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Review

The role of focal adhesion Kinase in early development

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Summary. FAK is a tyrosine kinase enzyme demonstrated to play an important regulatory role in several basic cellular activities. Scientific evidence have suggested that FAK possessing a central position in the integrin signaling cascade, is responsible, at least in part, for the modulation of cellular proliferation, protection from apoptosis, adhesion, spreading and migration. The role of FAK in the development of different species, including human, is under study. Various published data supported the role of the molecule in the development of the placenta, as well as of several organ systems, like the musculoskeletal, nervous, cardiovascular, genitourinary and respiratory organ systems. Additionally, FAK has been shown to be implicated in the pathophysiology of pregnancy related disorders and congenital neonatal diseases and defects. The purpose of this article is a comprehensive review of the existing literature with a view to the future and the potential conclusions that can be drawn by the study of FAK signaling on the events of early life and species development.

Key words: FAK, Placenta, Embryogenesis

Introduction

Integrins are molecules known to mediate the bidirectional signaling of the cell with the extracellular matrix (ECM) (Schoenwaelder and Burridge, 1999; Schwartz, 2001). An important regulator of integrin signaling is Focal Adhesion Kinase (FAK). FAK was first described in 1992 as a member of the nonreceptor protein tyrosine kinases (PTKs) subfamily (Hanks et al., 1992; Lipfert et al., 1992; Zachary et al., 1992). The cDNA of FAK encodes a protein with a predicted molecular weight of 119-121 kDa, depending on different species, though on the basis of its migration in gels it is known as p125*FAK* (Zachary, 1997). FAK is expressed in a variety of species (Cary and Guan, 1999; Schlaepfer et al., 1999), with different expression levels in various tissues and cell types (Schaller and Parsons, 1994; Zachary, 1997).

Many studies showed that FAK plays an important role as an early key modulator in the integrin signaling cascade. Integrin clustering results in FAK autophosphorylation and the binding of the kinase Src. Src phosphorylates FAK at several tyrosine residues potentiating its kinase activity. The FAK/Src complex binds and phosphorylates many downstream molecules, transducing signaling by distinct, complex pathways that interact with each other. Signaling through FAK regulates various basic cellular functions, such as cell proliferation and growth, protection from apoptosis, adhesion and spreading, invasive and migratory properties (Chatzizacharias et al., 2008). More specifically, in vitro and in vivo studies on benign and malignant cell lines and tumours demonstrated that increased expression and/or FAK activity have been held responsible for the acquisition by cells of a more aggressive phenotype, resulting in increased proliferation rates, protection from apoptosis and

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Abbreviations. BMP: bone morphogenic protein, CNS: central nervous system, CTBs: cytotrophoblasts, d.p.c.: days post coitum, ECM: extracellular matrix, EphB: Ephrin-B, Erk: Extracellular-regulated kinase, FAK: focal adhesion kinase, FAs: focal adhesions, FGF2: fibroblast growth factor-2, HGF: Hepatocyte Growth Factor, MMPs: matrix metalloproteinases, MTJ: myotendinous junctions, PCNA: proliferating cell nuclear antigen, PD-Ia/ATX: phosphodiesterase-Ia/autotoxin, PI3K: phosphatidylinositol 3-kinase, PP2: protein phosphatase-2, PTKs: protein tyrosine kinases, PTP: protein tyrosine phosphatase, Pyk2: Proline-rich Tyrosine Kinase-2, TGFß: tumor growth factor-ß, VSD: ventricular septal defect.

increased adhesive, migratory and invasive capability.

With regards to development, several studies supported a possible role of FAK in the regulation of embryonic cellular differentiation, organogenesis and morphogenesis, as well as its implication in developmental defects and anomalies. Since most data are available from in vitro studies on cell lines, reduction of the conclusions to in vivo is a difficult and often risky endeavor that frequently leads to contradictory theories. This is further complicated by the fact that no satisfying in vivo counterpart for the FAs has been proposed yet, even though epithelial adherent junctions share some common features (Ridyard and Sanders, 1999; Ridyard and Sanders, 2000).

The purpose of this review is to summarize the available in vitro and in vivo evidence, revealing the potential role of FAK in development.

FAK and its functions

A feature of FAK is its subcellular localization to the focal adhesions (FAs), specialized integrin-based submembranous multiprotein complexes that participate in cellular signaling (Zachary, 1997). FAK is unique among PTKs in comprising of a central catalytic (kinase) domain flanked by two very large non-catalytic regions, consisting of approximately 400 amino acids each, the NH_2-terminal region, which has homology with the band 4.1 protein, as well as with ezrin, radixin and moesin, and thus is called FERM domain, and the COOH-terminal region (Schlaepfer et al., 2004; Cox et al., 2006). The kinase domain at the center of FAK molecule includes the tyrosine 576 and 577 domains, which are phosphorylated by Src and positively regulate FAK kinase activity (Cary and Guan, 1999). Unlike many other PTKs, FAK does not have SH2 or SH3 domains, but it does have SH2 and SH3 domain-interacting regions (Cary and Guan, 1999), by which, when activated, interacts with various molecules (Chatzizacharias et al., 2008).

Integrin clustering may promote the binding of FAK via the FERM domain, exposing tyrosine 397. These conformational changes can lead to the unmasking of the FAK active site and/or allow the catalytic domain to adapt active conformation, permitting trans-phosphorylation of the enzyme at tyrosine 397 and the subsequent recruitment of Src. Subsequently, Src phosphorylates FAK at tyrosine residues 576 and 577 in the activation loop, resulting in full catalytic FAK activity and recruitment of its downstream effectors, such as p130*Cas*, Extracellular-regulated kinase (Erk), phosphatidylinositol 3-kinase (PI3K) and paxillin. By activating its substrates, FAK propagates signals to different complex pathways that control basic cellular activities, like regulation of the cell cycle, cellular growth, proliferation and apoptosis; lamellipodia formation, matrix metalloproteinases (MMPs) secretion, adhesion, spreading and migration; regulation of the cytoskeleton and epithelial to mesenchymal transition (Ridyard and Sanders, 2000; Chatzizacharias et al., 2008). A detailed description of FAK signaling cascade is beyond the purpose of this article, but it can be easily understood that regulation of all these fundamental cellular activities may have a significant impact on the events of gestation and embryonic development.

Specifically for embryogenesis, evidence suggested that integrin signaling has a role in cellular embryonic development, regulating all the above-mentioned fundamental cellular activities within the embryo, but the role of FAK has not been clarified yet (Ridyard and Sanders, 1999). This is further complicated by the high level of complexity of the FAK-dependent signaling pathways, as well as by enzymes, such as Proline-rich Tyrosine Kinase-2 (Pyk2), which are proposed to compensate in cases when FAK signaling is downregulated or blocked (Ridyard and Sanders, 2000; Charlesworth et al., 2006; Kim et al., 2007; Weis et al., 2008).

The role of FAK in placental development and implantation

Two of the most important early events in pregnancy are the implantation of the embryo and the formation of the placenta. Implantation is a series of interactions between the embryo and the endometrium, including hatching, attachment and outgrowth. The differentiation of trophoblasts towards an invasive phenotype is an indispensable process of outgrowth and consists of the formation of anchoring villi, proliferation of cell columns and invasion of extravillous cytotrophoblasts (CTBs) into the maternal residual stroma, spiral arteries of the decidua and upper part of the myometrium. CTBs replace endothelial cells, acquire a vascular adhesion phenotype and help in the transformation of spiral arteries into low resistance vessels, which ensures an adequate blood supply to the fetus (Pollheimer and Knofler, 2005).

Early evidence suggested that FAK activation played an important role in embryonic implantation. The role of the molecule in the epithelial-to-mesenchymal transformation, a process critical for implantation, has been well supported by in vitro and in vivo studies (Ridyard and Sanders, 1999). Data also supported that FAK phosphorylation and consequent activation in human decidual cells in vitro was induced after attachment to fibronectin and preceded cell spreading (Shiokawa et al., 1998). Additionally, evidence was provided that the use of herbimycin A, a tyrosine kinase inhibitor, inhibited FAK activation, FA formation and the outgrowth phase of implantation in a dose-dependent manner in mice, but not the attachment. These results were supported by more recent data suggesting that even though the use of FAK antisense oligonucleotides did not affect the hatching or the attachment of the blastocysts, it inhibited outgrowth (Hanashi et al., 2003).

Throughout the pregnancy the uninhibited nutrient supply to the developing fetus depends on the normal

development of the placenta. Several data have been published suggesting that the regulatory molecule FAK and its downstream molecules Erk and PI3K, controls the extravillous trophoblast differentiation and cell invasion by modulating degrading and adhesive activities of the ECM, while human trophoblast cells have been found to express all four FAK isoforms (four products of fak gene transcription) (Daoud et al., 2008). FAK was highly detectable between the 5th and 8th week of gestation, localized at both villous stem cytotrophoblast cells, particularly of the developing column in anchoring villi, and extravillous trophoblast cells, and markedly decreased thereafter (MacPhee et al., 2001), while tyrosine phosphorylation was markedly decreased after 10-12 weeks gestation. Cell cultures under hypoxic conditions exhibited low levels of activated FAK and limited cytotrophoblast invasion in vivo and in vitro (Ilic et al., 2001; MacPhee et al., 2001). Further investigations showed that FAK targeting molecules resulted in limited cellular migratory and invasive capabilities (Ilic et al., 2001; MacPhee et al., 2001) and reduced trophoblast outgrowth, which was attributed to decreased cell proliferation and not to increased apoptosis (MacPhee et al., 2001). This is further supported by evidence that FAK expression regulated cellular motility through the arrangement of the FAs and the cytoskeleton, but not apoptosis, in chick embryo cells in vitro (Ridyard and Sanders, 2001). Additionally, studies suggested that the known downstream effectors of FAK, Erk and PI3K (Chatzizacharias et al., 2008), promoted chorionic cells to acquire an adhesive and migrative phenotype when activated in vitro (McKinnon et al., 2001; Cartwright et al., 2002; Kabir-Salmani et al., 2002; Chakraborty et al., 2003; Kabir-Salmani et al., 2004; Qiu et al., 2004). Furthermore, laboratory data supported the hypothesis that FAK signalling also has a role in the processes of vasculogenesis and angiogenesis during early pregnancy. Conditional knockout of FAK in mice endothelial cells was found to correlate with embryonic intrauterine growth retardation, attributed to impaired vasculogenesis and angiogenesis of both the embryo and the placenta (Shen et al., 2005). Finally, in chorionic cell line cultures, fibronectin and VEGF were found to promote MMPs expression and angiostatin to downregulate it, by interfering with the FAK signalling cascade (Zhang et al., 2003).

These observations suggest that FAK is a key mediator of the integrin-dependent cell activities during early embryogenesis and placental development (Table 1). Data suggesting the implication of disregulated trophoblast basic cellular activities in the pathogenesis of diseases of pregnancy, like preeclampsia and intrauterine growth restriction (Brosens and Renaer, 1972; Khong et al., 1986; McFadyen et al., 1986; Pijnenborg et al., 1991), signify the importance of

Table 1. The role of FAK in placental development and implantation.

Macropathological level			
Activity	Species	Conditions of study	Reference
Role in the outgrowth phase of embryonic implantation	Murine	In vitro	Shiokawa et al., 1998; Hanashi et al., 2003.
No role in embryonic attachment	Murine	In vitro	Shiokawa et al., 1998; Hanashi et al., 2003.
No role in embryonic hatching	Murine	In vitro	Hanashi et al., 2003.
Role in trophoblast differentiation	Human	In vitro	Daoud et al., 2008.
Role in trophoblast invasion	Human	In vivo and in vitro	MacPhee et al., 2001; Ilic et al., 2001.
Role in trophoblast outgrowth	Human	In vitro	MacPhee et al., 2001.
Role in vasculogenesis and angiogenesis	Murine	In vivo	Shen et al., 2005.
	Human	In vitro	Zhang et al., 2003.
Cellular level			
Activity	Species	Conditions of study	Reference
Role in epithelial-to mesenchymal transformation	Chicken	In vivo and in vitro	Ridyard and Sanders, 2000
Role in cellular proliferation	Human	In vitro	MacPhee et al., 2001.
No role in cellular apoptosis	Chicken	In vitro	Ridyard and Sanders, 2001.
	Human	In vitro	MacPhee et al., 2001.
Role in cellular adhesion	Human	In vitro	McKinnon et al., 2001; Cartwright et al., 2002; Kabir-Salmani et al., 2002, 2004; Chakraborty et al., 2003; Qiu et al., 2004.
Role in cellular spreading	Human	In vitro	Shiokawa et al., 1998.
Role in cellular invasion	Human	In vivo and in vitro	MacPhee et al., 2001; Ilic et al., 2001; Daoud et al., 2008.
Role in cellular migration	Human	In vivo and in vitro	MacPhee et al., 2001; Ilic et al., 2001; McKinnon et al., 2001; Cartwright et al., 2002; Kabir-Salmani et al., 2002, 2004; Chakraborty et al., 2003; Qiu et al., 2004.

further investigations in the field.

The role of FAK in embryonic morphogenesis and the development of the organ systems

Even though early data suggested that FAK was not essential for the differentiation of embryonic stem cells in vitro and in vivo (Ilic et al., 1995b), subsequent studies on several species presented evidence in the opposite direction.

It has been shown that FAK expression in chicken embryos remained relatively constant during early development, with different levels of activity, and thereafter declined, suggesting it was mostly active during the early remodeling of the embryo (Turner et al., 1993; Ridyard and Sanders, 1998). Phosphorylated FAK was also expressed in the amphibian embryo, together with FAK mRNA, from cleavage stages onwards (Hens and De Simone, 1995; Zhang et al., 1995). In Xenopus laevis maternal FAK mRNA was abundant throughout early cleavage stages, although very little of the protein could be detected prior to the midblastula stage (Hens and De Simone, 1995). At the onset of gastrulation FAK protein levels increased and the activated form was first detected, possibly due to the integrin-dependent adhesive interactions of the cells with fibronectin. A considerable amount was present in the cells of the marginal zone, suggesting that FAK translation may be a consequence of mesoderm induction. Furthermore, FAK mRNA was detectable from 7.5 days post-coitum (d.p.c.) in mouse embryos and continued to be expressed throughout the embryonic development, reaching maximum values around 9.5 and started declining after 11.5 d.p.c. (Polte et al., 1994). FAK protein expression closely followed that of the mRNA, with maximum values near 9.5 and a gradual decline after 12.5 d.p.c. More pronounced immunostaining was noted in the neuroepithelia, brachial arches, somites and vasculature, while staining in skeletal, cardiac and non-vascular smooth muscle, such as the GI tract, did not exhibit significant intensity (Polte et al., 1994). Developing tissues with increased vascularity and under stronger shear forces exhibited stronger immunostaining and, even though this can be attributed partially to the increased cell density, a role for FAK in the signaling transduction process for the development of the vascular smooth muscle was supported (Polte et al., 1994).

Additionally, published data suggested that deletion of FAK resulted in developmental deficiencies. Studies on FAK gene knockout mice showed defects in the development of the axial mesodermal tissues and cardiovascular system (Furuta et al., 1995; Ilic et al., 1995a,b; Shen et al., 2005), resulting in a lethal phenotype during early embryogenesis (Furuta et al., 1995; Ilic et al., 1995b; Shen et al., 2005). FAK null embryos were reasonably normal by E7.5, but abnormalities started to be noticeable from E8 and were clearly evident at E8.5 (Ilic et al., 1995b). The anteroposterior axis was dramatically retarded and mesodermal defects were noted, including involution of head mesoderm and retarded development of the lateral and extraembryonic mesoderm, as well as of the heart and the vascular system. These abnormalities were attributed to the impaired migratory properties of the embryonic cells due to the disorganization of the cytoskeleton, as was exhibited in cell cultures from E8 embryos (Ilic et al., 1995b), as well as to the cessation of mesodermal cell proliferation and p53 activation (Lim et al., 2008). More specifically, the selective inhibition of FAK signaling could lead to a narrower spectrum of developmental defects, while restoration of FAK signaling in totally FAK knockout mice could not rescue embryonic lethality (Shen et al., 2005).

Furthermore, several data have been published on the role of FAK in the developmental process of most of the organs and major organ systems (Table 2).

Musculoskeletal system

During embryogenesis the development of the components of the musculoskeletal system plays a vital role in the process of morphogenesis and the overall normal embryonic development. Osteoprogenitor cells may differentiate into osteoblasts, chondrocytes, fibroblasts, myocytes and adipocytes. Data showing that FAK is present in areas affecting mechanotransuction in osteoprogenitor cells support the role of FAK in the process of bone formation. Furthermore, studies also implicated the enzyme in the processes of chondrogenesis and muscular development.

With regards to osteogenesis, evidence was presented that FAK colocalizes with integrin-$\alpha_v\beta_3$ to the plaque-like areas on the surface of human preosteoblast cells, being more localized to the boundaries of the plaques (Wozniak et al., 2000). These areas were shown to mediate interactions of the cells with the ECM and were suggested to represent subcellular domains of bone formation responding to mechanical strain rather than the FAs. Additionally, early studies presented evidence that FAK activation contributed to the survival and differentiation of osteoblastic cells (Takeuchi et al., 1997; Tamura et al., 2001), while FAK signaling has been implicated in the process of osteoblastic differentiation (Suzawa et al., 2002), through Erk and Runx2 (Xiao et al., 2000), a kinase important for this process (Lai et al., 2001). In vitro studies on human mesenchymal stem cells showed that FAK tyr-397 phosphorylation, as well as Erk activation, osteogenic genes transcription and matrix mineralization, were induced with adhesion to collagen-I, vitronectin, laminin-5 and laminin-332 (Kundu and Putnam, 2006; Salasznyk et al., 2007a,b; Klees et al., 2008). Specifically, osteogenesis on vitronectin was also correlated with increased FA formation (Kundu and Putnam, 2006). On the contrary, FAK siRNA treated cells exhibited reduced FAK expression and phosphorylation, as well as reduced Erk1/2 phosphorylation and Runx2/Cbfa-1 activation

Table 2. Key evidence in favour and against the role of FAK in the development of the organ systems.

Musculoskeletal system development			
Activity	Species	Conditions of study	References
No macroscopic skeletal anomalies	Murine	*In vivo*	Kim et al., 2007.
Role in mechanotransduction during osteogenesis	Human	*In vitro*	Wozniak et al., 2000.
No role in mechanotransduction during osteogenesis	Murine	*In vitro*	Manduca et al., 2005.
	Canine	*In vivo*	Moalli et al., 2001.
Role in the survival of osteoblastic cells	Murine	*In vitro*	Takeuchi et al., 1997; Tamura et al., 2001.
Role in the differentiation of osteoblastic cells	Murine	*In vitro*	Takeuchi et al., 1997; Tamura et al., 2001; Suzawa et al., 2002.
	Human	*In vitro*	Kundu and Putnam, 2006; Salasznyk et al., 2007a,b; Klees et al., 2008.
No role in the differentiation of osteoblastic cells	Murine	*In vitro*	Kim et al., 2007.
Role in the differentiation of chondrocytes	Bovine	*In vitro*	Vinall et al., 2002.
	Murine	*In vitro*	Vinall et al., 2002.
	Chicken	*In vitro*	Bang et al., 2000.
Role in the condensation of chondrogenic cells	Chicken	*In vitro*	Jin et al., 2007.
Role in the formation of intersomitic furrows and myotomes	Zebrafish	*In vivo*	Henry et al., 2001.
Role in the differentiation of myoblasts	Murine	*In vitro*	Clemente et al., 2005; Woo et al., 2006.
Role in mechanotransduction in myogenesis and costamerogenesis	Murine	*In vitro*	Quanch and Rando, 2006; Zhang et al., 2007.
Role in myofibrilogenesis	Murine	*In vitro*	Quanch and Rando, 2006.
Role in MTJ formation	Xenopus laevis	*In vitro*	Baker et al., 1994.
Nervous system development			
Activity	Species	Conditions of study	References
Role in axon outgrowth and pathfinding	Murine	*In vitro*	Liu et al., 2004.
	Chicken	*In vitro*	Mangoura, 1997.
	Chicken	*In vivo*	Li et al., 2004.
No role in axon outgrowth and pathfinding	Murine	*In vitro*	Charlesworth et al., 2006.
No role in synapse formation	Murine	*In vitro*	Charlesworth et al., 2006.
Role in myelination	Murine	*In vivo* and *in vitro*	Fox et al., 2004.
Role in dendritic spine morphogenesis	Murine	*In vitro*	Moeller et al., 2006.
Role in brain sexual differentiation	Murine	*In vivo*	Speert et al., 2007.
Eye development			
Activity	Species	Conditions of study	References
Role in the differentiation of ocular tissue	Murine	*In vivo*	Kokkinos et al., 2007.
Role in the morphogenesis of optic stalk and proliferation of surface glial cells	Drosophila	*In vivo*	Murakami et al., 2007
Cardiovascular system development			
Activity	Species	Conditions of study	References
Role in vasculogenesis	Murine	*In vivo*	Furuta et al., 1995; Ilic et al., 2003; Shen et al., 2005; Sirois et al., 2006; Braren et al., 2006; Peng et al., 2008.
	Murine	*In vitro*	Shen et al., 2005.
Role in angiogenesis	Murine	*In vivo*	Furuta et al., 1995; Ilic et al., 2003; Shen et al., 2005; Braren et al., 2006; Peng et al., 2008.
	Murine	*In vitro*	Shen et al., 2005.
Role in cardiac morphogenesis	Murine	*In vivo*	Black and Olson, 1998; Shen et al., 2005; Sirois et al., 2006; Hakim et al., 2007; Peng et al., 2008.
	Murine	*In vitro*	Hakuno et al., 2005.
Role in the differentiation of smooth muscle cells	Murine	*In vitro*	Berk and Corson, 1997; Xiao et al., 2007.
Role in the growth of smooth muscle cells	Murine	*In vitro*	Xiao et la., 2007.
No role in the differentiation of endothelial cells	Murine	*In vitro*	Ilic et al., 2003.
No role in the proliferation of endothelial cells	Murine	*In vitro*	Ilic et al., 2003; Braren et al., 2006.
Role in the apoptosis of endothelial cells	Murine	*In vitro*	Shen et al., 2005.
	Murine	*In vivo*	Furuta et al., 1995.
Role in the migration of endothelial cells	Murine	*In vitro*	Shen et al., 2005.
No role in the migration of endothelial cells	Murine	*In vitro*	Braren et al., 2006.
Role in the spreading of endothelial cells	Murine	*In vitro*	Braren et al., 2006.
Role in the development of the cellular organs of cardiac myocytes	Murine	*In vivo*	Peng et al., 2008.
No role in the differentiation of cardiac myocytes	Murine	*In vivo*	Peng et al., 2008.
Role in the proliferation of cardiac myocytes	Murine	*In vivo*	Peng et al., 2008.
No role in the proliferation of cardiac myocytes	Murine	*In vivo*	Hakim et al., 2007.
No role in apoptosis of cardiac myocytes	Murine	*In vivo*	Hakim et al., 2007; Peng et al., 2008.
Role in the migration of cardiac myocytes	Murine	*In vitro*	Hakim et al., 2007.
Respiratory system development			
Activity	Species	Conditions of study	References
Role in bronchiole branching	Murine	*In vivo*	Gill et al., 2006.
Role in the proliferation of the epithelial cells	Murine	*In vivo*	Gill et al., 2006.
Genitourinary system development			
Activity	Species	Conditions of study	References
Role in the proliferation of renal cells	Murine	*In vivo*	Wen et al., 1997; Sorenson and Sheibani, 1999.
Role in the migration of renal cells	Murine	*In vivo*	Wen et al., 1997; Sorenson and Sheibani, 1999.
Role in prostatic acinar morphogenesis	Human	*In vitro*	Bello-DeOcampo et al., 2001.
Role in the migration of apical cells during ovarian morphogenesis	Drosophila	*In vivo*	Cohen et al., 2002.

(Salasznyk et al., 2007a,b). Additionally, transfected cells expressed reduced levels of the hallmarks of ECM-induced osteogenic differentiation, bone sialopretein-2, osteocalcin and osterix, as well as reduced calcium deposition, alkaline phosphatase activity and mineral:matrix ratio (Kundu and Putnam, 2006; Salasznyk et al., 2007a,b). These results supported the fact that ECM-induced FAK activation promoted osteogenic differentiation through Erk1/2 and Runx2/Cbfa-1 signaling cascades *in vitro*, and suggested that FAK knockdown could interfere with bone formation in vivo, even though FAK signaling may be far more complicated than in the *in vitro* studies.

On the other hand, opposing theories and data have been also published. Evidence suggested that mechanical stimulation induced a rapid activation of FAK and increased association with Src, limited to the stromal cells and fibroblasts in the bone marrow area of woven bone in vivo, while osteoblastic cells on trabecular surfaces or within osteolytic lacunae did not respond (Moalli et al., 2001). Additionally, FMS Calciumfluor, a known promoter of osteogenic differentiation, has been shown not to cause alterations in FAK activity (Manduca et al., 2005). In the same direction were the results of a study on neonatal and adult murine calvarian cells (Kim et al., 2007), which showed that while most of the FAK null cells could not complete the differentiation in vitro, a minority of 30% could. Furthermore, FAK mutant embryos had an intact skeleton, with no differences in the onset, rate and extent of bone formation when compared with controls, as investigated with the use of histological staining, whole mount analysis, X-rays and micro-CT from E13.5 through postnatal and adult life. Additionally, the growth of the skeleton, as well as the architecture of the growth plates, exhibited no differences between mutants and controls. Similar were the results of studying bones being formed through intramembranous ossification. These observations suggested that FAK was not essential for the process of osteoblast differentiation in vivo. Another possibility might be that FAK signaling was required only transiently and that other molecules, like Pyk2, compensated, at least in part, for the loss of its function (Kim et al., 2007).

Furthermore, apart from the process of bone formation, FAK has also been suggested to participate in chondrogenesis, the process of cartilage formation consisting of the migration of mesencymal cells, the condensation of the progenitor cells and the differentiation and maturation of chondrocytes (Olsen et al., 2000; Goldring et al., 2006). FAK has been shown to mediate the signaling for the transition of chondro-progenitor cells to dedicated chondrocytes (Bang et al., 2000; DeLise et al., 2000), while evidence also supported that FAK mediated the phase of chondrogenic cell condensation (Jin et al., 2007). The knockdown of MMP-2 in chicken embryonic cells resulted in increased precartilage condensation and chondrogenesis, as well as increased fibronectin and integrin-αvß1 expression and FAK tyrosine phosphorylation. On the contrary, the use of bafilomycin A1, an MMP-2 activator, resulted in decreased FAK activity. The study proposed that FAK played an important role in the phase of condensation, regulated by the effect of MMP-2 on the integrin-mediated interaction between FAK and fibronectin. Additionally, data suggested that FAK also participated in the differentiation and morphogenesis of murine and bovine cartilage cells in vitro (Vinall et al., 2002). Initially, FAK staining was localized within the chondrocytes throughout the tissue depth, being more intense in the deep zone (adjacent to the subchondral bone) and mainly punctuate and membrane associated, suggesting the presence of FAs. Additionally, it was suggested that FAK expression was linked with collagen type II expression, a marker of chondrocyte phenotype. Adding bone morphogenic protein-7 (BMP) resulted in increased FAK and collagen type II expression. These effects were inhibited by cytochalasin-D, suggesting the involvement of the actin cytoskeleton. On the other hand, the catabolic cytokine IL-1 reduced FAK and collagen type II expression in a dose-dependent manner. These data suggested that extracellular signals, such as BMP-7 and IL-1ß affected chondrocyte phenotype, through an actin-dependent process that included FAK expression and localization in vitro. The reduced amount of FAK in the surface zone compared to the deep one, implied this role also in vivo (Vinall et al., 2002).

With regards to musculogenesis, immunohisto-chemical studying of zebrafish embryos (Henry et al., 2001) showed that FAK mRNA expression gradually increased in the paraxial mesoderm during the formation of somites. In the developed somites FAK mRNA expression was persistent, with the area of maximum staining being slightly variable. Both FAK and the activated form of the molecule were found to persist at the intersomitic furrows. Furthermore, phosphorylated FAK was observed at the recently formed somite boundaries and at the borders of cells within the myotomes. These data supported the role of FAK in the formation and maintainance of the intersomitic furrows and the formation of myotomes. It was suggested that FAK regulated the differentiation and adhesive properties of the cells, stabilizing and maintaining the intersomitic furrows through an integrin-mediated interaction between the cell and the ECM (Henry et al., 2001).

Skeletal muscle differentiation is a multistep process and a proposed model for the role of FAK is that initial myoblast differentiation is dependent on a transient decrease in FAK activity, but terminal differentiation with formation of myotubes is dependent on FAK tyr-397 phosphorylation and activation (Clemente et al., 2005). This model resulted from the process of data on murine C2C12 skeletal muscle cell differentiation. FAK phosphorylation levels were found to be transiently reduced when the cells were transferred from proliferation to differentiation medium. However, after 5 days in culture they increased to an even greater level

than those of cells in the proliferating medium. On the contrary, overall FAK protein levels remained unchanged. With regards to FAK localization, increased staining was exhibited in the lamellipodia and filopodia of the proliferating cells, while in the differentiating cells FAK staining was more prominent at the FAs. Furthermore, FAK tyr-397 phosphorylation blockage with use of mutant-FAK resulted in decreased cyclin-D1 and increased myogenin (myogenic regulatory factor) expression in both proliferating and differentiating myoblasts. Additionally, attenuation of the expected increase in creatine kinase activity after the change to differentiating culture medium was also observed, while myoblast fusion and formation of myotubes were significantly inhibited, even with increased levels of myogenin. Comparable were the results from the use of genistein, a known tyrosine kinase inhibitor, on rat skeletal myoblasts, which inhibited myoblast differentiation by disrupting FAK signaling (Woo et al., 2006). Genistein reversibly blocked both myoblast proliferation and fusion in a dose-dependent manner, impaired the synthesis and/or accumulation of the differentiation markers myogenin and creatine kinase, and resulted in a reversible reduction of FAK tyrosine phosphorylation with minimal effect on the protein concentration. Additionally, it was shown that genisteine-induced FAK inhibition resulted in reduced PI3K activity and impaired calcium influx into the cells. These data suggested a signaling pathway downstream of FAK, through PI3K, which regulated the calcium influx into the myoblasts, essential for their differentiation (Woo et al., 2006).

Furthermore, the role of FAK in the mechanical stimulated development of the musculoskeletal system has also been under study. Zhang et al. studied the role of FAK in the mechanotransduction signaling after stimulation of integrin-ß^{1D} in murine C2C12 myoblasts (Zhang et al., 2007). The cells cultured under conditions of stress exhibited an increased rate of Z line formation and a significantly larger myotube diameter compared to controls cultured under static conditions. Additionally, cells under stretch conditions had increased levels of integrin-ß^{1D} and phosphorylated FAK, which were correlated with the formation of myotubes. Silencing integrin-ß^{1D} with the use of siRNA led to a significant decrease in the formation of myotubes, which was compensated when integrin expression was restored, while the levels of activated FAK remained unchanged. These data suggested the role of FAK in the mechanical transduction-induced integrin signaling, essential for myoblast differentiation.

In the process of mechanotransduction the costameres, electron-dense formations rich in FA proteins located between the plasma membrane and the myofibrils, have an important role (Pardo et al., 1983; Shear and Bloch, 1985). Costameres have been proposed to function as transmitters of contractile force from the myofibrils to the ECM to maintain the spatial organization of the myofibrils and the integrity of the muscle fibers (Pardo et al., 1983; Shear and Bloch, 1985). The role of FAK in costamerogenesis was suggested by an in vitro murine skeletal myoblast model (Quanch and Rando, 2006), as FAK was localized at the FAs of myoblasts and costameres of mature cells, as well as at the respective sites of their differentiating stages. The transfection of cells with the FAK-inhibitor FAT resulted in the disruption of FAK localization and activity at the FAs of myoblasts, but it did not affect the biochemical differentiation of the skeletal muscle cells. On the contrary, in these cells costameres appeared less organized and the number of cells containing mature costameres was dramatically decreased. Additionally, FAK signaling also affected myofibrilogenesis, as FAT transfection resulted in decreased numbers of normal myofibrils in the developing cells compared to controls. Similar results were reproduced using FAK siRNA, further supporting that FAK signaling played an important role in the processes of myofibrilogenesis and costamerogenesis in the developing skeletal muscles in vitro, and predicting the same effects during the in vivo development (Quanch and Rando, 2006).

Finally, FAK is believed to also mediate the formation of the myotendinous junctions. Data on the the myotendinous junctions (MTJ) of Xenopous dissociated and leg muscle fibers (Baker et al., 1994), suggested that a significant concentration of FAK immunolabeling was detected at the MTJ, localized along the characteristic invaginations of the junction into the tendon, while FAK concentration dramatically dropped with moving away from the junction. When cultured myotomal muscle cells were labeled, FAK was colocalized with achetylcholine receptor clusters at sites of cell-substratum contact, while accumulation of FAK could be induced by beads in the cultured muscle cells in a spatial and temporal manner. These observations supported a role for FAK also in the formation of the MTJ.

Nervous system

Several data have been published on the potential role of FAK in the development of the nervous system. Studies have shown that the expression of FAK, as well as that of its active phosphorylated form, were high in the embryonic rat brain and decreased postnatally, suggesting the role of the enzyme in the developing neural system (Burgaya et al., 1995; Stevens et al., 1996; Serpente et al., 1996). In *Xenopus laevis*, during neuronal development, FAK was expressed in several cranial nerves, the lobes of the developing brain and the neural tube (Hens and DeSimone, 1995).

Axonal outgrowth and pathfinding are crucial for the development of the nervous system. The role of netrin receptors in the process has lately been established. Netrins are known to stimulate axon growth by either attractive or repulsive signals (Culotti and Merz, 1998). Even more interestingly, data suggested the role of FAK downstream of the netrin receptor activation (Liu et al.,

2004). FAK was found phospharylated in several residues after the treatment of embryonic cerebellar cortex cells with netrin, along with the Src family kinase Fyn, while use of the Src family kinase inhibitor PP2 inhibited axonal outgrowth and attraction. On the other hand, in cells from FAK knockout mice netrin failed to produce axon outgrowth and attraction. These results suggested the role of FAK in netrin-induced axonal outgrowth and attraction, through interaction with Src family kinases. Furthermore, in vivo studies on dorsal spinal and cortical chicken neurons showed an increase in DCC, a known-netrin receptor, and FAK tyrosine phosphorylation, while the use of an antibody against DCC significantly blocked FAK phosphorylation (Li et al., 2004). FAK and DCC were shown to form a complex under physiological conditions, with the C-terminal domain of FAK interacting with the C-terminal half of the P3 domain of DCC. The study suggested that netrin binding to DCC resulted in the recruitment of FAK and its autophosphorylation. The subsequent FAK/Src complex formation with the stimulation of FAK activity are well described events of FAK signaling cascade (Chatzizacharias et al., 2008) and resulted in the phosphorylation of DCC by the FAK/Src complex and the propagation of further downstream signaling. One of the molecules that participate in the cascade is UNC5, a netrin receptor known to mediate cell migration and axonal repulsion (Li et al., 2006). FAK and Src were able to directly phosphorylate UNC5 in vitro, but the proposed in vivo model is that the activated FAK/Src complex is responsible for the phosphorylation of UNC5 in the DCC-UNC5 receptor complex.

Additionally, the m-opiod receptor, known to regulate neuritic outgrowth (Sakellaridis et al., 1986; Vernadakis et al., 1990), when activated, resulted in the activation of FAK in chick embryonic neurons (Mangoura, 1997). The use of met-enkephalin to induce the receptor was shown immunohistochemically to result in increased FAK and vinculin phosphorylation, while the use of naloxone abolished these results. When the cells were cultured, by day 5 FAK was expressed in all neurons. High levels of expression were detected in fascicles interconnecting neuronal aggregates by day 7, while the lowest expression was noted in the filopodia of the growth cones. These results clearly supported the role of FAK downstream of the m-opiod signaling cascade and constituted an attractive hypothesis for the mechanism of the spectrum of disorders in neonates that have been exposed to excessive opioids during their neonatal development (Mangoura, 1997).

On the other hand, in vitro studying on murine neurons produced from FAK-null embryonic stem cells exhibited no differences between FAK deficient neurons and controls with regards to the length or the complexity of the processes and the formation of synapses (Charlesworth et al., 2006). Additionally, NMDA receptor gated channels and voltage sensitive calcium currents were unaltered in FAK-null neurons. The absence of morphological and electrophysiological phenotype differences in these neurons was attributed to the complexity of the FAK signaling cascade and the rescue effects of other kinases, such as Pyk2. The possibility that FAK might not be essential for the development of neurons in vitro, even though it is for their development in vivo, was also suggested (Charlesworth et al., 2006).

Furthermore, FAK has also been proposed to have a critical role in the development of the glial cells of the nervous system and the central nervous system (CNS) myelination. CNS myelination involves oligodendrocyte morphogenesis and changes in their interaction with the ECM, regulated by oligodendrocyte-released phosphodiesterase-Ia/autotoxin (PD-Ia/ATX) (Fox et al., 2004). In rat cell cultures, in the presence of PD-Ia/ATX, FAK phosphorylation at tyr-397 remained unchanged, while tyr-925 was significantly decreased. Further investigations showed that PD-Ia/ATX reduced FAK tyr-925 phosphorylation in postmigratory oligodendrocytes, but not in their migratory progenitors. The in vivo study of FAK phosphorylation showed that tyr-925 phosphorylation was reduced at the onset of myelination. Both failure of recruitment of an Src-family kinase or the action of a phosphatase can be held responsible for the described effect. Nonetheless, these results suggested a role for FAK in the process of oligodendrocyte morphogenesis and the myelination of CNS. Additionally, FAK has also been shown to play a role in the dendritic spine morphogenesis initiated by Ephrin-B (EphB) receptor activation (Moeller et al., 2006). Data on murine hippocampal neurons confirmed that FAK, as well as RhoA, became phosphorylated after the activation of the EphB receptor and formed discrete clusters along the dendritic cell surfaces. On the contrary, FAK knockout resulted in the disruption of the shortening of the dendritic filopodia. These observations suggested that FAK, through the activation of RhoA, regulated the contractility of microfilaments, thus playing an important role in the process of filopodia shortening, a vital process for the formation of the dendritic spines (Moeller et al., 2006).

Finally, evidence suggested the possible role of FAK in sexual differentiation of the brain. Data were presented that FAK protein levels exhibited sex related differences in rat hypothalamic cells (Speert et al., 2007). FAK levels were significantly elevated in hypothalamic cells on the day of birth in females when compared to those noted in males, following an incremental pattern until postnatal day 6, when they started falling, while in males FAK levels remained relatively constant. On the contrary, no differences were identified in the cortex and the thalamus. High FAK levels were suggested to correlate with sexual differentiation of the brain, since they were noted only in the hypothalamus, an area known to actively undergo changes during this process (Speert et al., 2007). This hypothesis was further supported by the fact that treatment with large estradiol doses resulted in reduced FAK expression and increased neurite branching, a

pattern consistent with diminished FAK expression (Rico et al., 2004), even though this was observed only during a restricted time window. Finally, inhibition of endogenous estradiol significantly reversed the low levels of FAK in male cells, but not in female ones.

The eye

The development of the eye can be schematically categorized in the development of the optic apparatus, responsible for receiving the signal-light, and of the neurological apparatus, responsible for the transformation of the light to action potential and its propagation to the brain. FAK has been implicated in the development of both. For example, in the developing eye of *Xenopus laevis*, FAK was expressed in the lens, as well as in the neuronal layer of the retina (Hens and DeSimone, 1995).

Lens development is comprised of a series of interactions, culminating in the contact of the neuroepithelium of the forming retina and the ectoderm of the forming lens, and is regulated by several genes and signaling pathways downstream of growth factors and ECM molecules (Fisher and Grainger, 2004). During early stages of lens development in mice, FAK was expressed ubiquitously in most ocular tissues, with those undergoing morphogenetic differentiation exhibiting the strongest FAK mRNA expression (Kokkinos et al., 2007). At E11.5 strong FAK expression was detected in the apical lens pit, while at E13.5 FAK expression became more intense in the elongating fibers and the anterior epithelium of the differentiating lens vesicle. At P1, FAK mRNA was detected in the apical cytoplasm of both equatorial and anterior epithelial cells and was strongly localized in the basal cytoplasm of differentiating lens fibers, while at P21 expression was initiated in the posterior region of the lens germinative zone and became more strongly expressed in the transitional zone. Throughout ocular development, FAK was expressed in the optic cup, with strong reactivity at the inner optic cup at E11.5, the peripheral optic cup at E13.5, the ganglion cells layer and the ciliary body at P1 and the ciliary body at P21. FAK protein localization followed FAK mRNA expression patterns as demonstrated by immunofluorescence. Further analysis exhibited phosphorylated FAK expression throughout the lens epithelium and in the transitional zone at E14.5. At E17.5, FAK reactivity was predominately detected in the cytoplasm of epithelial cells, while in the transitional zone it was predominately associated with cell membranes. On the contrary, in differentiating fibers phosphorylated FAK was localized in the nuclei. Similarly, at P21 phosphorylated FAK immunoreactivity was predominately detected in the cytoplasm in the germinative zone and in the nucleus in the transitional and cortical fibers. This rather obscure observation, since FAK is not known to have a nuclear targeting domain, has been rarely reported (Levkau et al., 1998; Fernandez-Valle et al., 1998; Ridyard and Sanders, 2000) and is thought to be associated with an interaction of the enzyme with an intermediate protein (Ridyard and Sanders, 2000). Interestingly, Src has also been described localized in the FAs and the nucleus of cultured cells (Moszczynska and Opas, 1993). Furthermore, exposure of lens epithelial explants to fibroblast growth factor-ß2 (FGF2) induced a gradual increase in FAK tyrosine phosphorylation, starting at 24 hours and up to the 6th day of culture, while the developing cells became more elongated and migratory. On the contrary, cells expressing a dominant negative tumor growth factor-ß (TGFß) receptor, which are known to have defective migratory responses to FGF2 and abnormal actin cytoskeleton dynamics (De Iongh et al., 2001), exhibited decreased FAK phosphorylation starting form P2. These observations suggested the role of FAK in lens differentiation induced by FGF, as also during the terminal differentiation mediated by TGFß family members. As FAK is known to mediate survival signals, it was suggested that the different levels of expression, activation and localization of the molecule regulate apoptosis in order to ensure denucleation without cytoplasmic blebbing of the fibres (Kokkinos et al., 2007).

With regards to the retina, FAK has been implicated in the development of the two retinal epithelia, the neural retina and the retinal pigment epithelium. The regulation of their phenotypic expression is dependent upon interactions between the cells and the extracellular environment, and characterized by transdifferentiation (Moszczynska and Opas, 1994b). Furthermore, transdifferentiation is dependent upon soluble and attachment factors, such as growth factors and cell-to-cell or cell-to-ECM interaction. In retinal cultures, FAK, which was abundant in FAs of flat undifferentiated cells, was downregulated in cells that had differentiated and packed into an epithelial sheet, and a switch from predominantly cell-ECM adhesions associated with FAs to cell-cell adhesions associated with zonulae adherence was observed (Moszczynska and Opas, 1994a). Additionally, FAK has been proposed to play a key role in the integrin-bound photoreceptor phagocytosis, a daily process critical for the optimal retinal function, involving the shedding of the distal outer segmental tips (Finnemann, 2003). FAK phosphorylation at tyr-861, presumably by Src, promoted the direct interaction of FAK with the integrin cytoplasmic tail and the beginning of the signaling cascade, which continued with the activation of $p130^{Cas}$ and the participation of the molecule MerTK and ended with the phagocytosis of the photoreceptors by macrophages, dendritic cells and fibroblasts. Furthermore, evidence supported the role of FAK in the invasion of collagen by retinal epithelial cells in vitro (Van Aken et al., 2003), a process related to proliferating retinopathy in vivo (Grierson et al., 1997). Hepatocyte growth factor (HGF) was detected in the conditioned medium, while N-cadherin was the major cadherin expressed by the cells (Van Aken et al., 2003). FAK, along with c-Met, was found activated and its

phosphorylation was inhibited by the use of antibodies against HGF and N-cadherin, which also inhibited collagen invasion. The study concluded that FAK participated in the autocrine HGF/c-Met pathway, stimulating retinal epithelial cell invasion, as well as in the N-cadherin induced invasion of the cells.

Finally, evidence supported the role of FAK in the morphogenesis of the optic stalk in Drosophila, which is comprised of the photoreceptor cells axons (R axons) and two kinds of glial cells, wrapping and surface glial cells, and innervates the optic ganglia (Meinertzhagen and Hanson, 1993; Perez and Steller, 1996; Hummel et al., 2002). Surface glial cells are organized into a monolayer tubular structure and ensheath the R axon bundle during optic stalk development. Data supported that optic stalk morphogenesis is a process that is primarily or wholly regulated by FAK-dependent surface glial cell proliferation, independently of the R axons (Murakami et al., 2007). FAK mutants exhibited defective optic stalks in terms of length and diameter, an observation that became clearer as development proceeded, indicating the role of FAK during optic stalk expansion rather than in its initial establishment. The expression of FAK in the surface glial cells rescued optic stalk development, while no such evidence was reproduced with the expression of the enzyme in the photoreceptor cells. Finally, investigation of the role of FAK in the cellular functions of the surface glial cells showed that FAK affected migration, with mutant cells failing to form a tubular structure due to a significant failure to disperse to the same distance as the wild type controls, but not proliferation, as no differences were detected between mutant cells and controls.

Cardiovascular system

During embryonic development, blood vessels are formed by two processes, vasculogenesis and angiogenesis. In the first one, a primitive vascular network is formed from the pluripotent mesenchymal progenitors and in the second one, new capillaries are formed from pre-existing vessels (Yancopoulos et al., 1998). In both, endothelial cells play a central role, acting both as transducers and effectors of local signals. The role of FAK in the process of cardiovascular development has been clearly established.

Immunohistochemical investigation of FAK expression during mouse embryonic development exhibited the strongest reactivity to the vasculature from 10.5 – 17.5 d.p.c., first being evident in the dorsal aorta (Polte et al., 1994). Similar staining was noted in the arterial vasculature, but veins exhibited little or no distinctive reactivity. The staining of vessel walls appeared to arise from the medial layer, which is much thicker in arteries than veins. In vitro studying of the levels of FAK phosphorylation in rat aortic smooth muscle cells suggested that increased levels of activated FAK were noted after interaction with fibronectin and angiotensin II (Berk and Corson, 1997), signifying a role in signaling transduction and cellular differentiation and growth. Additionally, evidence supported a possible role of FAK in the cellular endeavor for counteraction of the detrimental effects in embryonic signaling of vinculin null embryos (Xu et al., 1998). These embryos exhibited increased mortality rates, attributed to heart maldevelopment, but also increased phosphorylated FAK levels. Vinculin is not known to be a direct substrate for FAK, but both bind paxillin, and thus FAK over-reactivity might be one way of compensating for the diminished vinculin signaling.

Maldevelopment of the cardiovascular system was considered as one of the causes of early embryonic lethality in FAK knockout mice (Furuta et al., 1995; Ilic et al., 1995a; Braren et al., 2006; Peng et al., 2008). Both vasculogenesis and angiogenesis were impaired and neither the heart nor the blood vessels were normally developed in the embryos. Early in embryonic life no gross abnormalities were detected, but progressively a proportion of the mutants either suffered from severe vascular defects or died (Braren et al., 2006; Peng et al., 2008). For the rest that did not deviate significantly from the normal phenotype, a compensatory role of Pyk2 has been proposed by observations in in vivo and ex vivo models (Weis et al., 2008). Investigation of the mutants showed slightly smaller size compared to controls (Braren et al., 2006), as well as hemorrhages in the yolk sac and the amniotic cavity (Ilic et al., 2003; Braren et al., 2006; Peng et al., 2008) and dilated vessels, especially in the upper trunk and head regions (Braren et al., 2006). Additionally, all the vasculature exhibited distorted architecture. Furthermore, no vessels were present in the neuroepithelium, indicating defective sprouting angiogenesis (Braren et al., 2006). The dorsal aorta was formed in some regions only, suggesting poor survival and defective morphogenesis of the endothelium (Furuta et al., 1995). Addidtionally, the omphalomesenteric artery was not detected in mutants, compromising normal embryonic circulation (Furuta et al., 1995). The dilatation of the capillaries and the intercapillary spaces was suggested to be the primary defect, confirmed by the study of embryonic explants ex vivo (Braren et al., 2006). It was also suggested that FAK deletion resulted in decreased vessel growth and increased regression and contraction (Braren et al., 2006). Even though the lack of FAK activity did not seem to prevent the differentiation of the endothelial cells, tubulogenesis seemed to correlate both with FAK levels of expression and distribution, as was observed in cell cultures obtained from the FAK deficient embryos (Ilic et al., 2003). With regards to the cellular effects of FAK deletion, it has been proposed that increased mortality and abnormal spreading with defective lamellipodia formation could be held responsible, although significant changes in the proliferation rate and the migratory capacity of the cells were not demonstrated (Braren et al., 2006).

Furthermore, FAK deletion also compromised the cardiac development. Histological analysis of the hearts

of surviving embryos revealed significantly thinner ventricular walls in FAK null embryos when compared to controls, with most of those, which grossly appeared normal, having ventricular septal defect (VSD) (Hakim et al., 2007; Peng et al., 2008) with associated malalignment of the outflow tract, including an overriding aorta and a double-outlet right ventricle (Hakim et al., 2007). Less penetrating phenotypes included embryos with persistent truncus arteriosus and thickened valve leaflets (Hakim et al., 2007). These results suggested the possibility that structural defects during cardiac development were responsible for the in utero mortality. All survived embryos had a normal life span, but autopsy showed dilated right ventricles with no apparent reason being revealed after histological examination, suggesting that FAK inactivation caused spontaneous right ventricular hypertrophy in the surviving mice (Peng et al., 2008). Coming to the cellular level electron microscopical analysis showed that FAK deficient cardiac myocytes presented a dilated rough endoplasmic reticulum, more in the atria, irregularly spread ventricular mitochondria with disrupted cristae and partially lucent matrix and thinner and disorganized myofibrils (Peng et al., 2008). Additionally, it was supported that the downregulation of MEF2a, a transcription factor known to play an important role in cardiac development (Black and Olson, 1998), by FAK deficient activity through a Src-dependent pathway, could be held responsible for the demonstrated cardiac abnormalities. With regards to the FAK-regulated cellular activities the results were contradictory. In one study (Hakim et al., 2007) no significant differences in proliferation were noted, while in another (Peng et al., 2008) reduced cell proliferation was demonstrated by the analysis of phosphorylated histone-3 and Ki67 labeling. On the contrary, differences in the apoptotic rates (Hakim et al., 2007; Peng et al., 2008) or cellular differentiation (Peng et al., 2008) were not noted. However, decreased myocardialization of the endocardial cushions was noted, resulting in outflow tract malfusion to the interventricular septum. FAK deficient cardiac myocytes exhibited reduced migration in vitro, correlated with reduced tyrosine phosphorylation of Cas (Hakim et al., 2007), a known downstream molecule of FAK signaling cascade controlling cellular migratory properties (Chatzizacharias et al., 2008).

Similar were the results of Shen et al. (2005), confirming that FAK inactivation led to embryonic lethality in early embryogenesis, but also suggesting that the selective inhibition of FAK signaling could lead to a narrower spectrum of developmental defects. Furthermore, restoration of FAK signaling in endothelial cells of totally FAK knockout mice, could not rescue embryonic lethality. The selective conditional deletion of FAK in endothelial cells, produced by flanking of the gene by two loxP sites, impeded vascular development in mouse embryos in vivo. In contrast to generalized FAK knockout phenotypes, the selective deletion of FAK in endothelial cells did not lead to the death of the embryo in the early developmental period. However, later on some of the embryos started to show developmental retardation, but the majority was found normal. During the subsequent stages, increasing embryo numbers exhibited various defects and embryonic mortality started to increase. Thorough examination of the embryos revealed variably sized hemorrhages and oedema, attributed to the absence of normal superficial vasculature and amniotic blood vessels. Even more specific investigations revealed markedly decreased vasculogenesis and angiogenesis in the FAK knockout embryos during late embryogenesis, when compared to the control group. Histological analysis showed endothelial cells with pyknotic, karyorrhectic changes and evidence of apoptosis and collapsed blood vessels. Further in vitro studies on endothelial cells from these embryos suggested decreased ability to change their morphology and form capillaries, a process mimicking the in vivo sprouting and tube formation. Additionally, increased apoptotic and decreased survival rates were noted when compared to the control group, as well as decreased cell cycle progression. With regards to cellular migration, reduced migration was noted in response to VEGF, but not to fibronectin (Shen et al., 2005).

The role of FAK in cardiovascular embryonic development was also supported by evidence linking the enzyme with the action of phosphatases. Deletion of the protein tyrosine phosphatase (PTP) PEST in mouse embryos resulted in several developmental failures, including impaired vascularisation and decreased cardiac region (Sirois et al., 2006). The amount of phosphorylated FAK in these embryos was increased, and since evidence exists that FAK may be a substrate of PTP-PEST (Lyons et al., 2001), increased FAK signaling can be held responsible for the described defects. Additionally, FAK has been implicated in the in vitro differentiation of stem cells to smooth muscle cells (Xiao et al., 2007). Differentiating cultured murine stem cells on collagen-IV expressed high levels of several integrins, including α_1, α_5, $ß_1$ $ß_3$, and PI3K activity. Integrin blocking significantly decreased the expression of differentiation markers and FAK tyrosine phosphorylation, as well as the activity of its downstream molecules PI3K, Erk1/2, JNK and c-Jun. Differentiation was also inhibited by the use of cytochalasin-B and the consequent disruption of the actin filaments, as well as the use of FAK inhibitors, such as genistein and protein phosphatase-2 (PP2). These data suggested that an integrin-initiated signaling pathway, through FAK and via PI3K, Erk1/2, JNK and c-Jun, was involved in the differentiation of murine stem cells into smooth muscle cells in vitro. Finally, treatment of murine embryonic stem cells in vitro with PP2 showed that the promotion of selective cardiogenesis was attributed, at least in part, to the inhibition of adhesion-induced FAK activation (Hakuno et al., 2005). PP2 was suggested to direct cells towards cardiac

progenitors rather than promoting the proliferation of the cardiac progenitors, since it did not affect cardiogenesis after the point that stem cells had already been directed to cardiac progenitors. On the contrary, activation of FAK by cell adhesion during the early stages of development inhibited cardiogenesis. Therefore, it was suggested that inhibition of cellular migration, but not proliferation, by downregulation of FAK signaling, may promote cardiogenesis.

Respiratory system

The development of the respiratory system depends on the full and uninhibited bronchiole branching. Evidence supported the central role of FAK in regulating this morphogenetic process in mice (Gill et al., 2006). By studying embryonic lungs of MMP-null mice, reduced bronchiole branching was observed. Additionally, reduced expression of phosphorylated FAK was noted during the initial stages of bronchiole branching when compared to controls. On the contrary, treatment of the embryos in utero with a synthetic MMP inhibitor led to an increase in FAK activation and localization in epithelial cells of branching bronchioles. Both these observations coincided with the distribution of fibronectin in the basement membrane of the cells examined. These data supported the hypothesis that a reduction in FAK signaling may be responsible for the inhibition of bronchiole branching. This was further supported by the fact that downregulation of FAK using siRNA resulted in a significant reduction of peripheral terminal bronchiole buds when compared to controls. Furthermore, the use of siRNA also led to a decrease in the number of epithelial cells, attributed to decreased cell proliferation since a significant reduction in the number of positive cells for the proliferating cell nuclear antigen (PCNA) was detected. Finally, analysis of cells presenting active MMPs suggested that the number of proliferating epithelial cells was reduced, while in utero treatment with an MMP inhibitor significantly enhanced the proliferation rates. All this evidence suggested that FAK signaling was required for bronchiole branching and that this signaling could be modulated by the enzymatic activity of MMPs (Gill et al., 2006). The role of the enzyme was proposed to be most likely the signal transduction between the ECM and the cells, which is a well recognized presupposition for lung morphogenesis (Cardoso, 2000).

Genitourinary system

Several published data suggested the role of FAK in the development of the kidneys and ureters, as well as in the development of the internal male and female genitalia. Staining in the glomerulus of the kidney appeared to be confined to the capillary loops and the smooth muscle-like mesangial stalk, with particular intensity from the afferent and efferent arterioles, while strong staining of the germ cells of the gonads in both sexes was observed as early as the 13.5 d.p.c. (Polte et al., 1994). Additionally, FAK tyrosine phosphorylation, regulated by the cytoplasmic and transmembrane domains of CD98, has been proposed to control branching morphogenesis of inner medulary collecting duct cells (Cai et al., 2005).

The investigation of FAK expression in the kidneys of normal mice from E13 to P20 (Sorenson and Sheibani, 1999), by Western blot analysis, detected high levels of FAK in utero, but decreased postnatally to a low level at P20. This decline corresponded with an increase in the levels of the FAK fragments p77 and p85, known to be generated by the cleavage of FAK by caspases (Wen et al., 1997). Additionally, the fragment FRNK, truncated COOH-form of FAK, was detected being phosphorylated (Sorenson and Sheibani, 1999). The level of tyrosine phosphorylated FAK also followed the same distribution throughout the period, being highest from E15 to P0, but marking a decline from P10 when the formation of new nephrons decreased to virtually undetectable levels following kidney maturation at P20. Again, levels of the phosphorylated p77 fragment were noted to increase throughout renal maturation. These observations were consistent with a distinctive role of FAK during nephrogenesis, with increased levels of the active enzyme being expressed during the proliferative and migratory phase and decreased during the latter stages and in the mature kidney when stable FAs are formed. Furthermore, evidence was presented that cystic kidneys from bcl2 -/- mice exhibited sustained FAK phosphorylation. This observation would be consistent with epithelial cells being near, but unable to reach, full maturation. Deletion of Bcl2, a gene known to facilitate cellular proliferation and believed to participate in the FAK signaling cascade (Chatzizacharias et al., 2008), would lead to sustained FAK activation resulting in increased cellular migration, proliferation and focal adhesion formation, thus interfering with the formation of cell-cell adhesions during renal maturation with consequent formation of cysts (Sorenson and Sheibani, 1999).

Furthermore, published data supported that FAK has an important role in prostatic and ovarian morphogenesis. The use of three dimensional models in the study of prostate development is a relatively new practice, giving information more closely associated to the in vivo process. In the developing prostate the budding urogenital epithelial cells use the ECM to migrate into the mesenchyme and form acini and ducts. Essential for this process is the binding of the cells to the ECM proteins via the integrin receptors. The study of human prostatic epithelial cells in three dimensional cultures (Bello-DeOcampo et al., 2001) suggested the use of the integrin-$\alpha_6\beta_1$ receptor for the observed cellular polarization and acinar morphogenesis on laminin-1. FAK was found colocalized with integrin-β_1, while the use of a MEK-1 inhibitor, an inhibitor of a FAK-mediated pathway, resulted in a significant decrease in acinar formation, supporting a FAK-

mediated signaling pathway downstream of integrin-$\alpha_6\beta_1$ that regulated acinar morphogenesis. With regards to ovarian development, evidence suggested that FAK mediated the secreted glycoprotein DWnt4-induced regulation of the process in Drosophila (Cohen et al., 2002). Ovarian structure was disrupted in DWnt4 mutants that exhibited impaired apical cellular migration. Normal apical cells initially exhibited primarily filamentous staining coincidental with actin fibers, but at later stages FAK staining had a more spotty appearance. In cells carrying the DWnt4 mutation, the overall levels of FAK were reduced. Additionally, the large FAK spots that were observed during the initial phases in the wild phenotype were not present, while at later stages FAK appeared as small spots, as in the normal cells, even though the overall FAK staining was diffuse and reduced. These observations suggested a signaling pathway through FAK for the regulation of the migratory properties of apical cells during the DWnt4-regulated female gonadal development in Drosophila (Cohen et al., 2002).

Conclusions

The role of FAK in integrin signalling is well established. Due to its central location in the signalling cascade, FAK mediates several basic cellular activities, such as proliferation, adhesion, spreading, migration and apoptosis. Published data supported the role of FAK in the events of early pregnancy and embryonic development in several species. In vitro and in vivo studies suggested that FAK participates in the implantation of the embryo and the formation of the placenta in mice and humans. Additional data, from in vitro and in vivo studies on several species including human, have been published on the role of the kinase in the development of the musculoskeletal, nervous, cardiovascular, respiratory, genitourinary systems and of the eye (Table 2). Furthermore, evidence exists supporting theories of a possible implication of abnormal FAK signalling in the pathogenesis of diseases and conditions, such as preeclampsia, intrauterine growth restriction, cardiovascular defects, renal cysts, proliferating retinopathy and even death in utero (Table 3).

Perspectives

Several scientific data supported the role of FAK in animal and human development, yet the specific pathways remain vastly unspecified. Since FAK is known to mediate and regulate basic cellular functions, as already mentioned, future studies should concentrate on specifying the cascade of the complex signalling pathways that control these activities. This would clarify even more the role of the enzyme and illuminate aspects that still remain unclear, such as the crosstalk among the pathways, the compensating signalling adaptations and the role of the nuclear localization of the kinase. Specifically, more experiments are needed in order to obtain solid evidence for the in vivo role of FAK, since theories and assumptions based on in vitro results are often ambitious and do not provide solid and adequate explanations to the in vivo observed phenomena.

The role of the enzyme in human development is yet equivocal, since studying is limited to in vitro staining investigations of cell lines and preparations. The ethical boundaries against in vivo studies on humans are preventive of a direct observation on the results of either normal FAK signalling or the deficiency or blocking of its enzymatic activity. Therefore efforts should be made in the direction of interpretation of the role of FAK signalling in embryonic development of vertebrates and other species that can lead to useful and potentially valid assumptions on the role of the molecule in human development. In this way, not only will we expand our

Table 3. Possible implication of FAK in disease.

Pathology		Species	Conditions of study	References
Diseases of pregnancy (preeclampsia, intrauterine growth restriction)		Human	*In vivo*	Brosens and Renaer, 1972; Khong et al., 1986; McFadyen et al., 1986; Pijnenborg et al., 1991.
		Murine	*In vivo*	Braren et al., 2006.
Fatality with embryonic FAK-null phenotype		Murine	*In vivo*	Ilic et al., 1995b, Furuta et al., 1995; Shen et al., 2005.
Disorders from intrauterine exposure to opioids		Chicken	*In vitro*	Mangoura, 1997.
Severe vascular defects (malformation of aorta and omphalomesenteric artery)		Murine	*In vivo*	Furuta et al., 1995; Peng et al., 2008.
Cardiac defects:	- VSD with associated malalignment of the outflow tract including overriding aorta and double-outlet right ventricle	Murine	*In vivo*	Hakim et al., 2007; Peng et al., 2008.
	- Persistent truncus arteriosus and thickened valve leaflets	Murine	*In vivo*	Hakim et al., 2007.
	- Spontaneous right ventricular hypertrophy	Murine	*In vivo*	Peng et al., 2008.
Renal cysts and polycystic kidney disease		Murine	*In vivo*	Sorenson and Sheibani, 1999.
Proliferating retinopathy		Human	*In vitro*	Van Aken et al., 2003

knowledge on the biological development of animal species, but also form and assess possible theories on the pathophysiology of congenital diseases.

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