CHAPTER 1

General introduction: matrix adhesion, cell migration and breast cancer

Cell migration is a central process in development, as well as in many physiological and disease states such as cancer progression. Metastasis, the dissemination of cancer cells from the primary tumor to a distant organ, is the most frequent cause of deaths for patients with cancer. Matrix adhesions are cytoplasmic substructures that attach the cells to the extra-cellular matrix (ECM). This attachment is mediated by the receptors integrins and adaptor proteins that include structural and signaling molecules. Matrix adhesions are dynamic structures that play a crucial role in diverse biological processes and among them, cell maintenance and migration. This chapter will describe the role of matrix adhesions in relation to cell migration and breast cancer metastasis formation.

1. Molecular and cellular measurements of matrix adhesions dynamics

Matrix adhesion complexes are cytoplasmic structures that have been originally identified by electron microscopy[1] and by interference reflection microscopy[2]. Upon integrin ligation to the ECM, the adhesion receptors will cluster and link the cell to the ECM. This induces the recruitment of structural and signaling adhesion proteins, forming large protein complexes bound both to clustered integrins and to actin filaments. Integrins are the principal cell surface adhesion receptors mediating cell-matrix adhesions[3,4].

1.1 Molecules and structure

In the last years, considerable information has been accumulated on the molecular composition of matrix adhesions. Approximately 150 proteins, which form the so-called 'integrin adhesome', have been to date retrieved to be part of the matrix adhesions including kinases, phosphatases and structural proteins (for review see[5]). Upon attachment, integrins will cluster and promote local recruitment of structural proteins like vinculin, paxillin, talin, α-actinin and tensin, and signaling molecules including tyrosine kinases such as focal adhesion kinase (FAK), serine/threonine kinases and various adapter proteins (Fig. 1). The molecular complexity of cell-matrix adhesions enables them to fulfill their dual role as modulators of both mechanical cell anchorage and transmembrane signaling[6]. Integrins are not the unique transmembrane receptors that have been described to control and regulate adhesion signalling; syndecans can also bind directly to the ECM while growth factors such as EGFR can crosstalk with the integrins to regulate the recruitment of cytoplasmic proteins to adhesion sites (for review see[7]).

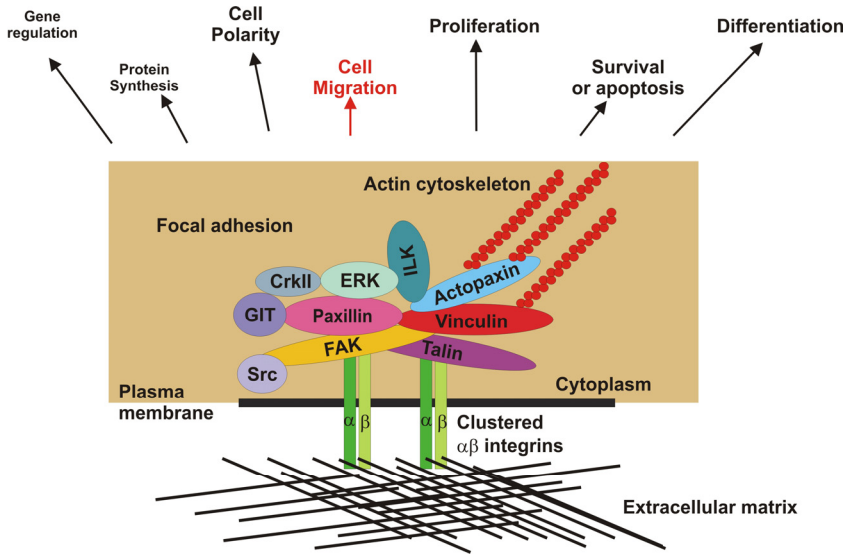

Figure 1: Focal adhesions provide both a structural and a signaling link between the ECM and the actin cytoskeleton. The adhesion of a cell to the ECM, via transmembrane β-integrin heterodimers, leads to integrin activation and the recruitment of numerous intracellular proteins to the plasma membrane. Focal adhesions are now known to comprise over 150 protein species (only selected examples are depicted), which include both structural proteins (which mediate a physical link to the actin cytoskeleton) and regulatory proteins (which have a major role in the modulation of actin dynamics for productive cell migration). Proteins such as paxillin serve as scaffold proteins to facilitate the functional integration of these different categories of focal-adhesion proteins. Adapted from[4].

Matrix adhesions are diverse in molecular composition, size and shape. Their distribution over the whole cell body is also heterogeneous and depends on cell type and environment. Detailed analysis based on morphology, molecular composition and method of formation of mainly fibroblasts and epithelial cells allowed those adhesions to be classified in three different classes. The most common forms of integrin-mediated cell-matrix adhesions in cultured cells are focal adhesions (FA), fibrillar adhesions (FB) and focal complexes (FC)[8]. Focal adhesions are oval structures, usually a few μm^2 in area, and are associated with the termini of actin stress fibers. Fibrillar adhesions, which are derived from FAs, are elongated contact sites, associated with fibronectin fibrils. Focal complexes are small, dot-like adhesions that are mainly found at the cell edge and apparently nucleate FA formation (reviewed by[6]). Furthermore, matrix adhesions are heterogeneous in their molecular composition which can be studied using fluorescence ratio imaging. For example, FC do not contain zyxin whereas FB do not contain β_3 integrin or phosphorylated FAK[9] but is highly enriched with tensin[8]. Matrix adhesions are mechanosensors that sense the matrix physical properties whether it is rigid or soft and whether it is two- or three dimensional[10].

1.2 Molecular and structural dynamics

The molecular and structural diversity of the matrix adhesions is regulated by a wide variety of mechanisms, including competition between different partner proteins for the same binding sites, interactions triggered or suppressed by tyrosine phosphorylation, and conformational changes in component proteins, which can affect their activity[9,11,12]. The dynamic changes in structure and molecular properties are driven by mechanical force generated by the actin- and myosin-containing contractile machinery of the cells, or by external forces applied to the cells, and regulated by matrix rigidity (for review see[6,10]). Typically, in migrating cells, adhesions are newly formed at the leading edge of the cells, will mature along the cell body and disassemble at the rear of the cell. Overall, the matrix adhesion turnover include assembly, maturation and disassembly[13,14].

1.3 Dynamic imaging of matrix adhesions during tumor cell migration

Understanding movement of single tumor cells in tissue requires the analysis of matrix adhesion turnover. Ideally, we would like to measure movement and shape changes of cells and correlate these with the spatio-temporal dynamics of the matrix adhesion elements and the extra- and intracellular signaling pathways controlling these behaviors. Nowadays, imaging is the most suitable method to obtain such data that can be collected at high spatial and temporal resolution. Through the advances of genetically encoded labels, it is now possible to label many of the cellular and cytoskeletal components[15,16]. This allows direct visualization of the dynamics of matrix adhesion and cytoskeleton components

during cell migration. Furthermore, using photobleaching approaches, it is also possible to obtain information on protein turnover within the matrix adhesions[17,18]. In **Chapter 4**, we present a methodology that makes use of an adapted photobleaching approach together with computer simulation to understand the turnover of matrix adhesion proteins. Activity of signaling components that control the assembly/disassembly of matrix adhesions such as FAK, Src, RhoGTPases can be measured through changes in the cellular distribution of these components, changes in intramolecular conformation or complex formation with other signaling molecules using Fluorescence resonance Energy Transfer (FRET)[19]. Finally, those different fluorescence imaging techniques, which are reviewed in details in **Chapter 3,** can be performed either in migrating cell in a two-dimensional (2D) or in a three-dimensional (3D) environment including *in vivo* tumor environment.

2. Breast cancer progression and metastasis formation

Breast cancer is the most common cancer among women. In The Netherlands, about 11,000 cases of breast cancer patients are retrieved per year: it means that 1 over 9 women will get breast cancer in her life. Each year, still more than 3,500 women die from breast cancer (http://www.rivm.nl/vtv/object_document/o1492n17276.html). When the tumor is still restricted to its primary site, the disease can be relatively easily treated by surgical removal of the tumor. Indeed, the majority of cancer mortality is attributed to metastasis, which is the spread of tumor cells to a secondary site.

2.1 Metastasis

2.1.1 Processes involved in metastasis

The metastatic process consists of a large number of steps (Fig. 2) which are very much independent from each other[20-22]. As a primary tumor grows due to several mutations, it needs to develop a blood supply that can support its metabolic needs, a process called angiogenesis. The blood and lymphatic vessels can provide an escape route by which cells can leave the tumor and enter into the body's circulatory system, known as intravasation. Tumor cells might also enter the blood circulation indirectly via the lymphatic system (Fig. 2). The cells need to survive in the circulation until they might extravasate from the circulation into the surrounding tissue and arrest in a new organ. Once in the new site, cells must initiate and maintain growth to form micrometastases; this growth must be sustained by the development of new blood vessels in order to form secondary tumors. Tumor cell-intrinsic alterations include the loss of cell polarity and alterations in cell-cell and cell-matrix adhesion as well as de-regulated receptor kinase signaling, which together support detachment, migration and invasion of tumor cells. On the other hand, the tumor stroma, including endothelial cells,

fibroblasts and cells of the immune system, is engaged in an active molecular crosstalk within the tumor microenvironment. Subsequent activation of blood vessel and lymph vessel angiogenesis together with inflammatory and immune-suppressive responses further promotes cancer cell migration and invasion, as well as initiation of the metastatic process (for review see[23,24]).

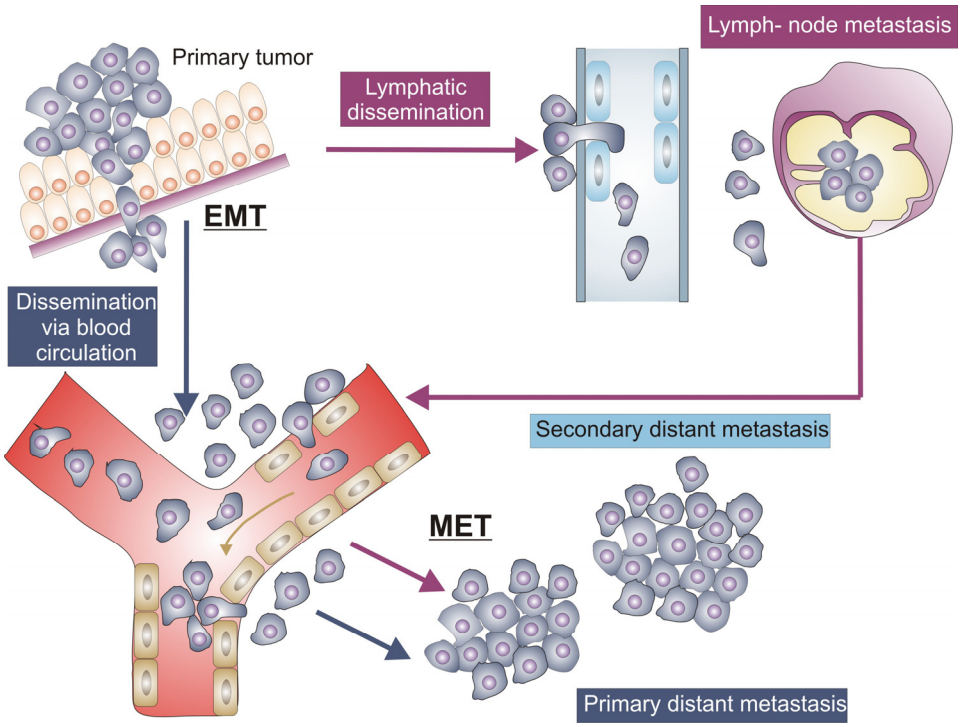

Figure 2: Models of the metastatic cascade. The metastatic process starts with the uncontrolled growth of cells (primary neoplasm), followed by epithelial to mesynchymal transition (EMT) to migrate through the extracellular matrix (ECM). Cancer cells can disseminate from the primary site via lymphatic routes (red arrows) and the blood circulation (blue arrows). Secondary haematogenous dissemination also occurs from overt metastases to other distant sites (black arrows). In the first model, disseminated tumor cells proliferate in the lymph nodes to form solid metastases, whereas the tumor cells at distant sites die or remain dormant. At later stages, tumor cells disseminate from the established lymph-node metastases to distant sites, where they form secondary metastases. In the second model, tumor cells primarily undergo dissemination through blood vessels to form distant metastases. This occurs in patients who develop metastases at other organs, whereas the lymph nodes remain tumor free, such as in patients with breast cancer. Haematogenous dissemination seems to start at the earliest stages in tumor progression. Adapted from[21].

2.1.2 *Mechanisms of metastasis*

Metastasis is a very complicated, not yet totally elucidated, process. It requires both the appropriate cues from the environment as well as the right expression and activity of distinct proteins in the cancer cells [25]. In order to progress through the different steps of metastasis, several classes of proteins are overexpressed in cancer cells (reviewed in[20]). Some of the important proteins involved in the separate steps are summarized in table 1. The uncontrolled cell proliferation is mainly triggered by deregulated growth factor signaling (e.g. EGFR and TGFβ). Furthermore, cancer cells have to undergo so-called epithelial-to-mesenchymal-transition (EMT) to be able to escape the primary tumor. This means that the tumor cells loose contact with their neighbouring cells (loss of E-cadherin) and are then able to migrate (overexpression of FAK and paxillin). During the migration/invasion process the cell excretes enzymes, including so-called matrix metalloproteinases's (MMPs), that degrade the extracellular matrix that is present in, and surrounds the tumor.

	Metastasis processes	**Pathways/molecules involved**
Tumor cell-intrinsic changes	Proliferation	C-Met and hepatocyte growth factor (HGF) Wnt signaling ErbB signaling Insulin-like growth factor 1 receptor (IGF1R)
	Epithelial-Mesenchymal transition (EMT)	TGF-β, Wnt and IGF1R signaling Protein Tyrosine Kinases (PTKs) Loss of E-cadherin function
	Migration	Integrins, Immunoglobulin family adhesion molecules (NCAM and L1), matrix-metalloproteinases (MMPs), and PTKs
	Survival	p53 and PTKs
	Invasion	MMPs, Chemokines receptors
Metastasis-promoting activities of the tumor microenvironment	Tumor angiogenesis	VEGF, uPA, uPAR
	Tumor-induced lymphangiogenesis	
	Carcinoma-associated fibroblasts	
	Contribution of the immune (innate and adaptative) system to tumor metastasis	

Table 1: Signaling pathways involved in the steps of the metastasis process. These proteins become activated/overexpressed and thereby activate a number of downstream targets.

2.1.3 Models to study metastasis

Breast cancer is not a single disease. It is instead a collection of mammary gland diseases that have diverse histopathologies, genetic and genomic variations, and clinical outcomes. A major challenge in advancing our understanding of the biology of breast cancer is the availability of experimental model systems that recapitulate the many forms of this disease. Because of this complexity and heterogeneity no single model mimics all aspects of the disease (for review see[26]). For breast cancer and most other solid cancers, it is necessary to develop models to evaluate treatments for metastatic disease and to enhance our understanding of the mechanisms that underlie metastatic progression, which is the principal cause of mortality[27,28]. Transgenic mice are often used in the study of breast cancer. In these, oncogenes (HER2) or tumorsuppressors (p53) can be overexpressed (or downregulated), either constitutively or inducibly, and spontaneous or chemically induced tumor / metastasis formation can be studied. Although studies involving transgenic mice provided information on tumorigenesis, they have been less successful in replicating advanced cancer. On the other hand, orthotopic xenografts of tumor cell lines in immunodeficient mice reproduce the histology and metastatic pattern of most human tumors at an advanced stage[29,30]. *In vitro*, the tumor cell lines can be genetically manipulated and subsequently xenograft models can be used to molecularly dissect the metastatic process. In **Chapter 5** and **6** we described an improved mouse model[31] that can be used for intravital imaging[32,33] and which is suitable for the study of the cell autonomous processes of metastasis formation[34]. Nevertheless, this approach is not useful in the study of the initial stages of tumorigenesis or the contribution of the immune system in this process. To investigate the role of the immune system, a third model that can be used is the syngeneic model[35]. In this model a tumor cell line is isolated from a spontaneous or chemically induced tumor, studied or manipulated *in vitro* and subsequently injected to animals with the same genetic background. By combining the *in vitro* and *in vivo* data of the cell-line of interest, these different models can be used to molecularly dissect the metastatic process, including the contribution of the immune system.

2.2 Signaling pathways in cancer progression

The selective process of metastasis requires that cancer cells successfully complete several sequential, rate-limiting steps. They must detach from the primary tumor, invade the host stroma, intravasate into lymphatic or blood vessels, spread to the capillary bed of distant organs, extravasate and proliferate in the receptive organ parenchyma[20,22,23]. Several key signaling pathways play crucial roles in this process.

2.2.1 *Growth factor receptors and cell migration*

Many metastatic human carcinomas are characterized by the overexpression or constitutive activation of ErbB tyrosine kinase receptor (EGFR) family members involving activating mutations of the receptor kinases or an autocrine loop with EGF family ligands. High expression of EGFR and ErbB2, as well as another tyrosine kinase receptor c-Met are associated with bad prognosis of breast cancer patients. In addition to stimulating cell differentiation and proliferation, EGF promotes tumor cell motility, invasion and metastasis[31]. *In vivo* studies show that ErbB1 expression can enhance invasiveness most probably through increased chemotaxis to gradients of EGF[36]. Indeed, there is a paracrine loop that takes place between macrophages and tumor cells which enhances invasiveness in response to EGF[37,38].

Interestingly, ErbB receptors and Wnt signaling cooperate during tumorigenesis, which may be also critical for metastasis formation the β-catenin/ErbB interaction correlates with the incidence of pulmonary metastases, indicating a functional role of these interactions in metastasis formation[39]. Tyrosine phosphorylation of β-catenin by ErbB1 and by other receptor tyrosine kinases is known to destabilize its binding to E-cadherin. As a result, adhesion junctions disassemble, the liberated β-catenin may accumulate in the cytoplasm, transfers to the nucleus and, in combination with Tcf/Lef1 transcription factors, modulates gene expression. In the absence of Wnt ligands, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β), ubiquitinated and degraded by proteasom system[40]. Such activation of the Wnt pathway may result in increased tumor cell migration, invasion and metastasis (reviewed in[41]).

2.2.2 *MAP kinase and cell migration*

Mitogen-activated protein kinase (MAPK) pathways involve kinases that control cell growth, proliferation, differentiation, migration and apoptosis[42,43]. MAPK pathways function in a typical cascade in which a MAPK is activated upon phosphorylation by a mitogen-activated protein kinase kinase (MAPKK), which in turn is activated when phosphorylated by a MAPKKK. To date six distinct groups of MAPKs have been characterized in mammals; extracellular signal-regulated kinase (ERK)1/2, ERK3/ 4, ERK5, ERK7/8, Jun N-terminal kinase (JNK)1/2/3 and the p38 isoforms a/b/g(ERK6)/d (see review[44,45]). Abnormalities in MAPK signalling impinge on most, if not all processes that are involved in tumorogenesis, and play a critical role in the development and progression of cancer[46-48]. Especially, in this thesis (**Chapter 7**) we focus on the JNK-paxillin pathway previously described to be involved in tumor cell migration[49,50].

2.2.3 The focal adhesion-associated tyrosine kinase focal adhesion kinase (FAK)

One of the first integrin signalling molecules to be identified was focal adhesion kinase (FAK), which acts as a phosphorylation-regulated signalling scaffold and is important for adhesion turnover, Rho-family GTPase activation, cell migration and cross-talk between growth-factor signalling and integrins[51,52]. This ubiquitously expressed, essential protein contains an N-terminal FERM domain, a central kinase domain, proline-rich regions and a C-terminal focal-adhesion-targeting (FAT) domain that interacts with paxillin and talin. The FAK homologue Pyk2 shares many of these features, but Pyk2 and FAK have unique activities and are only partially redundant. In response to integrin clustering, the autophosphorylation of FAK generates docking sites for SH2-domain-containing proteins; these include Src kinases, which in turn become activated and phosphorylate FAK, promoting its kinase activity and its interaction with other proteins. Structural analyses have revealed the mechanism of interaction between FAK and paxillin, and how FAK is inhibited by interactions between its FERM and kinase domains; they have also elucidated a role for PtdIns(4,5)*P*2 in FAK activation[51,53,54].

Considerable evidence implicates FAK in the regulation of cell migration. Most notably, FAK-deficient cells spread more slowly on extracellular matrix proteins, exhibit an increased number of prominent focal adhesions and migrate poorly[13,55-58]. Overexpression of FRNK also inhibits the rate of cell spreading and migration and metastasis formation in a breast cancer *in vivo* model, presumably by sequestering key regulatory proteins required for efficient FAK signaling[59-61]. Finally, overexpression of FAK in Chinese hamster ovary (CHO) cells enhances cell migration[62]. Furthermore, FAK is shown to be involved in tumor progression (reviewed in[63-66]). Recent reports (see also **Chapter 2**) review that FAK is a potential target for cancer therapy[67-69].

2.2.4 The focal adhesion scaffold protein paxillin

Paxillin, is an essential signalling scaffold that is recruited early to integrin adhesions and interacts with signaling components such as FAK and ILK[70]. Since paxillin is extensively studied in this thesis in the context of breast cancer progression, it will be discussed in detail below.

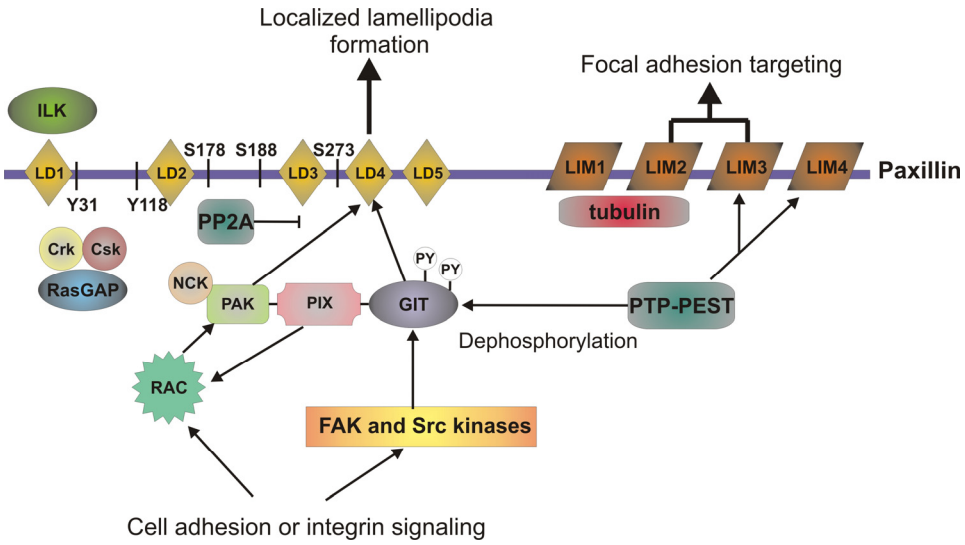

Figure 3: The scaffold protein paxillin interacts with other focal adhesion-related proteins through the LD and LIM domains. Paxillin contains several protein-protein interaction modules (leucine-rich repeats, a prolinerich region and LIM domains) and its numerous phosphorylation sites provide additional regulated sites of protein-protein interaction. Together, they mediate the binding of kinases (e.g. FAK, Src and ILK), phosphatases (e.g. PTP-PEST), actin-binding proteins (e.g. vinculin and the parvins) and regulators and effectors of the Rho family of small GTPases (e.g. the CrkII-DOCK180-ELMO complex and PIX). Adapted from[70].

2.2.4.1 Paxillin structure and paxillin 'interactome'

Paxillin (Fig. 3) is a molecular adaptor or scaffold protein which primary function is to serve as a scaffold to coordinate, integrate and facilitate efficient cell signaling, through direct and indirect interactions with multiple signaling and structural proteins that constitute the paxillin 'interactome'. Paxillin contains various domains that mediate the interactions with other structural and signalling proteins[71,72].

The C-terminal half of paxillin contains four LIM domains, which are double-zinc-finger motifs that mediate protein-protein interactions[73].The LIM2 and LIM3 domains of paxillin are essential for targeting the protein to focal adhesions[74]. It has been established that phosphorylation of these domains contributes to the regulation of focal-adhesion targeting of paxillin[75]. The LIM domains of paxillin also serve as binding sites for several structural and regulatory proteins, including tubulin and the tyrosine phosphatase PTP-PEST[76,77], and these interactions have important roles in controlling focal-adhesion dynamics[78,79].

The N-terminus of paxillin controls most of its signaling activity. It contains five leucine- and aspartate-rich LD motifs (LD1-LD5)[80]. LD motifs were originally identified when the binding sites on paxillin for vinculin and FAK were

mapped[79,81]. LD motifs interact with actin-binding proteins (e.g. vinculin and actopaxin) and signaling proteins such as FAK, integrin-linked kinase (ILK), and the family members of ADP ribosylation factor/GTPase activating proteins (ARF/GAPS) including G-protein-coupled receptor kinase interacting protein/paxillin kinase linker (GIT1/PKL)[82,83]. Between LD1 and LD2 there is a proline-rich motif, which binds to SH2 domain of Crk and p120Ras GAP[84].

Multiple tyrosine, serine and threonine phosphorylation sites exist throughout the paxillin molecule[81,84,85], these sites are targeted by diverse kinases that are activated in response to various adhesion stimuli and by growth factors. These include p21-activated kinase (PAK)[86], FAK-Src[87], receptor for activated C kinase 1 (RACK1)[88], Jun N-terminal kinase (JNK)[49,50], extracellular-signal-regulated kinase (ERK)[89,90], p38 mitogen-activated protein kinase (p38 MAPK)[89,90], cyclin-dependent kinase 5 (CDK5)[91] and Abl[92]. The phosphorylation of paxillin contributes to the complexity of its interactome by regulating the interactions of various proteins with its protein binding modules or, as in the case of tyrosine phosphorylation, by providing additional docking sites for other structural and signaling components[84].

2.2.4.2 Paxillin in cell migration

Paxillin is one of the earliest proteins that is recruited to nascent focal adhesions and is necessary for the turnover of focal adhesions during cell migration. Assembly of paxillin LD4-PKL-PIX-PAK-Nck complex and sequential activation of paxillin (Y31/118)-Crk-p130Cas-DOCK180 cascade are two major ways to regulate cell motility and Rac activation. The LD4 motif of paxillin is an important region of the protein for the regulation of Rho GTPase signaling and focal adhesion turnover. The motif recruits a molecular complex that comprises the proteins G-protein-coupled receptor kinase interacting protein [GIT1 or GIT2; the latter is also known as paxillin kinase linker (PKL)], PAK-interacting exchange factor (PIX), p21-activated serine/threonine kinase (PAK) and NCK to adhesion sites at the leading edge of migrating cells[82,83,93]. GIT2 and the closely related GIT1 bind directly to the paxillin LD4 motif[82,83,93] and are members of the ArfGAP family[93]. Importantly, fibroblasts that express a paxillin mutant that lacks the LD4 motif, and therefore cannot recruit the GIT1/2-PIX-PAK-NCK complex to focal adhesions, exhibits abnormal membrane-protrusion dynamics that are caused by sustained global Rac1 activity[83]. These cells are also defective in polarized cell migration[83,84] and focal adhesion turnover[79]. The recruitment of the GIT-PIX-PAK-NCK complex to paxillin and the activity of the complex are tightly regulated, which is consistent with the crucial role of paxillin in coordinating Rac1 signaling.

Upon integrin clustering, paxillin becomes tyrosine phosphorylated, primarily on tyrosine residues 31 and 118 (Y31 and Y118, respectively)[94-96] in a FAK and Src-dependent manner[97]. The CrkII-DOCK180-ELMO complex, which regulates Rac1 signaling[98], can interact with the phosphorylated Y31 and Y118

residues of paxillin via the SH2 domain of CrkII[96,99,100] and, by means of DOCK180[101], activates Rac1 to promote cell migration. Interestingly, the phosphorylation of Y31 and Y118 has also been shown to regulate RhoA activity[102]. Therefore, paxillin that is phosphorylated at Y31 and Y118 can indirectly activate Rac1 and inhibit RhoA, and both of these activities are necessary for efficient leading-edge protrusion during cell migration. Furthermore, tyrosine phosphorylation of Y31 and Y118 indirectly enhances the binding of FAK to the adjacent LD motifs of paxillin and has been implicated in focal-adhesion maturation[103]. In addition to its role in the assembly of focal adhesions, paxillin also regulates cell migration by contributing to efficient focal-adhesion disassembly, as indicated by the stabilization of adhesions in cells that are devoid of paxillin[79].

Paxillin is also phosphorylated at serine residues in many systems in response to adhesion to fibronectin and growth factor stimulation[49,94,104,105]. Paxillin is a JNK substrate[49]. JNK efficiently phosphorylates paxillin at Ser178, and expression of JNK promotes the specific phosphorylation of Ser178 *in vitro*. Moreover, EGF stimulates phosphorylation of Ser178 in MDA-MB-231 human breast cancer cells and a JNK inhibitor SP600125 blocks this effect. Interestingly, expression of a Ser178 to Ala mutant of paxillin (PaxS178A) significantly inhibits the migration of NBT-II cells, MDA-MB-231 human breast cancer cells, and Chinese hamster ovary (CHO-K1) cells, whereas expression of wild-type paxillin has no obvious effect on cell migration. JNK-mediated phosphorylation of paxillin regulates focal adhesion disassembly and thus cell migration[79,93].

2.2.4.3 Paxillin in cancer

Paxillin is potentially involved in several processes of tumor development. So far most studies have focused on paxillin-related FA signaling in cytoskeleton organization, cell motility and survival. Paxillin is a target of many oncoproteins, such as Src, BCR/ABL and E6. Moreover, the role of paxillin in regulating gene expression[84] through its interactions with ERK[89], poly- A-binding protein[106], Abl and steroid receptors[107], as well as through its own ability to undergo nucleocytoplasmic shuttling[84], should not be overlooked. The exact role of paxillin in tumor progression is still largely unknown. The few studies done on paxillin expression in cancer patients are very contradictive: depending on the type of cancer, either low paxillin expression favorites tumor progression[108-110] or high paxillin expression correlates with tumor aggressiveness[111-113]. The deficient activation or presence of paxillin might have functions on cell migration, proliferation and survival in cancer development. Paxillin expression is higher in metastatic human osteocarcinoma sub-cell line than less metastatic sublines and knock down of paxillin reduces cell motility[114]. This is associated with tyrosine phosphorylation of paxillin. Yet, no research about the role of paxillin and its diverse phosphorylable residues in breast tumor cell migration, survival or breast

cancer progression has been published. In **Chapter 7**, we aim to investigate the role of Ser178 residue of paxillin in breast cancer progression.

AIM AND OUTLINE OF THE THESIS

Cell adhesion and migration are crucial steps in various biological processes and especially in breast tumor progression. Understanding metastasis formation which involves enhanced cell migration and invasion still remain a challenge for further drug development. Recent advances in imaging techniques and *in vivo* model for breast cancer progression are now available to investigate the molecular mechanisms that control and regulate tumor cell migration. In this thesis, our overall goal is to set up imaging approaches and *in vivo* model that allow us to better understand the role of matrix adhesion signaling in relation with breast cancer progression and metastasis formation. We established a systems microscopy approach (different microscopy techniques combined with adapted data analysis methods) that enables us to understand the role of matrix adhesions in breast cancer metastasis formation by studying the molecular, structural and cellular levels in both *in vitro* and *in vivo* of a particular protein. In **Chapter 2**, we review the role of FAK in cancer progression and underline that FAK is a potential target for cancer therapy. In **Chapter 3**, we review the various systems microscopy approaches that are nowadays available to get a better insight into the role of matrix adhesion in migrating cells. In **Chapter 4**, using an epithelial cell-line with visible prominent matrix adhesions, we establish a new technique, the FLIP-FRAP technique that provides quantitative measurements about protein dynamics at focal adhesions. In **Chapter 5**, we establish an improved mouse model for studying breast metastasis formation. In **Chapter 6**, we describe a methodology that use this *in vivo* model together with an inducible cell-line and multicolor two photon microscopy to investigate cell autonomous properties in metastasis formation. In **Chapter 7**, we focus on the specific role of JNK-paxillin pathway in tumor cell migration and metastasis formation. In **Chapter 8**, we briefly summarize and discuss the developed strategy to study the role of matrix adhesions in breast cancer progression.

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