Focal adhesion signaling in breast cancer treatment

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CHAPTER 5

The serine178 residue of the focal adhesion-associated scaffold protein paxillin determines EGF-induced cell migration of metastatic breast tumor cell MTLn3

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ABSTRACT

The focal adhesion-associated scaffold protein paxillin plays a prominent role in cell migration. Recent data indicate JNK-mediated phosphorylation of paxillin at serine178. Here we investigated the role and mechanism of paxillin-Ser178 in the control of EGF-induced cell migration of the highly metastatic rat mammary carcinoma MTLn3 cells. EGF induced transient activation of JNK in MTLn3 cells, which was associated with JNK-dependent Ser178-paxillin phosphorylation. MTLn3 cells expressing a GFP-tagged Ser178Ala (S178A)-paxillin mutant had a phenotype with reduced proliferation, slower attachment and wound healing rate, compared to GFP-wt-paxillin expressing cells. Furthermore, the mutant cells did not undergo EGF-induced random cell migration sufficiently. EGF treatment resulted in limited phosphorylation at Ser178 of endogenous paxillin in S178A-paxillin MTLn3 cells. Moreover, EGF was unable to efficiently activate the PI3K/AKT and ERK pathways in these cells, compared to wt-paxillin cells. This suggests that Ser178-paxillin is important to control the EGF-induced signaling. Since paxillin phosphorylation at Ser178 is important to control cell migration of breast tumor cells, this phosphorylation event may also be important for breast tumor progression and metastasis formation.

INTRODUCTION

Breast cancer represents the most common worldwide type of cancer among women. The occurrence of distant metastases, or secondary tumors, is related to a very poor disease prognosis. The formation of secondary tumors involves distinct steps at the cellular level, including detachment, migration, invasion, extravasation and proliferation (1). To combat breast cancer metastasis more efficiently, improved insights into biological mechanisms in metastasis formation are of great importance. Focal adhesions, the closest contacts between cells and ECM, are important sites for signaling events (2-4). At focal adhesions, structural and enzymatic molecules act together to facilitate growth factor-stimulated and cell adhesion-dependent signaling, which are crucial in the different steps of the metastatic process (5,6).

Paxillin, a 68 kD multidomain adaptor protein, is associated with focal adhesions, where it functions as a scaffold to integrate multiple signaling pathways (4). At the N-terminus paxillin contains five leucine-rich LD domains (consensus LDXLLXXL) and several SH2 and SH3-binding domains; at the C-terminus paxillin contains four double zinc-finger LIM domains. LD domains facilitate the binding of a large array of binding partners, including integrin-linked kinase (ILK), actopaxin, G-protein coupled receptor kinase-interacting protein (GIT), focal adhesion kinase (FAK) and vinculin (7-10). The LIM domains of paxillin mediate the interaction with PTP-PEST and tubulin (11). LIM3, together with LIM2, targets paxillin to focal adhesions (11).

Throughout the paxillin molecule are many potential phosphorylation sites, including tyrosine, serine and threonine, which have all been mapped by mass spectrometry (12-14). Growth factor and integrin-mediated phosphorylation of paxillin at Tyr31 and Tyr118 induces the formation of a paxillin-Crk complex at focal adhesions and is essential for cell migration (15-17). Subsequently Crk-DOCK180 mediates the activation of Rac to enhance migration through lamellipodial extension (18-20). In NBT-II bladder tumor cells, induction of paxillin Tyr 31/118 phosphorylation and its association with CrkII are involved in cell adhesion, spreading and motility (20). Also Ephrin B1-stimulated cell migration requires phosphorylation of paxillin Tyr31/118 as well as the LD4 domain in a variety of cell types (21).

Serine/threonine phosphorylation of paxillin is observed by growth factor-mediated signaling, cellular stress and during mitosis (22-25). For example, adhesion stimulates the phosphorylation at Ser188/190 by an unknown kinase, as well as serine and threonine residues within LIM domains 2 and 3 (26). In addition, phosphorylation of paxilin at Ser273 has also been reported to regulate cell adhesion and protrusion dynamics via enhancing paxillin-GIT1 binding and promoting localization of a GIT1-PIX-PAK signaling module near the leading edge (12). Furthermore, p38 MAP kinase targets serine 85 in the process of neurite outgrowth (23). Finally, phosphorylation of paxillin at serine 178 is involved in EGF-stimulated cell migration via JNK (26,27) as well as in microtubule disruption condition which is also in association with JNK activation (see chapter 4). Many cell processes in tumor development depend on growth factor-mediated signaling, including EGF and HGF, which involves the activation of different MAPK family members. Therefore, it is important to further explore and understand the exact role of Ser178-paxillin phosphorylation by growth factors in cell migration and proliferation. Here we studied the role of Ser178-paxillin in the highly metastastic breast tumor cell line MTLn3.

We generated MTLn3 cell lines stably expressing either wt paxillin or Ser178Ala (S178A) mutant paxillin. S178A-paxillin significantly decreased cell proliferation and adhesion. Cell migration under control conditions (serum starvation) or after EGF stimulation was inhibited. S178A-paxillin suppressed JNK-mediated phosphorylation of endogenous paxillin under control and EGF conditions. The data indicate that Ser178 phosphorylation of paxillin after growth factor stimulation is essential to control cell migration and efficient activation of downstream signaling events including the PI3K/AKT and MEK/ERK signaling pathways. Altogether we conclude that the serine 178 residue of paxillin is an important player in cell proliferation and migration of metastatic breast tumor cells, and may be important for *in vivo* metastasis formation.

MATERIALS AND METHODS

Chemicals and Antibodies- Alpha modified minimal essential medium without ribonucleosides and deoxyribonucleosides (α-MEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin and geneticin (G418 sulphate) were from Life Technologies. Rat tail collagen type I was from Upstate Biotechnology. LipofectAMINE Plus transfection reagents were from Invitrogen. Bradford protein assay was obtained from Bio-Rad and polyvinylidene difluoride (PVDF) membranes were from Millipore. Primary antibodies were anti-paxillin (BD), anti-tubulin, anti-GFP (Sigma, St. Louis, MO), anti-pT183/pY185-JNK (Promega), anti-Phospho-Thr202/Tyr204 ERK1/2, anti-pSer473-AKT (Cell signaling), anti-pSer178-paxillin (Abcam, Cambridge, UK). All secondary antibodies were from Jackson (GαRb-AP, GαM-HRP, GαRb-HRP or GαM-CY5). The Western-Star immunodetection system (Tropix kit) was from Applied Biosystems and ECL Plus reagent was from Amersham. Hoechst 33258 and rhodamine-phalloidin were from Molecular Probes and Aqua Poly/Mount was from Polysciences. All other chemicals were of analytical grade.

Cell Culture- MTLn3 cells were cultured as before (28). To generate stable cell lines, MTLn3 rat mammary carcinoma cells were transfected with GFP-paxillin or GFP-paxillinS178A along with empty vector pcDNA3 using LipofectAMINE plus reagents according to manufacturer's procedures. Stable transfectants were selected using G418 at a concentration of 500 μg/ml. Individual clones were picked and maintained in α-MEM supplemented with 5% (v/v) FBS containing 100 μg/ml G418 (complete medium). Clones were regularly analyzed for the expression of GFP constructs by flow cytometry analysis, western blotting and immunofluorescence. Cells were used for up to 8 passages and expression levels of GFP tagged proteins remained stable during experiment period. For stimuli experiments, 80-90% confluent cells were starved for 4hr and stimulated with EGF (10 nM) or HGF (5 ng/ml) for indicated time periods.

Proliferation, Attachment and Wound Healing Assay- For proliferation assay, cells were seeded in complete medium on 6 well-plates for 24, 48, 72 or 96 hrs. Cells were detached and the amount of cells was determined by counting. For cell attachment assay, cells were starved for 1 hr in serum-free medium and detached. Equal amounts of cells were plated in complete medium on collagen-coated 6-well plates. After 30, 60, 90 and 120 minutes, attached cells were trypsinized and counted. For wound healing assay, monolayer cells were scratched using a pipette tip to generate a wound. Plates were then washed with medium and wounds were photographed using phase contrast microscopy with a Nikon Coolpix digital camera. Plates were then incubated in α-MEM supplemented with 1% (v/v) FBS for 20 hrs. Wounds were photographed again and wound closure was determined using Image J software.

Live Cell Imaging- Cells were cultured in glass-bottom plates overnight and starved for 4 hrs followed by visualization with high throughput microscopy for 1 hr on a Nikon TE

2000-E microscope equipped with perfect focus system in a humid climate of 37°C and 5% CO_2. Subsequently, cells were treated with EGF or HGF and the exactly same fields were visualized for 1 hr. Movies were captured five minutes per frame with 20x objective. Cell speed was determined by tracking cell center and calculating the distance between two sequential frames. Cell dynamics were measured with cell surface area change between two sequent frames by homemade macro adopted in Image-Pro Plus (version 5.1, Media Cybernetics Inc., Silver Spring, MD).

TIRF and FRAP- Total internal reflection fluorescence (TIRF) microscopy was performed on GFP-paxillin MTLn3 cells and GFP-S178A-paxillin in a climate control chamber. TIRF movies were captured on a Nikon TIRF microscope system (Eclipse TE 2000-E, Nikon with automated stage) with framing every 5 minutes for 4 hrs using NIS-elements AR software (Nikon). To determine the turnover of GFP-tagged paxillin in individual focal adhesions, fluorescence recovery after photobleaching (FRAP) was performed as follows: photobleaching was applied to a small area covering a single focal adhesion for 1 s with laser intensity of 50 μW. Redistribution of fluorescence was monitored with 100 ms time intervals at 7.5μW starting directly after the bleach pulse. Approximately 20 focal adhesions (each in distinct cells) were averaged to generate one FRAP curve for a single experiment. All measurements were performed at 37 °C using a heating stage with feedback temperature control and the experiment was performed on at least three different days. Images were analyzed with Image software (Zeiss). The relative fluorescence intensity of individual focal adhesion was calculated at each time interval as follows: $Irel(t) = (FA_t / FA_0)$, where FA_t is the intensity of the focal adhesion at time point t after bleaching, FA_0 is the average intensity of the focal adhesion before bleaching. The fluorescent curves were analyzed with non-linear regression analysis (GraphPad Prism 5).

Gel Electrophoresis and Immunoblotting- Western blot analysis was performed as before (29). Cells were scraped in ice-cold TSE (10 mM Tris, 250 mM sucrose, 1 mM EGTA, pH 7.4) plus inhibitors (10 μg/ml aprotinin, 1 mM dithiothreitol, 10 μg/ml leupeptin, 50 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride). After sonication, protein levels were determined using the Bradford protein assay with IgG as a standard. Equal amounts (25 μg) of cellular lyses were separated on 7.5% polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked in either 0.2% (w/v) casein in TBS-Tween20 (for phospho-state specific antibodies) or 5% (w/v) BSA in TBS-T (for other primary antibodies) and probed with primary antibody overnight followed by sufficient washes and incubation with secondary antibodies. Alkaline phosphatase (AP)-conjugated secondary antibodies for phospho-proteins were detected with the Western-Star immunodetection system. For detection of horseradish peroxidase (HRP)-conjugated antibodies, ECL Plus reagent was used and followed by visualization on a Typhoon Imager 9400 (520nm, blue laser).

Immunofluorescence- Cells were seeded on collagen coated glass coverslips. Cells were briefly washed in PBS, followed by fixation in 3.7% formaldehyde for 10 min at room

temperature. After washing, coverslips were blocked in TBP (0.1% (w/v) Triton X-100, 0.5% (w/v) BSA in PBS, pH 7.4). Incubation with primary antibodies diluted in TBP containing 0.05% (w/v) NaN_3 was carried out overnight at 4°C. Primary antibodies were against paxillin. Coverslips were incubated with secondary antibodies conjugated to Cy5. After sufficient washing, coverslips were mounted on glass slides using Aqua Poly/Mount. Cells were visualized using a Bio-Rad Radiance 2100 MP confocal laser scanning system equipped with a Nikon Eclipse TE2000-U inverted fluorescence microscope and a 60X Nikon objective.

Statistical Analysis- Student's t test was used to determine significant differences between two means ($p<0.05$).

RESULTS

Mutation of Paxillin Serine178 Inhibits MTLn3 Cell Proliferation and Wound Healing Capability and Attenuates Cell Attachment- Paxillin is implicated in EGF-stimulated cell migration of rat bladder tumor epithelial cells (NBT-II) through JNK-mediated phosphorylation at paxillin Ser178 (27). Firstly we determined the involvement of JNK in EGF-induced migration of MTLn3 mammary adenocarcinoma cells. Exposure to EGF caused a rapid onset of random cell migration in MTLn3 cells, which was inhibited by an inhibitor of JNK, SP600125, indicating the requirement for JNK in cell migration (Fig. 1A). Next we determined the involvement of paxillin in this process. Treatment with EGF induced the transient phosphorylation of paxillin at Ser residue 178 in association with JNK activation. Importantly, this paxillin phosphorylation was dependent on JNK, since the inhibitor SP600125, prevented the phosphorylation at Ser178 (Fig. 1B). These data indicate that in MTLn3 cells EGF-induced phosphorylation of paxillin at Ser178 is mediated by JNK.

To further investigate the role of paxillin Ser178 in cell migration and proliferation, we generated MTLn3 cell lines stably expressing either GFP-tagged wt-paxillin or mutant GFP-paxillin in which the serine residue 178 was replaced by alanine (further referred to as S178A-paxillin). Clones of MTLn3 cells stably expressing GFP-wt-paxillin or GFP-S178A-paxillin were evaluated by flow cytometry (data not shown), western blotting and immunofluorescence (data not shown). Three wt-paxillin clones and three S178A-paxillin clones were selected for further experiments. Expression levels were equal in all three wt clones; one S178A clone had a lower expression level most likely due to reduced number of GFP-positive cells (Fig 2A). Expression levels of recombinant proteins remained stable for at least 8 passages (data not shown). Next, we determined the effect of S178A-paxillin expression on cell proliferation. S178A-paxillin expression significantly reduced the growth rate of MTLn3 cells (Fig.2B). This suggests that S178A-paxillin disturbs the essential cell proliferation signaling.

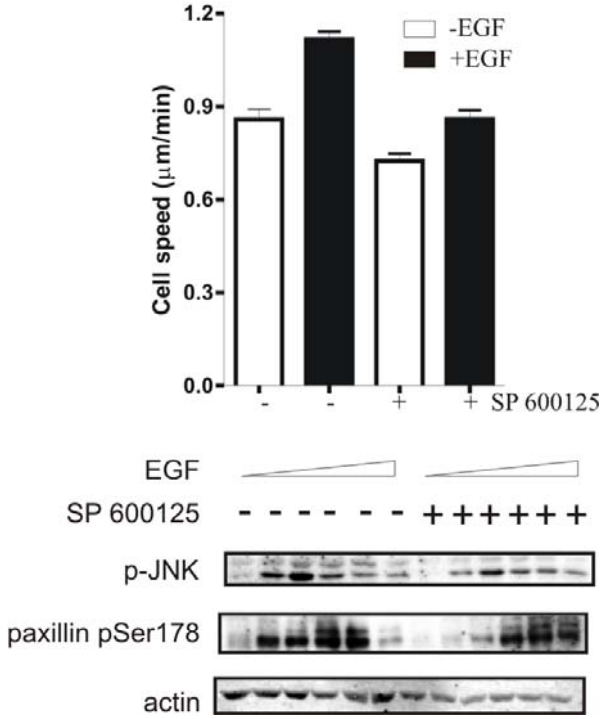

Figure 1: EGF signaling induces JNK-paxillin involved cell migration. MTLn3 cells grown in collagen-coated glass-bottom plate were pretreated with or without JNK inhibitor SP600125 for 30min and stimulated with EGF. Live cell migration was visualized on Nikon Eclipse TE2000-E PFS microscope (Nikon). Cell migration speed was analyzed as described in Materials and Methods section (A). Monolayer cells were pretreated with SP600125 for 30 min and followed with indicated incubation of EGF for 0, 5, 10, 20, 30, 60 min. Cell lyses were collected and separated with SDS-PAGE, and probed with pSer178-Paxillin and pJNK antibody (B).

Given the prominent role of paxillin in focal adhesion formation and dynamics, we next examined the effect of S178A-paxillin expression on MTLn3 cell attachment and spreading. For this purpose cells were seeded on collagen-coated dishes and allowed to attach for 30, 60 90 and 120 minutes. Significantly less S178A-paxillin cells, compared to wt-paxillin cells, attached. Importantly, while most of wt-paxillin cells had already spread, most of the S178A-paxillin cells remained round and presented a smaller surface area even when they attached and spread (Fig. 2C). This indicates that Ser178 of paxillin is essential for efficient attachment and spreading of MTLn3 cells to collagen. Next we determined the effect of S178A-paxillin on cell migration in an artificial wound healing assay. The closure speed of artificial wounds was determined after 20 hrs (Fig. 3). While wt-paxillin cells had closed the wound by 83 %, in sharp contrast, S178A-paxillin cells had only closed 25% of the wound. In conclusion, these above results indicate that S178A-paxillin affects different aspects of cell adhesion and migration.

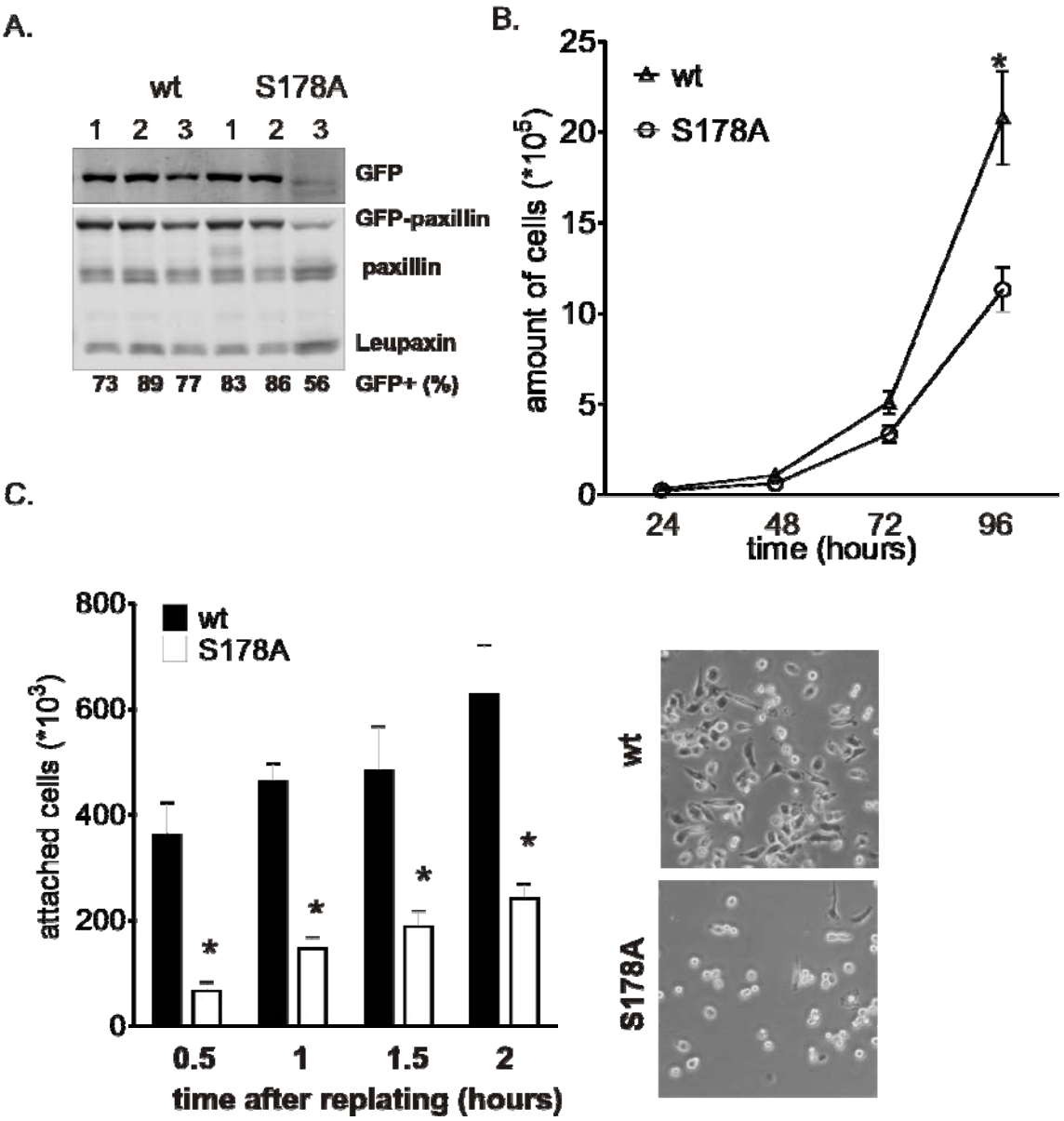

Figure 2: Ser178 residue of paxillin regulates cell proliferation and attachment. 3 different colonies for wt and S178A cells were picked for further research. Cell lysates for each cell line were collected and analyzed for GFP-paxillin expression by western blotting (A). Equal amounts for each clone (3 wt-paxillin clones and 3 S178A-paxillin clones) were cultured for indicated time periods and cell proliferation was determined by cell counting (B). Cell adhesion assays were performed as described in Materials and Methods section for all the wt and S178A clones. Note that S178A-paxillin cells are defective in efficient cell spreading (right panel) (C). Data shown are results from three independent experiments (mean ± SD; n=3).

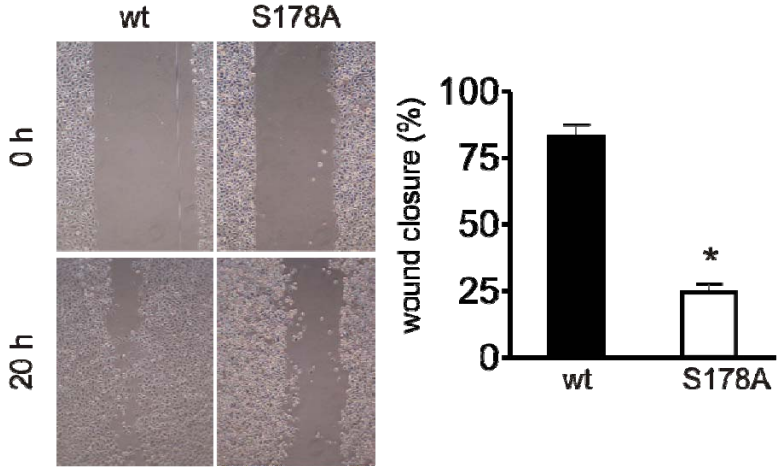

Figure 3. GFP-S178A-paxillin reduces wound closure rate. The wound healing assay was performed as described in Materials and Methods. The wounds at indicated time points were photographed (Left). The length of wounds was measured in Image J and migration was expressed as the percentage of wound closure (Right).

Reduced Dynamics of GFP-S178A-paxillin at Focal Adhesions- To investigate the mechanism of the inhibitory effect of S178A-paxillin on cell migration, next we determined the dynamics of S178A-paxillin at focal adhesions. First, we evaluated the localization of GFP-wt-paxillin and GFP-S178A-paxillin in MTLn3 cells. With normal confocal microscopy, clear localization at focal adhesion was difficult to determine, although both GFP-wt-paxillin and GFP-S178A-paxillin were present at membrane ruffles and co-localized with paxillin in the same staining pattern indicating spatial functionality of both wt and mutant GFP paxillin constructs (Fig. 4A). To discern localization at focal adhesions we used TIRF microscopy, which allowed the detection of GFP signal in cells at the focal plane where cells make direct contact with the coverslip. TIRF microscopy indicated that both wt-paxillin and S178A-paxillin were localized at focal adhesions (Fig. 4B), thus indicating that the localization of S178A-paxillin at focal adhesions does not disturb the formation of focal adhesions. Next we investigated the dynamics of wt-paxillin and S178A-paxillin at focal adhesions. For this purpose we performed fluorescence recovery after photobleaching (FRAP) experiment with GFP-wt-paxillin and GFP-S178A-paxillin cells. Interestingly, GFP-S178A-paxillin cells showed the same recovery rate as GFP-wt-paxillin under control conditions. EGF stimulation increased the florescence recovery rate in GFP-wt-paxillin cells but not in GFP-S178A-paxillin cells (Fig. 4C). Thus, Ser178 at least in part determines the turnover of paxillin at focal adhesions and this residue mutant disturbs the cell response to EGF.

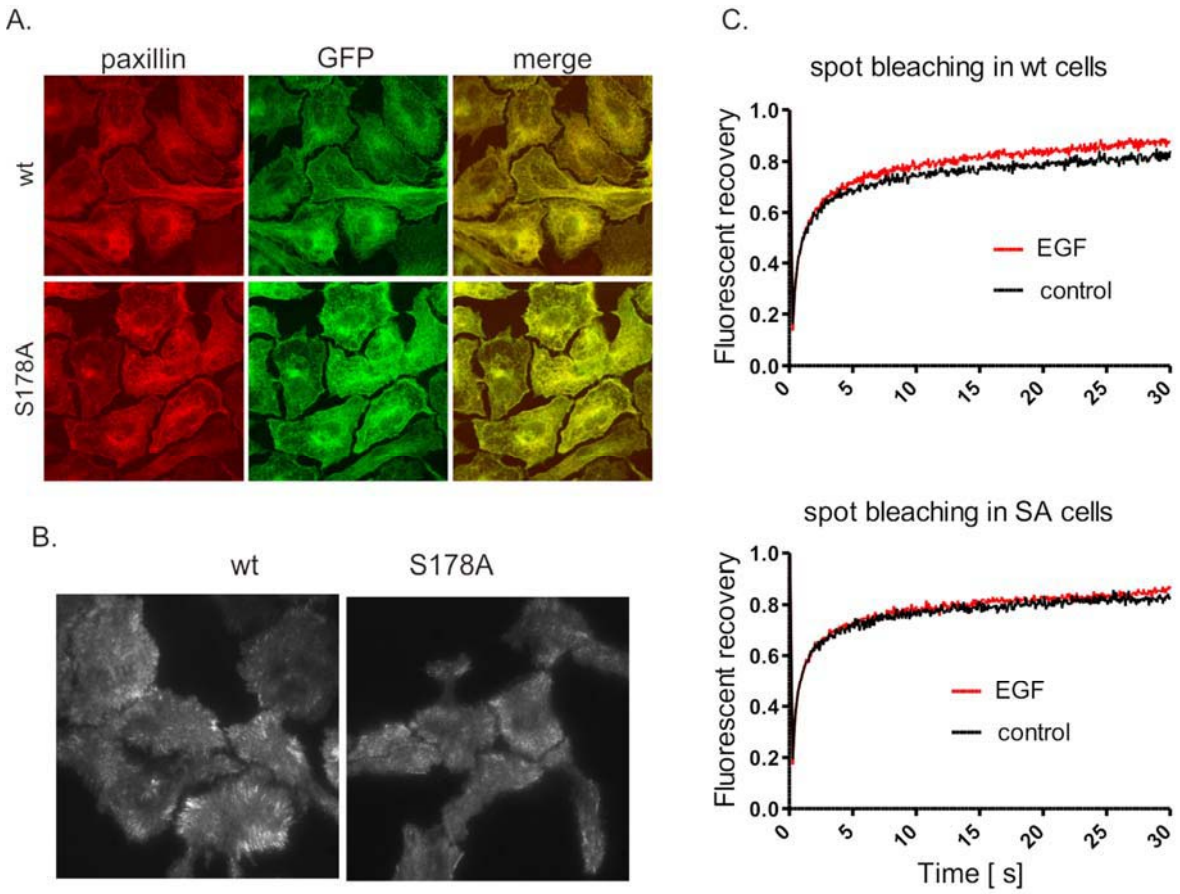

Figure 4: GFP-S178A-paxillin competitively co-localizes with endogenous paxillin at focal adhesion sites and decreases EGF-induced turnover of focal adhesions. GFP-wt-paxillin and mutant Ser178Ala cells were fixed for immunofluorescent assay and stained with mouse anti-paxillin antibody (A). A single frame from TIRF movies for GFP-wt-paxillin and mutant Ser178Ala cells (B). FRAP assay with GFP-wt-paxillin cells and mutant Ser178Ala-paxillin cells. Cells were treated with or without EGF for 20 mins before FRAP (C). Data shown are representative results from three independent experiments.

S178A-paxillin Inhibits Cell Motility in Serum Starved and EGF-Treated Conditions, but HGF Stimulates More Sufficiently- Next we investigated the effect of S178A on EGF-induced cell migration. Both wt-paxillin and S178A-paxillin cells were treated with EGF (10 nM) followed by the analysis of random cell migration. While wt-paxillin cells rapidly formed lamellipodia and became highly motile, this did not happen for S178A-paxillin cells (movie not shown). Since S178A-paxillin acts as a dominant negative construct in these cells, S178A-paxillin would compete for the localization of endogenous paxillin at focal adhesions and we reasoned that endogenous paxillin should lose either the basal phosphorylation level of Ser178 and/or the capability of being phosphorylated by JNK at Ser178. Indeed, EGF stimulation hardly activated phosphorylation of endogenous paxillin; as expected GFP-S178A-paxillin in these cells was not phosphorylated at all. In wt-paxillin cells, both endogenous and GFP-wt-paxillin were phosphorylated at Ser178 after EGF treatment (Fig. 6 top).

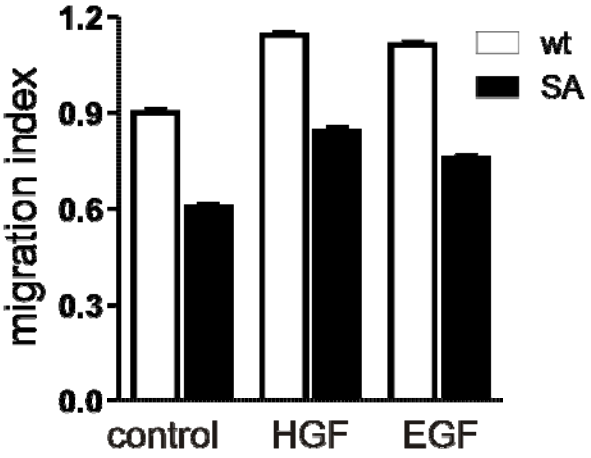

Figure 5: **EGF and HGF induce cell migration and dynamic differently in GFP-wt-paxillin and mutant Ser178Ala cells.** Random migration assay was done as mentioned in Materials and Methods.

Cell scattering is also induced by other growth factors, including hepatocyte growth factor/scatter factor (HGF) (30,31). HGF also induces activation of JNK in various cell types (32). We wondered whether HGF would be able to induce cell scatter in mutant cells. Treatment with HGF (5 ng/ml) induced cell migration in wt-paxillin cells. While S178A-paxillin did hardly migrate under serum starvation conditions, HGF stimulated cell migration better than EGF in S178A-paxillin cells (Fig. 5). These data indicate that, apparently, the cell migration machinery is functional in S178A-paxillin cells. Interestingly, in S178A-paxillin, HGF was capable of phosphorylating endogenous paxillin at Ser178 to a similar extent as in wt-paxillin cells; again S178A-paxillin was not phosphorylated after HGF treatment (Fig. 6 bottom). This suggests the phosphorylation of Ser178 is crucial for cell migration.

S178A-paxillin MTLn3 Cells have Reduced EGF-induced Activation of AKT and ERK- Finally, we determined the possible mechanism by which S178A-paxillin affects EGF-induced cell migration. Since EGFR signaling is regulated and trans-activated at focal adhesions by both integrins (33,34) and FAK (35-37), we reasoned that possibly S178A-paxillin would disturb the downstream signaling of the EGFR. To investigate this, both wt-paxillin and S178A-paxillin cells were treated with EGF and downstream activation of both AKT and ERK was determined by western blotting. While EGF caused activation of AKT in wt-paxillin cells, proper AKT activation by EGF was inhibited in S178A-paxillin. Also ERK activation was slightly reduced in S178A-paxillin cells compared to wt-paxillin cells after EGF treatment, albeit less significant than that for AKT (Fig. 7 top). We then evaluated whether S178A-paxillin cells responded normally to HGF. Indeed, HGF treatment resulted in similar levels of phosphorylated ERK in both wt-paxillin and S178A-paxillin cells (Fig. 7 bottom). While the activation of AKT was reduced in S178A-paxillin cells, the overall activity of AKT by HGF stimulation was higher, compared to EGF stimulation (compare Fig. 7 top and bottom panels). These data suggest

that S178A-paxillin affects EGFR-mediated signaling and interferes with downstream signaling events that are essential to initiate and/or mediate cell migration.

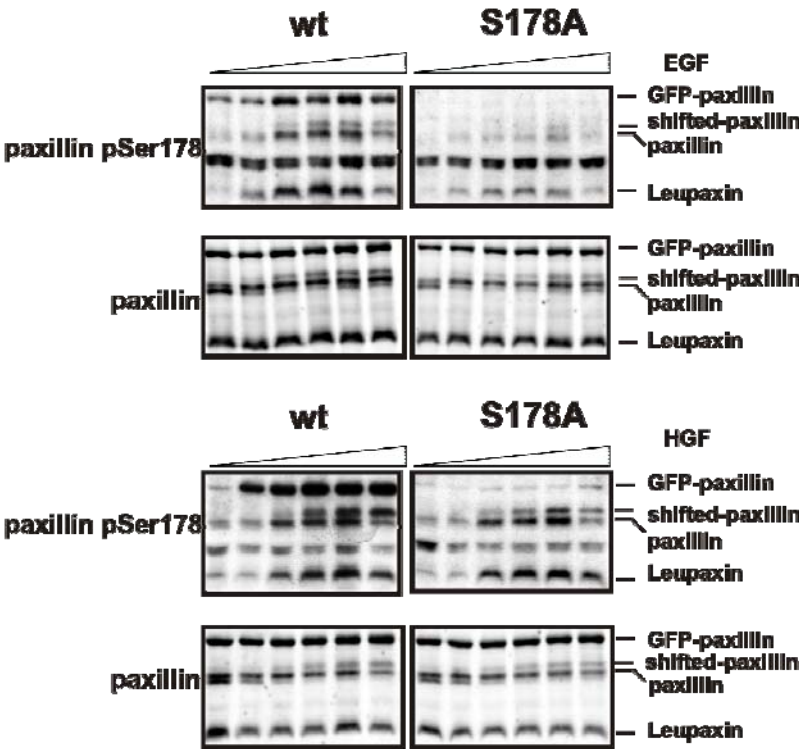

Figure 6: GFP-wt-paxillin and mutant Ser178Ala cells display different activation of signal pathway to EGF and HGF. 90% confluent cells were starved for 4hrs and stimulated with EGF (10nM, top panels) or HGF (5ng/ml, lower panels) for 0, 5, 10, 20, 30, 60 mins. Cell lyses were collected and western blot assay were performed to probe with antibodies against pSer178 paxillin and paxillin as mentioned in Materials and Methods.

DISCUSSION

The scaffold protein paxillin at focal adhesions regulates cell motility by regulating FA assembly and disassembly processes (3,17). In this study, we investigated the role of JNK-mediated phosphorylation of paxillin at Ser178 by generating cell lines stably expressing GFP-tagged paxillin in which the Ser178 residue was replaced by non-phosphorylatable alanine. Using these cell lines we were able to demonstrate that: 1) Ser178 phosphorylation is essential for efficient cell migration; 2) Ser178 phosphorylation determines the rate of cell proliferation; 3) Ser178 affects the efficiency of EGF-induced downstream signaling.

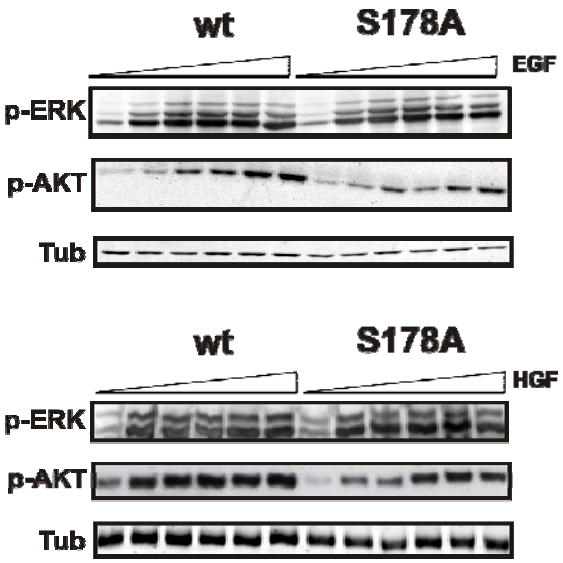

Figure 7: MTLn3 mutant Ser178Ala cells display a delayed and deficient activation of p-AKT compared to wt-paxillin cells in response to EGF. 90% confluent cells were starved for 4 hrs and stimulated with EGF (10 nM, top panels) or HGF (5 ng/ml, lower panels) for 0, 5, 10, 20, 30 or 60 min. Cell lysates were collected and analyzed by western blotting with antibodies against activated pERK and pAKT.

EGF is an important growth factor in the tumor metastasis process of MTLn3 cells (38). Our data indicate an important role for Ser178 of paxillin to control efficient EGF-induced signaling. We have performed Affymetrix cDNA microarray analysis of GFP-paxillin and GFP-S178A-paxillin (all clones depicted in Fig. 2). Interestingly, the preliminary data analysis indicate that the stable mutant S178A-paxillin cell lines express reduced levels of EGFR compared to wild type cell lines. Although further analysis of EGFR at the protein level is required, these data might explain both the reduced EGF-induced activation of AKT and ERK, in association with reduced EGF-induced cell migration. Since EGFR signaling is crucial for tumor progression, more *in vivo* work concerning the role of paxillin Ser178 and EGFR expression in tumor formation and progression should be carried out. In addition to EGFR, the levels of some cytoskeleton-associated proteins and matrix components were affected by S178A-paxillin expression, including secreted phosphoprotein 1 (fold change (mutant cells/wild type cells) =15), Rho GTPase activating protein 18 (FC=2), MMP3 (FC=6). These proteins may provide a clue for the cytoskeletal differences as well as the different capabilities in cell adhesion and migration observed in S178A-paxillin cells.

We have showed that JNK mediates the phosphorylation of paxillin Ser178 after EGF treatment. JNK is also activated by cellular stress conditions such as oxidative stress or microtubule disruption. As indicated in chapter 4, the microtubule disrupting agent vincristine induces a drastic and sustained activation of JNK in MTLn3 cells, which is associated with the modification of paxillin by phosphorylation of Ser178 as well as by an alternative modification of paxillin resulting in a mobility shift by SDS-PAGE. Apparently, the JNK activation by growth factors does not cause the same mobility shift of paxillin (see appendix figure at the end of this chapter). This suggests a more complex

regulation of paxillin by JNK, which may be dependent on either the transient JNK activation after growth factor treatment, or alternative stress signaling pathways that are activated by stress conditions, such as microtubule disruption. Future work should establish the role of both of these paxillin phosphorylation events under in vivo conditions, and the relevance to both cancer progression and the sensitivity towards anticancer drug treatment, which typically involves the activation of JNK.

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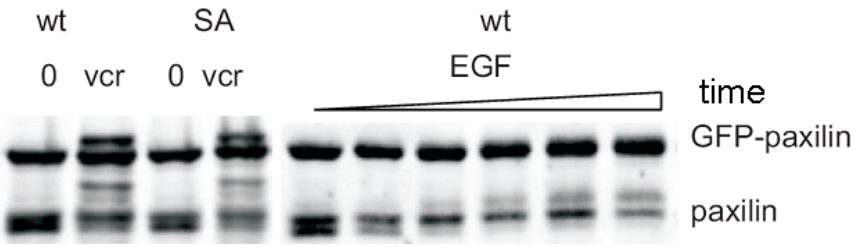
Appendix

The comparison of paxillin modulation induced by vincristine (VCR) and EGF (shown is the immunoblot with paxillin antibody). Note the mobility shift of both GFP-tagged paxillin and endogenous paxillin by VCR but not by EGF.



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