CHAPTER 8

Summary and discussion

Tumor cell migration and invasion are essential steps in cancer metastasis. Better understanding of the molecular mechanisms and function of the individual proteins this behaviour are essential to define potential novel drug targets to combat cancer. In this thesis, we set up technologies and investigated the molecular mechanisms of the matrix adhesions dynamics in relation to tumor cell behaviour both *in vitro* and *in vivo* situation. In general, cells in a normal tissue environment are attached to the extra-cellular matrix (ECM) and to each others. The interactions with the ECM are mediated through binding of the integrins while cell-cell contact is mediated through E-cadherin. Matrix adhesions are the physical link between the ECM and the actin cytoskeleton and are important for survival, proliferation, differentiation and migration. Matrix adhesions are classified in three groups: focal complex, focal adhesions and fibrillar adhesions[1]. These cytoplasmic structures are composed with various signaling (phosphatases and kinases) and structural proteins that form the so-called 'integrin-adhesome'[2]. The spatial and temporal regulations of those components determine the type of matrix adhesion, their behaviour and finally the fate of the cell. For instance, resting cells such as renal epithelial cells show enlarged and stable focal adhesions as well as tight cell-cell contact. In contrast, tumor cells which are able to invade and metastasize, lose their interactions with adjacent cells and show fast, small and highly dynamic matrix adhesions. Below the results from the different chapters will be discussed in a broader context and future perspectives will be lined out.

1. Methodology to understand tumor cell migration in 2D and 3D

1.1 Measurements of tumor cell motility

Chapter 3 reviews all the different microscopy techniques available to understand the role of matrix adhesions in migrating cells. Cell motility assays that quantify several parameters including cell speed, directionality and persistence are commonly used in a two-dimensional environment. Obviously along the years, this approach did provide a wealth of information on the mechanisms regulating tumor cell migration. Nevertheless, it is now becoming clear that it is not enough and that it is necessary to study tumor cell migration in both a 3D environment and *in vivo* environment. For instance, MTLn3 cells with increased expression of EGFR move at a speed of 1 µm/min in a Petri dish while *in vivo* the cells move at 10 µm/min (**Chapter 6**). Furthermore, while paxillin knock down in 4T1 cells increases their speed in a 2D environment, the same cells move much slower than its control in a 3D collagen gel and do not metastasize in the breast cancer mouse model (unpublished data). Indeed, in a 2D environment, cells are attached to stiff substrates such as glass or plastic. Most of all described assays are practically only feasible in a 2D environment. Nevertheless, the stiffness of this material does not represent the *in vivo* situation and most of the time, cells behave differently on substrate with different compliances. The matrix controls the cell fate[3-5]. In a 3D environment, tumor cells show distinct modes of migration such as mesenchymal or amoeboid which is not detectable in a 2D environment. Many mechanistic details associated with metastasis remain elusive due to the difficulty of studying cancer cells in relevant 3D microenvironments. P. Keely's group showed that breast epithelial cells sense the rigidity or density of their environment via ROCK-mediated contractility and a subsequent down-regulation of Rho and FAK function, which is necessary for breast epithelial tubulogenesis to occur[6,7]. *In vivo*, metastatic cells sense the rigidity of their environment as well and favorites a stiff support. The current data in this field provide compelling evidence for the importance of the mechanical features of the microenvironment, and suggest that mechanotransduction in the tumor cells occurs through a FAK–Rho–ERK signaling network. As such, it is proposed that increased matrix stiffness explains part of the mechanism behind increased epithelial proliferation and cancer risk in human patients with high breast tissue density[8]. Indeed, in the case of a primary tumor, the environment evolves in time with an increasing compliance due to enhanced collagen. Therefore, to draw any conclusion about the role of a protein in tumor cell migration, we need to study its function not only in 2D but also in a 3D environment which relevant to the *in vivo* situation.

1.2 Measurements of focal adhesion turnover

In **Chapter 3** we described the TIRF microscopy technique to quantify matrix adhesion assembly/disassembly (=turnover) in migrating cells. This is at this moment the most suitable dynamic imaging technique to analyze the fast matrix adhesion turnover of migrating tumor cells. Only TIRF and not with confocal microscopy allows us to visualize the adhesions that harbor for instance the fast moving MTLn3 cells. Currently this method is still limited to a 2D environment in which cells are cultured on glass coverslips. How can we measure matrix adhesion turnover in a 3D environment and even more complex in a primary tumor i*n vivo*? Till now, nobody has been able to visualize matrix adhesion type structure *in vivo*. In our group we did perform some intravital imaging using the MTLn3-GFP-paxillin cells but no detectable structures were visible using the technology available. To reach that gap, improved sensitivity of the hardware is required. Alternatively 2D approaches that better mimic 3D migration strategies may be an option. This may involve the pharmacological-induced switch of mesenchymal cell migration to amoeboid type of cell migration[9-13], or cell migration of single cells on patterned substrates[14,15].

1.3 Measurements of protein dynamics

A major challenge of cancer biology is to observe the dynamics of the proteins involved in a process in their functional and physiologic context. Here, in this thesis, I have used FRAP photobleaching approaches to compare the mobility of cell adhesion proteins *in vitro* normal and transformed epithelial cells which was performed on cells cultured in 2D on glass coverslips. Yet, since as indicated in the above paragraph 2D is an artificial situation, one can wonder about the protein dynamics in a 3D environment and in particular in tumors grown in mice (*in vivo*)? Recently, Serrels and co-workers found clear differences between *in vitro* and *in vivo* recovery dynamics of two key molecules, the tumor suppressor E-cadherin and the membrane-targeting sequence of H-Ras[16]. E-cadherin dynamics are significantly faster *in vivo* compared to cultured cells. Their results show for the first time the utility of photobleaching and photoactivation in the analysis of dynamic biomarkers of cells in the living animal. Furthermore, this work highlights critical differences in molecular dynamics *in vitro* and *in vivo*, which have important implications for the use of cultured disease models as surrogates for living tissue. A challenge for the future will be to analyze matrix adhesion protein dynamics in migrating tumor cells in a primary breast tumor environment. The use of MPCLSM that can image effectively to depths of 400–1000 mm will allow cell biology studies that can be performed on cells within relevant 3D matrice as well as *in vivo* with intravital imaging.

2. Methodology to understand tumor cell invasion in the *in vivo* situation

2.1 Which in vivo model is the most suitable for studying breast cancer metastasis formation?

Because of the complexity and heterogeneity of breast cancer no individual model recapitulates all aspects of this disease. Thus, an integrated and multi-systems approach is currently the best way to model this disease (see for review[17]).

2.1.1 Breast cancer cell-lines

In **Chapter 5, 6** and **7**, we described the use of rat (MTLn3) and mouse (4T1) mammary carcinoma cell-lines. Breast cancer cell lines have been the most widely used models to investigate how proliferation, apoptosis and migration become deregulated during the progression of breast cancer. Certain breast cancer cell lines may provide useful tools for high-throughput screening of small molecules and siRNA (RNA interference) libraries as a first line of investigation to identify the interacting pathways that are important for tumor cell migration (unpublished data). Furthermore, we can use these cell-lines in 3D cultures which strengthen the preclinical utility of these models. To better understand the validity of observations in these two cell lines to the human situation, we are currently establishing the use of several human breast cancer cell-lines in our Rag2$^{-/-}\gamma^{-/-}$ immunodeficient mice model.

2.1.2 Genetically engineered mice (GEM)

Genetically engineered mouse models of breast cancer exhibit many features of human breast cancer and thus provide relevant models for investigating the biology and pathogenesis of this disease[17,18]. However, most tumors from GEM still do not recapitulate the most common subtypes of breast cancer. Despite these limitations, it is important to acknowledge the impact that these experimental models have on our understanding of the genetics and biology of breast cancer. P53, which is one of the most frequently mutated genes in breast cancer, and ERBB2, which is amplified in 25–30% of human breast cancers, have been studied most extensively in GEMs[19-23]. The *p53*-null mouse is thought to mimic human breast cancer the best among all GEMs[24]. In this respect, recent studies have shown that the transplantation of tumors from GEM is a highly effective way to generate large cohorts of mammary tumor-bearing mice in a well-defined molecular and genetic background with a determined rate of tumor development and frequency of metastasis. In our lab, we are breeding both mouse strains (p53 null, p53 mutant and Her2T) and we aim at using them in the near future. A further improvement could be the crossing of these different models with animals that can express GFP everywhere or locally such as the MMTV-GFP. This type of crossing would

facilitate the tracking with BLI, FLI or fluorescence microscope of the metastatic cells and this would enable us a better understanding of the metastatic process[25].

2.1.3 MECs transplantation model

Combining an optimal GEM with the mammary epithelial cells (MECs) transplantation model is a next step towards understanding breast cancer initiation and progression. Indeed, the mouse mammary gland regenerates completely upon orthotopic transplantation, making it ideally suited for *in vivo* gene function studies through viral-mediated gene delivery. An obstacle that has challenged the widespread adoption of this technique is the inability to transduce mammary stem cells effectively. Recently, Welm and coworkers have overcome this limitation by infecting total primary mammary epithelial cells in suspension with high-titer lentiviruses. Transduced cells gave rise to all major cell types of the mammary gland and were capable of clonal outgrowth[26]. In our lab, we did set up the transplantation technique successfully[27] but still need to apply successfully the transduction protocol of Welm and coworkers. A relevant *in vivo* model could be as follows: i) isolation of the primary p53 mutant MECs, ii) efficient lentiviral transduction to knock out a protein of interest, iii) transplantation of the knockout cells in cleared mammary fat pads of recipient mice to evaluate the role of this protein in development, differentiation and transformation of the mammary gland. Furthermore, 3D culture methods for breast epithelial and cancer cells has provided invaluable models to dissect the complex signalling interactions that are difficult to investigate *in vivo*[28,29]. An emerging application of the GEM of breast cancer is the examination of primary mammary epithelial cells or tumor cells that are derived from these mice in 3D cultures or other *in vitro* assays, allowing the delineation of signaling mechanisms that are not easily investigated *in vivo*[27]. What is learned in these culture studies can then be validated *in vivo.*

2.1.4 Direct visualization of the tumor growth: intravital imaging or bioluminescence?

Fluorescent imaging technologies has improved tremendously in the last decade. Fluorescent probes are continuously improved for their brightness and photostability. Imaging techniques have increased with respect to speed, resolution and sensitivity. In **Chapter 6**, we described an adapted fluorescent intravital imaging technique that permits us to visualize in the intact living animal how tumor cells escape from a primary tumor, interact with its environment and eventually intravasate through a blood vessel. This technology provides us with information on the type (single or collective, amoeboid or mesenchymal) and parameters (speed, directionality) of the cell migration. Nonetheless, this technique requires intense surgery and is still limited with respect to capabilities to follow up invading cells at the target organs such as lung and bone. Ideally, intravital

imaging of tumor cells invading the tumor tissue, intravasating and extravasating is performed on different days in the same animal without performing any surgery. Non-invasive fluorescent and bioluminescent imaging could be the answer to our need[30,31]. Unfortunately, the resolution remains too low to visualize metastatic events at the cell level. Right now a combination of both technique bioluminescence and multiphoton intravital imaging is the most optimal research approach to address the role of matrix adhesions in breast tumor progression. The introduction of a mammary imaging window (MIW) would greatly help with microscopy and most importantly allows the investigator to follow in time the tumor development[32]. In combination with a photoconvertible fluorophore (e.g. Dendra), it is possible to delineate a certain region in the primary tumor and follow days later where the tumor cells did migrate[33]. Although, the use of the MIW is limited in time due to practical reasons (up to 15 days for the MTLn3 cells while the tumor is mostly highly metastatic around 30 days), it does help reducing the number of test animals. We are planning to apply this technique in our further research.

3. Understanding FAK-paxillin behaviour and signaling in normal and transformed cells

3.1 The type of adhesion is determined by the protein residence time

Any type of normal or transformed cell harbors different types of matrix adhesions which differ in size, shape, localization and protein concentration. There is a need to better understand the molecular behaviour of individual proteins within these complexes to better understand the biological process of cell-matrix adhesion turnover. In **Chapter 4**, we describe an adapted photobleaching approach including Monte-Carlo simulation that enables us to quantify protein kinetics in any matrix adhesion contact site in the entire cell. We demonstrated that the residence time of both FAK and paxillin, two central functional components of cell adhesion structures, increases with the matrix adhesion size. Furthermore, this same residence time increases with the ECM density. However, the local molecular events that differentially regulate the kinetics of these proteins in relation with a certain type of matrix adhesion are still poorly understood. One of the mechanisms believed to be involved in matrix adhesion assembly and turnover is tyrosine phosphorylation of FA proteins such as focal adhesion kinase (FAK) and paxillin[34]. Inhibition of tyrosine phosphatase activity stimulates FAK and paxillin phosphorylation, and enhances focal adhesion assembly[35]. Recent work from Zaidel-Bar and colleagues reported that the proportion of phosphorylated paxillin is negatively regulated by mechanical force[36]. They show that the phosphorylation status of paxillin depends on the type (FX, FA, or FB) and localization (central or peripheral FA) of matrix adhesion. Furthermore, they could make a correlation between paxillin phosphorylation and dynamic properties of the three forms of

matrix adhesions. FX structures are considerably more dynamic than FAs, and both are more dynamic than FBs. In our study, we found a correlation between matrix adhesion size, paxillin/FAK density and paxillin/FAK residence time. This might be orchestrated by the phosphorylation status of both proteins, since increased phosphorylation of paxillin and FAK regulate their binding properties. A detailed study using phospho-specific antibodies would shed light on this probable correlation.

3.2 Paxillin immobile fraction at matrix adhesion is a marker for normal and transformed cell phenotype

While normal epithelial cells show prominent matrix adhesions attached to tense actin stress fibers in a 2D environment, transformed cells have an altered rigidity/tensional homeostasis with loose and punctuated matrix adhesions (Fig.1A). Indeed, many reports have linked transformation to profound alterations in cell shape and migration that depend on oncogenic tyrosine kinase signaling[37,38]. In transformed cells, stress fibres are usually absent and mature FAs can sometimes be replaced by more invasive infrastructures, such as podosomes that typify v-Src transformed cells[39]. Thus transformed cells generally develop weaker traction forces (decreased cell adhesion on the outside) and display increased adhesion dynamics that rely in part on deregulated Src-FAK signaling[40]. This corroborates our work on GFP-paxillin kinetics studied in epithelial and transformed cells using photobleaching technique. In **Chapter 4**, we show that the renal epithelial cells LLC-PK1 harbor very stabile matrix adhesions (half-life longer than 15 min) and that the percentage of immobile fraction of GFP-paxillin at matrix adhesions is more than 50% (Fig.1). In contrast, in the rat mammary carcinoma MTLn3 cell-line with small and dynamic matrix adhesions (half-life shorter than 1 min), GFP-paxillin immobile fraction was less 15% (**Chapter 7**, Fig.1B). We can conclude that paxillin dynamics and in particular the percentage of immobile fraction could be a sensor/marker for the matrix adhesion turnover and consequently for the cell phenotype. Recent work suggested that paxillin phosphorylation acts as a switch in adhesion assembly/disassembly in response to changes in actomyosin contractility and mechanical cues[36]. It was proposed that mechanical force-induced paxillin targeting to FA involves the dephosphorylation of paxillin tyrosine residues, which leads to dissociation of FAK from the complex, promoting stabilization and growth of the adhesion[36,41]. Consequently, the disengagement of FAK from FA is expected to inhibit turnover and increase adhesion stability[41,42]. So we propose that the de-phosphorylation of paxillin at least of its tyrosine residues induces protein stabilization, increases its residence time and decreases the matrix adhesion turnover. Other paxillin phosphorylation events are also important for matrix adhesion dynamics. Thus, as an alternative phospho-residue, in **Chapter 7** we now demonstrate that JNK-induced paxillin phosphorylation at serine residue 178 is also required for matrix adhesion

disassembly. Further insight in the different role of the various phosphorylation sites of paxillin is matrix adhesion assembly and disassembly in both normal and transformed cells is still needed. Our novel technology described in chapter 4 will allow this in an efficient and accurate manner.

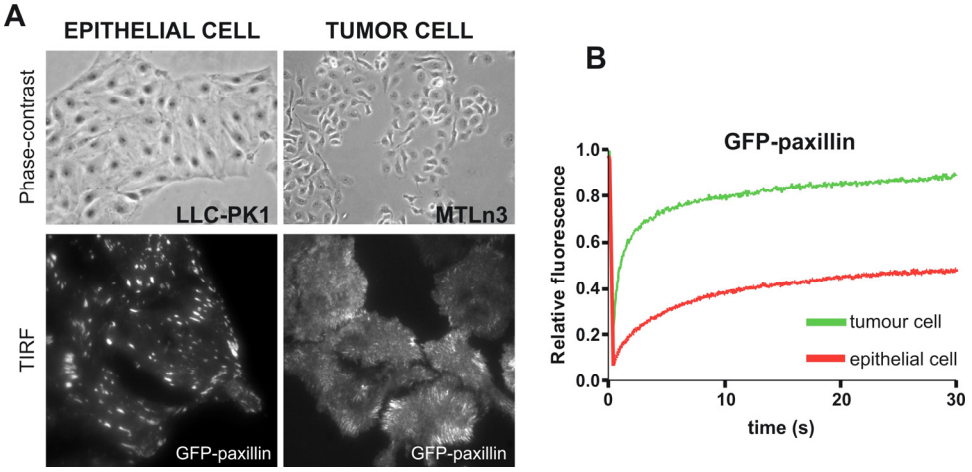

Figure 1: Differential protein dynamics correlates with the normal and transformed cell phenotype. A) Normal renal epithelial cell (LLC-PK1) show a spread phenotype with tight cell-cell contact and prominent matrix adhesions visualized with TIRF microscopy, while transformed MTLn3 tumor cells lost their cell-cell contact and harbor punctuated small irregular matrix adhesions. B) Fluorescence recoveries after spot photobleaching of GFP-paxillin expressed in both cell types show clearly a difference in immobile fraction percentage. The slower the matrix adhesion turnover, the larger the matrix adhesions the more stabile the paxillin is at adhesions.

3.3 JNK-paxillin signaling in transformed cells: an EGFR relationship.

As mentioned above, throughout its protein sequence, paxillin contains a number of serine/threonine and tyrosine phosphorylation sites[43]. Phosphorylation of these sites in response to extracellular stimuli recruits signaling molecules to the matrix adhesions and thus facilitates cell migration. Focal adhesion kinase (FAK) phosphorylates paxillin at Tyr31 and Tyr118 that generate binding sites for SH2-containing proteins such as CrkII[44]. JNK-mediated paxillin Ser178 phosphorylation has been found to be essential for cell migration of various cell types, including rat bladder tumor cells[45] and rat mammary carcinoma MTLn3 cells (**Chapter 7**). C-Jun N-terminal kinase is a member of the MAP kinase family which activity is shown to be involved in cancer progression. JNK is normally activated by diverse growth factors (e.g. EGF and HGF) or cytokines (e.g. TNF-alpha or interleukin-1). In MTLn3, engagement of EGF receptors by EGF triggers rapid activation of JNK, leading to the phosphorylation of paxillin on Ser 178 which facilitates adhesion turnover thus promoting rapid migration (**Chapter 7**). We generated MTLn3 cell lines that ectopically express GFP-paxillin-S178A mutants. In these mutant cells we

found that the EGF receptor is downregulated at both the protein and mRNA levels. Paxillin S178A mutant protein might associate with JNK preventing its activation and consequently affecting AP1 activity (c-Jun phosphorylation) and consequently EGFR expression (Fig. 2). In our model, still some links need to be further determined such as how exactly paxillin regulates via JNK EGFR expression. Our data suggest that the perturbed JNK signaling pathway in the mutant cells lowers the activity of the AP1 complex resulting in EGFR downregulation at mRNA level. In general phosphorylated JNK mainly localized at matrix adhesions[46-48]. Our preliminary data reveal that in the mutant cells p-JNK is kept at focal adhesions and probably is prevented to translocate to the nucleus reducing c-Jun activation. Another explanation could be that the assembly of paxillin-PKL-PIX-PAK complex is not efficient anymore in the mutant cells and do prevent proper JNK activation[49-51]. Recently, a crosstalk between JNK and WNT signaling has been reported as well as the existence of a direct interaction between c-Jun and β-catenin (see review[52]). This could be another direction to investigate the role of the JNK signaling in the regulation of the EGFR. Finally, there is increasing evidence that paxillin can be nuclear in certain conditions[53]. The role of nuclear paxillin is still largely unknown but we can suspect a potential activity at transcription level.

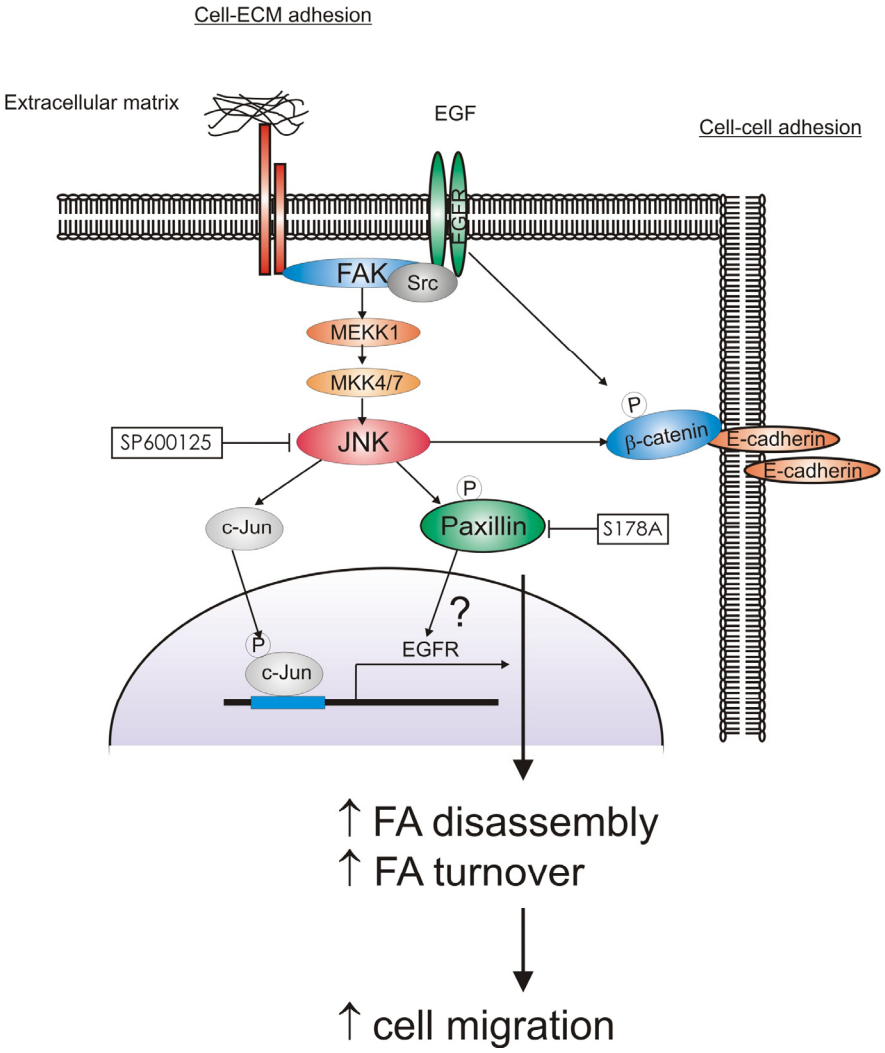

Figure 2: Diagram depicts the hypothesized signaling pathways for JNK-paxillin dependent cell migration. Engagment of EGF receptors by EGF triggers rapid activation of JNK, leading to the phosphorylation of paxillin on Ser 178 which might facilitate adhesion turnover thus promoting rapid migration. Upon EGF stimulation, JNK phophorylates also c-Jun which regulates EGFR transcription and β-catenin which delocalizes from the cell-cell contact breaking down the adherens junctions. Paxillin S178A mutant protein might associate with JNK preventing its activation and consequently affecting AP1 activity and EGFR expression. Inactive JNK as well as down-regulation of EGFR result in de-phosphorylation of β-catenin and formation of stabile cell-cell contact in MTLn3 cells.

4. Translation from model systems to novel breast cancer treatment

In this thesis, we touched upon different molecules that play crucial roles in the progression of breast cancer. We demonstrated that EGFR, a receptor tyrosine kinase is very important in metastasis formation. Further downstream, we highlighted the role of two signaling hubs proteins involved in the matrix adhesion dynamics: FAK and paxillin. Can those focal adhesion associated molecules be targeted for blocking breast metastasis formation?

4.1 FAK as an anticancer therapeutic target

Focal adhesion kinase (FAK) is a 125-kDa non-receptor and non-membrane protein tyrosine. FAK can function with integrins and growth factor receptors to promote cell survival dependent kinase activity and nuclear FAK promotes cell proliferation and survival through FERM (FAK, ezrin, radixin, moesin) domain-enhanced p53 degradation independent kinase activity. In **Chapter 1**, we briefly review the current literatue many studies which indicate that FAK plays a critical role in the biological processes of normal and cancer cells and we propose FAK as a potential target in cancer therapy. Small molecule inhibitors (PF-573,228; PF-562,271 and NVP-226) for use as potential cancer therapies have been developed and are currently tested in clinical trials. However, the detailed molecular mechanism of the role for FAK in tumor cell generation and progression remain unclear, so future work is needed to explore these issues. More inhibitors that can be effectively inhibit the function of FAK still need to be explored due to the low specificity, and resistance.

4.2 Paxillin a therapeutic target for breast cancer therapy?

In **Chapter 7**, we demonstrate a crucial role of the serine residue 178 of paxillin in breast cancer progression. In agreement with a recent study, we established an important role for the JNK-paxillin pathway in breast tumor progression[54]. Nevertheless, the few studies done on paxillin expression in cancer patients are contradictive. Depending on the type of cancer, low paxillin expression may favorite tumor progression[54-56] while high paxillin expression which correlates with tumor aggressiveness is found in other cancer types[57-59]. The exact role of paxillin in tumor progression is still unknown. Easy targeting paxillin as an anti-cancer therapy seems not feasible. It is a substrate of several important kinases and targeting those kinases would be an appropriate approach. One of them FAK is indeed a potential anticancer target therapy. JNK is another kinase that constitutes an interesting target for anticancer therapy. Indeed, the field of JNK inhibitors is rapidly moving, it is anticipated that several targeted therapies with new drugs will be successfully applied and used in the clinic in the near future[60-65]. Alternative approaches could be to inhibit the downstream signaling pathways of paxillin. In this context in can be expected that the different phosphorylation forms of paxillin

affect different downstream effectors. Thus, phosphorylated Tyr31 and Tyr118 lead to activation of DOCK and thereby affect actin dynamics and cell migration[66]. Finally, although paxillin does not contain domains with enzyme activity that might be targeted by small molecules, searching for molecules that would block the interactions of (phosphorylated) paxillin with crucial downstream effectors would be a feasible approach to inhibit cell migration and possibly metastasis formation under *in vivo* conditions.

4.3 EGFR as an anticancer therapeutic target

Aberrant signal transduction through activated growth factor receptors, for example the ErbB family of receptor tyrosine kinases (RTKs), is a common feature of many types of solid tumors. Dimerization of ErbB receptors leads to induction of the kinase activity. As a result, a number of tyrosine residues at the C terminal end of the ErbB molecules become phosphorylated. The phosphorylated tyrosine residues serve as docking sites for an array of signaling molecules that contain Src homology 2 (SH2) domains or phosphotyrosine-binding domains. The 2 major signaling pathways activated by ErbB receptors are the MAPK and PI3K-AKT pathways. In **Chapter 5** and **7**, we show that EGFR is crucial for lung metastasis formation in an *in vivo* breast cancer model, which is in agreement with findings by others[67,68]. Therapeutic reagents have been designed to target this receptor and selectively attack malignant cells but still the clinical trials are not as promising as expected. The development of cancer therapeutics targeting the ErbB receptor extra-cellular and tyrosine kinase domain is still ongoing[54,69-73]. Nevertheless, a better understanding of the various EGFR pathways involved in tumor progression is required due to the potential adverse effects when targeting this ubiquitous receptor. Further insight in EGFR downstream pathways that are distinct for cell migration using siRNA screen approach as described in **chapter 3**, could lead to new genes candidate for anticancer therapies.

5. Perspectives: towards understanding the role of matrix adhesion components in breast cancer progression

Matrix adhesions are clearly crucial for tumor development and the mechanisms behind are still poorly understood. A lot is now known about the role of focal adhesion kinase (FAK) in tumor progression. The fact that FAK is a potential drug target for cancer therapies is demonstrated in **Chapter 2**. The 'integrin-adhesome' count more than 160 components from several candidates might be relevant to study their role in breast cancer progression. Paxillin is a very important scaffold protein that is shown to be involved in tumor cell migration and cancer progression (**Chapter 7**). The various phosphorylation sites of this scaffold make it a crucial hub in processing different signaling pathways which lead to cell survival, proliferation, differentiation and migration. Preventing the

phosphorylation of paxillin could be a strategy to aim at. In our lab, we are performing different high through put screening using phosphatase, kinase and adhesome libraries to unravel the mechanism that control the matrix adhesion turnover responsible for cell migration. We hope to find other matrix adhesion gene candidates that could be drug targeted to prevent tumor cell migration and consequently metastasis formation.

6. Conclusions

In conclusion, we have set up various tools from *in vitro* to *in vivo* to investigate the role of matrix adhesions in breast tumor progression. With a newly developed photobleaching approach we can analyze the protein dynamics and relate it to the matrix adhesion behaviour and consequently to the cell fate. We also successfully characterized a suitable *in vivo* breast tumor model which allows us to get an insight into the cell autonomous processes that are required for a tumor cell to metasize. Furthermore we also set-up a multi-color multiphoton intravital imaging together with an inducible cell-model to get a detailed insight into the role of matrix adhesion associated proteins in tumor cell migration and metastasis formation. Indeed matrix adhesion proteins such as FAK and paxillin are crucial for breast tumor progression. Especially, the JNK-paxillin pathway is shown to be highly relevant in this process. Since paxillin through its numerous binding domains and phosphorylation sites seems to be a key regulator of matrix adhesions, we think that future research on the role of this scaffold protein and its downstream signaling pathways will help us to better understand the key pathways that define cancer metastasis formation.

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