the development of potent pharmacological inhibitors is a challenge. Unraveling the connections between FAK and other signaling pathways, like Wnt, could be of paramount importance both in our understanding of the disease and the future development of therapeutic strategies.

WNT-FAK INTERACTIONS IN EMBRYO DEVELOPMENT

Drosophila Ovarian Morphogenesis

The first in vivo study linking Wnt and FAK proteins investigated embryological ovarian morphogenesis in Drosophila melanogaster [12]. During early fly development, extensive cell migration occurs during ovary formation. Wnt4 protein is expressed in apical cells that migrate to form the ovarian sheath epithelium. In Wnt4 mutant flies, these cells fail to migrate and normal ovary morphology is severely perturbed, due to the loss of the sheath structure. FAK protein is expressed in focal adhesion “big-spots” at the leading edge of these migrating apical cells. In Wnt4 mutants, overall FAK protein levels are reduced, more diffuse, and the FAK “big-spot” structures are lost. While dishevelled protein was required for ovarian cell migration, TCF protein was not critical, suggesting that Wnt4 regulates FAK protein levels and activity via a non-canonical Wnt pathway. This non-canonical Wnt pathway appears to be dependent on endogenous PKC activity expressed in migrating apical cells. The mechanism of how Wnt4 ligand signaling acts to regulate FAK protein levels and sub-cellular organization is still entirely unclear. However, these genetic studies clearly show that Wnt4 signaling acts upstream of FAK to orchestrate apical cell morphogenesis in the developing Drosophila ovary.

Xenopus laevis Nervous System Development

A recent study in the frog, Xenopus laevis, has shown a strong interaction between Wnt and FAK in regulating early patterning events in the developing nervous system [13]. FAK protein was knocked down in whole embryos by antisense morpholino oligonucleotide (MO) microinjection. FAK MO injected embryos had a severe phenotype, in which posterior nervous system lineages such as primary neurons and the hindbrain were severely reduced, while more anterior neuroectodermal tissues like the forebrain were expanded. In addition, neural plate folding was severely perturbed. The nature of the FAK knock down phenotype suggested that posterior cell fate specification was strongly compromised. Zygotic FAK mRNA is specifically expressed in the developing neural plate in Xenopus embryos. Thus, FAK is expressed in a correct local and temporal manner to potentially regulate neural cell fate specification. The FAK knock down phenotype also strongly resembled the same phenotype seen for Xenopus embryos depleted of zygotic canonical Wnt-signaling components, by either ectopic Dkk1 protein (Wnt/β-catenin inhibitor) expression or protein knock down by a Wnt3a-specific MO [14]. Furthermore, the Wnt3a and FAK genes have overlapping expression patterns in the developing Xenopus neural plate. Endogenous FAK protein depletion in these FAK knock down embryos eliminated Wnt3a gene expression in the neural plate. Re-addition of canonical Wnt signaling to the FAK knock down embryos by ectopic expression of Wnt3 protein rescued the neural phenotype, thus placing FAK upstream of the Wnt/β-catenin pathway during Xenopus embryogenesis. These results also show that FAK protein, in addition to its role as a regulator of intracellular cell signaling pathways, can also have a critical role in regulating cellular nuclear functions. During early vertebrate development, FAK protein is critical for proper temporal expression of the Wnt3a gene in the neural plate, which is then required for induction of posterior neural fates.

WNT-FAK INTERACTIONS IN NORMAL TISSUE HOMEOSTASIS

Bone Remodeling

The bone remodeling response to mechanical loading is an essential adaptation mechanism of skeletal tissue and a unique model system for studying translation of mechanical forces into gene expression. Osteocytes are specialized bone cells that sense changes in mechanical force. They translate these mechanical force changes into a biological response by secreting signaling molecules to nearby bone forming osteoblasts or bone resorbing osteoclasts. One of the crucial components of the osteocyte sensing mechanism is Wnt/β-catenin signaling. It was shown that mechanical stimulation causes β-catenin protein stabilization, nuclear translocation and transcription of pathway target genes [15, reviewed in 16]. Immediately after mechanical stimulation, β-catenin protein is stabilized in a Wnt-independent manner, but later pathway activation maintenance is achieved through activation of Wnt ligand gene expression and a reduction in expression of Wnt antagonist proteins [17-21]. This initial mechanotransduction induces an increase in β-catenin protein levels that is dependent on FAK, since the FAK inhibitor-14 blocked this process in osteocyte cells [15]. In addition to FAK, AKT and PI3 kinase protein activities were also required for β-catenin protein stabilization by mechanical loading [15]. This initial stabilization of β-catenin protein appears to be Wnt ligand-independent, since Wnt3a ligand production in osteocytes occurs significantly later, a few hours after the primary mechanical stimulation [21]. An intriguing point not addressed in these studies, is whether the later Wnt ligand expression induced by mechanical loading is also dependent on FAK protein activity?

Mechanical stimulation of osteoblast and osteoclast cells also protects these cells from apoptosis. Apoptotic protection by mechanical force requires ERK protein activation. This anti-apoptotic ERK activation requires active FAK protein, since an auto-phosphorylation deficient dominant-negative FAK protein (Y397F) blocks cell survival induced by mechanical force [22]. It was suggested that a signaling-complex mediated by integrins, Src kinases and FAK activates this ERK pathway. The Wnt and FAK pathways also appear to be linked in this process. FAK/ERK activation induced by mechanotransduction is highly reduced when Wnt/β-catenin signaling is compromised [23]. The anti-apoptotic effects induced by ERK are lost in osteocyte cells by either ectopic expression of the Dkk1 or Axin proteins, which forcibly drive β-catenin protein degradation [23]. Thus, during bone mechanotransduction, both Wnt and FAK signaling pathways mesh to regulate the process.

Intestinal Regeneration

An intestinal regeneration model has been established in mice, in which the intestinal mucosa is damaged by irradiation, which subsequently triggers cell proliferation and architecture restoration after three days. In the damaged intestine, the Wnt/β-catenin signaling pathway induces c-myc gene expression that is crucial for its regeneration [24]. This study shows that while FAK protein is weakly expressed in the normal intestinal epithelium and its activity is dispensable for normal tissue maintenance homeostasis, it has a crucial role during the rapid proliferation following radiation damage or Wnt signaling deregulation. In this regeneration system, FAK expression is up regulated in a Wnt/c-myc dependent fashion. Ablation of FAK activity inhibits the regeneration of the intestinal epithelium, with almost a complete absence of the hyper-proliferating crypts that are the centers of tissue cell re-growth. The same FAK-dependency was also observed following treatment with...
cisplatin, a potent chemotherapy agent. The effects of FAK activation on intestinal regeneration were mediated through AKT phosphorylation. Thus during intestine regeneration, FAK lies downstream to Wnt/β-catenin signaling.

WNT-FAK INTERACTIONS: IMPLICATIONS IN CANCER

Intestinal and Colon Cancer

Wnt/c-myc activation of FAK is also required for induction of intestinal adenomas in APC knock out mice [24]. Acute APC deletion caused over-activation of Wnt/β-catenin signaling with subsequent unrestrained cell proliferation. In the homozygous and heterozygous APC knock out mice models of colorectal cancer, the role of FAK in Wnt-deregulation was examined. Double deletion knock out of both APC and FAK had smaller crypts and diminished short-term proliferation of the intestinal epithelium in comparison to APC knock out mice with functional FAK protein. Furthermore, heterozygous APC deletion mice lacking FAK protein did not develop intestinal tumors and had prolonged long-term survival in comparison to APC heterozygote mice that have normal FAK protein. Thus in intestinal cancer, Wnt-deregulation induces tumorigenesis in a FAK-dependent manner. Similar to intestinal regeneration, FAK functioned downstream of β-catenin/c-myc and upstream of AKT activation, as determined by unchanged β-catenin and c-myc nuclear localization in FAK deleted mice in comparison to FAK expressing mice. Given the lack of effect of FAK deletion on normal intestine homeostasis, FAK inhibition could be an attractive potential target for therapeutic use in colorectal cancer, since it acts downstream of Wnt-signaling to promote cell proliferation.

Interestingly, in FAK inhibitor treated colon cancer cells, the expression of LRP5 and Frizzled2 Wnt-pathway activating receptor genes are down regulated two-three fold, while expression of the Wnt-pathway inhibitor Dkk1 gene is up-regulated nearly four-fold (Golubovskaya, V., unpublished). These results suggest that FAK acts upstream of Wnt-pathway component gene expression, thus, suggesting that there may be multiple levels of interaction between the FAK and Wnt signaling pathways in colon cancer cells.

Another potential link between the FAK and Wnt pathways is the human, CAS family member, enhancer of filamentation 1 protein (HEF1). HEF1 was originally shown to be a necessary and specific downstream mediator of FAK in a model of highly invasive glioblastoma cell migration [25]. HEF1 down regulation by siRNA in glioblastoma cells inhibited FAK promoted cell invasiveness. FAK appears to activate HEF1 protein via protein phosphorylation. Phosphorylated HEF1 is enriched in focal adhesions that are likely involved in glioblastoma invasive cell migration.

Recently, HEF1 also was identified as a direct target of Wnt signaling in colorectal cancer [26]. Elevated HEF1 levels were found in human colon cancer cell lines, where it promotes cell proliferation. Higher levels of HEF1 were associated with advanced tumor grade in the tissue sample of colorectal cancer patients, pointing to its role in cancer progression. In HeLa and normal colon epithelia cells, HEF1 protein levels were up regulated in a dose-dependent manner by over expression of the Wnt3a, dishevelled 2 (Dvl2) or β-catenin genes. Reciprocally, shRNA knock down of β-catenin protein in the SW480 colorectal cancer cell line strongly reduced HEF1 mRNA and protein levels. Furthermore, genetic studies in mice complement the cell culture observations. Mice having APC mutations that activate Wnt signaling have increased HEF1 gene expression in the colon and small intestine in comparison to wild type mice. Analysis of the HEF1 promoter identified three functional β-catenin/TCF binding sites, establishing the HEF1 gene as a direct-target of Wnt β-catenin signaling in colorectal cancer. These findings suggest an additional link connecting the Wnt and FAK pathways to cancer progression, through common activation of the HEF1 target gene and protein.

Breast Cancer Cell Lines

In the MCF-7 human breast cancer cell line, FAK protein was depleted by RNAi, and microarray analysis showed reduced expression of a multitude of genes [10]. Similar to the observations in Xenopus embryos, human Wnt3a and Wnt3 mRNA levels were also strongly reduced in the FAK-depleted MCF-7 cells, yet expression of other Wnt ligands was not significantly lowered [13]. These FAK depleted MCF-7 cells also have reduced Wnt3 protein levels.

The human FAK gene promoter region contains two p53 binding sites and p53 protein represses FAK gene expression [27]. In various estrogen-dependent breast cancer cell lines, the repression of FAK gene expression by p53 is mediated by estradiol [28]. Perhaps not so coincidentally, estradiol was also shown to be involved in suppressing Wnt3 and Wnt3a gene expression in breast cancer cell lines [29]. However, it still needs to be determined if FAK/Wnt interactions downstream to estrogen play any functional role in breast cancer development.

Pancreatic Cancer

In pancreatic cancer, FAK/Wnt interactions could be potential targets for tyrosine kinase inhibitors. In recent years, tyrosine kinase inhibitors, like gleevec have become a focus for extensive research and development as treatments for various cancer types. One of these tyrosine kinase inhibitors, masitinib was recently shown to be an effective sensitizing agent in human pancreatic cancer cell lines and animal models [30]. Pancreatic cancer is one of the most aggressive human malignancies, with 5-year survival of only 5%. The treatment for unresectable and metastatic disease, which is the diagnosis of 80% of patients, is chemotherapy with gemcitabine. Unfortunately, many cancers develop resistance to this agent, which is often associated with over expression of FAK [31].

Masitinib is a novel tyrosine kinase inhibitor that specifically and selectively inhibits different targets, including various isoforms of the c-Kit receptor, PDGFRα/β, Lyn, and to a lesser degree, the FGFR3 and the FAK pathways [32]. It was recently shown that the addition of masitinib to gemcitabine significantly sensitized previously resistant human pancreatic cancer cell lines in vitro [30]. Masitinib also has a synergistic effect with gemcitabine on human pancreatic tumor growth in vivo, in Nog-SCID mice. Transcriptional analysis, using whole genome DNA microarrays identified that Wnt/β-catenin components were the most significantly down-regulated oncogenic pathway in cell lines re-sensitized by the masitinib/gemcitabine combined treatment [30]. While masitinib does not exclusively target FAK activity, other tyrosine kinase inhibitors, including gleevec, which also inhibit the c-Kit receptor and PDGFR, but not FAK, did not induce a sensitizing effect. In this system, perhaps masitinib’s effects are mediated via inhibition of FAK protein which leads to an eventual down regulation of the Wnt/β-catenin pathway. Activated Wnt/β-catenin has been associated with pancreatic carcinomas [33], suggesting the possibility for developing FAK-specific small molecule inhibitors that could trigger down regulation of the Wnt signaling pathway in pancreatic cancer.

FAK-WNT PATHWAY SYNERGISM IN HUMAN CELL LINES

FAK/Grb2/Wnt Interactions

Studies in the human embryonic kidney, HEK cell line reveal a point of interaction between the Wnt/β-catenin signaling and the integrin/FAK pathway [34]. In HEK cells, FAK protein, while not active on its own, synergizes with Wnt3a or its downstream cascade component, the Dvl2 protein, in activating transcription of β-catenin/TCF target genes. This effect was dependent on the activity of the Grb2 (Growth receptor bound 2) protein. Grb2 is an adaptor protein with one SH2 and two SH3 domains, which participates in multiple signaling inputs for various integrins and growth factor
receptors. Grb2 protein acting downstream to integrin/FAK activation has a strong potentiating effect on Wnt pathway components (Wnt3a, constitutively-active LRP6, Dvl2, LEF1, constitutively-active β-catenin) in transcriptional activation assays. These interactions probably take place at numerous regulatory levels along the pathway cascade. However, Grb2 directly binds the Dvl2 protein through its SH3 domains, and SH3 domain mutations convert Grb2 to a dominant-negative form, which inhibits Wnt pathway transcriptional activation by FAK protein. Furthermore, downstream to Grb2 protein, Rac1 and Jnk/c-Jun proteins mediate β-catenin nuclear translocation and transcriptional complex activity. There is still a missing link explaining how FAK and Grb2 proteins interact, thus leading to eventual Wnt activation, but it was suggested that this interaction may be indirect [35].

FAK-WNT SIGNALING IN LUNG DISEASE

IPF – Idiopathic Pulmonary Fibrosis

Both Wnt/β-catenin and FAK pathways were independently found to be implicated in idiopathic pulmonary fibrosis (IPF), a common and mostly fatal form of lung fibrotic disease of unknown etiology. IPF is a progressive disease and is characterized by increased fibroplastic proliferation and extracellular matrix remodeling. The result of these processes is the dramatic disruption of the lung's natural architecture.

Biopsies from IPF patients have shown nuclear accumulation of β-catenin in the involved pathologic lesion sites [36]. β-catenin target genes such as Cyclin D1 and matrixin/MMP-7 were also over expressed at these lesions. Interestingly, the Cyclin D1 gene is also a downstream transcriptional target of FAK signaling [4]. Consequently, inhibition of Wnt/β-catenin signaling either by ICG-001 inhibition of β-catenin/CBP protein interactions [37] or by β-catenin siRNA inhalation [38] strongly attenuated and even reversed fibrosis in a murine experimental IPF model system.

FAK phosphorylation and activation were also found in murine models of IPF, where inhibiting FAK activation protects against fibrosis [39, 40]. It was also shown that human fibroblasts obtained from IPF patients have increased levels of phosphorylated and activated FAK, in comparison to normal individuals [41]. This FAK over activation was accompanied by down regulation of the endogenous FAK dominant-negative protein, FRNK, and enhanced activity of the Rho and Rac GTPases. Perhaps, this emerging FAK/Wnt connection is important in regulating IPF disease progression. Further investigation of this novel connection should help to decipher the pathogenesis of this mysterious disease.

CONCLUSIONS

In summarizing FAK/Wnt dynamics, there seems to be a wide range of complex and diverse interactions, at many different regulatory levels (summarized in Fig. 1). In some systems, such as Xenopus CNS development, bone osteocytes, breast cancer and possibly pancreatic cancer cells, FAK protein appears to act upstream of the Wnt/β-catenin pathway. However, in these different systems, the upstream regulatory connection and downstream responses are not identical in nature. In other systems, such as in intestinal regeneration or tumorigenesis in mice, there is evidence that Wnt/β-catenin functions upstream to FAK. Additionally, there is also data in HEK cells, suggesting that the Wnt and FAK proteins work synergistically to regulate expression of downstream target genes, through the Grb2 adaptor protein. A plethora of FAK/Wnt interactions are possible, suggesting that the nature of the FAK/Wnt interactive relationship may be context-dependent in different species or cell types. It seems that there are multiple molecular pathways and components connecting Wnt and FAK activities, and it seems less likely that there is one simple unified mechanism of FAK/Wnt cooperation. Much more experimentation still needs to be carried out in cell culture, cancer, and in vivo animal model systems to fully understand the complex nature and physiological relevance of these FAK/Wnt interactions.

Mechanism of FAK/Wnt Interaction - Nucleus vs. Cytoplasm

How FAK protein regulates and controls the Wnt/β-catenin pathway raises many interesting questions concerning the mechanisms of activation. Observations in Xenopus CNS development and in the human MCF-7 breast cancer cells suggest FAK-dependent activation of Wnt ligand expression. This is a plausible option, since recent findings showed that FAK protein undergoes nuclear localization and appears to play a significant role in regulating gene expression by interacting with other nuclear proteins [42]. In initial studies, FAK N-terminal region fragments were found to be localized in the nucleus [43]. A nuclear localization signal has been identified in the FAK N-terminal FERM domain region [1]. More recent studies have showed that both full-length and C-terminal fragments of FAK protein can also undergo nuclear localization [44]. The FAK FERM domain appears to interact with other nuclear proteins, such as p53 and MDM2.

However, it is still not clear if FAK’s role as a transcription modulator is necessarily a direct consequence of its nuclear function, especially when concerning its interaction with the Wnt/β-catenin pathway. In addition to the nucleus, β-catenin protein is present at high levels in the cytoplasm, cytoskeleton and cell membrane, which are other potential sites for interfacing with FAK protein. β-catenin protein is not only a transcriptional activator of Wnt signaling, but also is a structural perimembrane protein in cadherin associated adherence junctions and in actin anchoring protein complexes. This potential site of direct interaction of FAK and β-catenin could link cell membrane adhesion status with the Wnt signaling cascade and subsequent nuclear activity. FAK/Wnt interaction could also be mediated by the more conventional FAK activities in the cytoplasm. These interactions could be mediated through PI3 kinase and AKT activation as shown in HEK or osteocyte cells, either directly or indirectly by FAK or its downstream mediators. Additionally, cytoplasmic FAK protein could modulate activity of intracellular Wnt/β-catenin pathway component mediators, like Frizzled, LRP5/6, APC, GSK3β or Dvl proteins.

Other Integrin Signaling Components and Wnt

Interestingly, another intracellular kinase, associated with integrin signaling and focal adhesions can also modulate canonical Wnt signaling. Integrin linked kinase (ILK) is a cytoplasmic serine-threonine kinase that also has adapter protein functions in the signaling mediated by integrins and different growth factors. ILK over expression causes nuclear translocation of β-catenin and activation of Wnt dependent gene expression; additionally, phosphorylation and subsequent inactivation of GSK3β is also observed [45, 46]. Down regulation or ILK inhibition decreases Wnt3a-dependent stabilization of β-catenin protein, thus inhibiting the pathway. Some activities of ILK are mediated through activation of PI3 kinase and AKT, also targets of FAK signaling. There are significant similarities between ILK and FAK, and potentially, some FAK/Wnt interactions could be very similar to these ILK/Wnt mechanisms.

Cancer Treatment

Linking FAK and Wnt pathways could potentially aid in creating new options for cancer treatment. FAK, being a kinase, is a very attractive target for development of small-molecule kinase inhibitors [11]. XAV939 (Fig. 2), a specific small-molecule chemical inhibitor of the Wnt/β-catenin pathway has recently been developed [47, 48]. XAV939 stabilizes the Axin protein by inhibiting poly-ADP-ribosylation Tankyrase enzyme isoforms. Since Tankyrase stimulates Axin protein degradation, XAV939 stabilizes Axin, thus triggering constitutive degradation of β-catenin protein and inhibition of the canonical Wnt-signaling pathway. Whether acting upstream or downstream to one another, the connection of these two
pathways in cancer could enable development of novel pharmacological strategies that target both pathways for more aggressive and specific chemotherapy treatments. The joint activation of FAK/Wnt pathways in specific cell or tissue types in cancers or other pathological states, suggests a potential dual sensitivity to both FAK and Wnt inhibitors for treatment. The involvement of both FAK and Wnt over-activation in different human cancers cells and the integration of these two signals in cancer pathology could help in finding more intelligent solutions for these often fatal diseases.

**FUTURE DIRECTIONS**

Only recently have the interactions between the Wnt and FAK pathways started to emerge (Fig. 1). There are at least two main directions for future investigation. First, there needs to be a greater understanding of the molecular mechanisms of these already identified FAK/Wnt connections. How does FAK regulate Wnt transcription and pathway activation during embryo development, cancer cell progression, or bone mechanotransduction? The link between FAK/Pi3K/AKT and β-catenin nuclear translocation in osteocytes

Fig. (1). FAK and Wnt/β-catenin pathways interact in diverse ways to regulate gene expression, neural cell fate specification, intestinal regeneration, bone metabolism and cancer cell formation.

Fig. (2). The chemical structure of XAV939 (3,5,7,8-tetrahydro-2-{4-(trifluoromethyl)phenyl}-4H-thiopyran[4,3-d]pyrimidin-4-one). Taken from Wang et al. 2010 [48].
should be evaluated, by examining the potential role of mediator proteins like Grb2. FAK1 protein's role in FAK/Wnt interconnection should also be further investigated in other cellular systems. A second approach is to look at additional fields of research through the eyes of FAK/Wnt interactions. In the example of IFP, there is much correlative information that there is indeed a causative FAK/Wnt link in disease progression, but there are few clues as to the true role of this interaction in disease etiology. There may be many overlooked biological and disease systems in which Wnt and FAK proteins are interacting, but not enough attention has been focused to detect these connections in existing experimental paradigms. An active future search to integrate these two signaling pathways in diverse model systems may reveal a better understanding of disease etiology and normal biological processes.

ACKNOWLEDGEMENTS

We thank Mr. Pavel Antokolsky for the graphics. D.F. was supported by a grant from the Israel Science Foundation (6389/09). D.F. and Y.F. were supported by a grant from the Israel-Niedersachsen Fund (ZN2319).

REFERENCES


