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Differences in Membrane Composition and Organization of Crucial Molecules Define the Invasive Properties of MCF-7 Breast Cancer Cells

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1. Introduction

Breast cancer is the leading cause of cancer deaths among women in the United States and Europe [Jemal et al., 2011]. Most of these women die from their metastases, also known as the spread of primary breast cancer cells, to distant organs such as lung, brain and bone [Mehrotra et al., 2004]. Metastatic development comprises a complex series of linked, sequential steps. These steps include disconnection of intercellular adhesions and separation of single cells within a primary tumour, interaction with the surrounding extracellular matrix, local migration and invasion, followed by intravasation, transit through blood vessels and extravasation at distant organs, where they establish secondary tumours or metastases. In other words, the most lethal aspects of breast cancer are the processes of tumour cell migration and invasion, both prerequisites for the formation of metastases [Yilmaz & Christofori, 2010]. At present, however, the mechanisms responsible for the acquisition of invasive and metastatic potential of tumour cells are poorly understood. Thus, a better understanding of the biology and molecular interactions that regulate and coordinate the steps of tumour cell migration and invasion, is required to effectively treat metastatic breast cancer and control the devastating nature of breast cancer. It is widely accepted that cancer cells, capable of initiating metastases acquire specific features and exert activities that are not shared with the primary tumour cells [Liotta & Kohn, 2001]. Over the past years, a number of particular complex invasion-associated cellular activities have been recognized and characterized, including variations in expression levels of cell-cell and cell-matrix adhesion molecules and proteases that degrade the surrounding extracellular matrix, along with changes in expression or activity levels of a variety of cellular proteins in multiple branched signalling pathways [Mareel & Leroy, 2003]. Additionally, previous studies have highlighted the importance of aberrant glycosylation as a crucial event in the induction of invasion and metastasis [Hakomori, 2002], including altered glycosylation of cell-surface glycoproteins [Kim & Varki, 1997] and GSLs (glycosphingolipids) [Hakomori, 1998]. GSLs are common components of cell membranes. In malignant cells, they have been identified as tumour-associated antigens as defined by specific monoclonal antibodies [Hakomori, 1996]. Essentially all GSLs, in tumour and normal cells, can cluster and assemble

with specific membrane proteins and signal transducers. Of importance is that clustering of particular GSLs may affect cellular activities associated with tumour cell migration and invasion, since they are recognized to mediate cell-cell and cell-matrix adhesion and initiate signal transduction, induced by stimulation of GSLs [Hakomori et al., 1998]. Roles for GSLs in invasion were demonstrated recently for GM3 (monosialoganglioside 3) in mouse melanoma B16 cells [Iwabuchi et al., 1998; Iwabuchi et al., 1998] and in human bladder KK47 cells [Mitsuzuka et al., 2005], for GM1 in mouse Lewis lung cancer cells [Zhang et al., 2006], for DSGG (diasyl-GalNAcLc4) in renal cell carcinoma [Satoh et al., 2000; Ito et al., 2001] and for GD3 (disialoganglioside 3) in melanoma cells [Hamamura et al., 2005]. The glycosphingolipid, MSGb5 (monosialyl-Gb5), also known as SSEA-4 (stage-specific embryonic antigen-4) [Kannagi et al., 1983], is found maximally expressed in human renal cell carcinomas and correlates with metastases [Saito et al., 1991; Saito et al., 1997]. The molecular mechanisms regulating synthesis of MSGb5 have been studied previously [Saito et al., 2003]; however, no clear functional role of MSGb5 in the invasive and metastatic behaviour of tumour cells has been demonstrated. In this chapter, we present data on a functional role of the glycosphingolipid MSGb5 in the human mammary carcinoma variant cell line MCF-7/AZ, showing increased invasiveness and motility in response to stimulation of MSGb5 by its monoclonal antibody RM1 or through induced clustering of MSGb5 by ET-18-OMe. ET-18-OMe is a synthetic ether lipid analogue shown to induce loss of cell-cell adhesion and to stimulate invasion of MCF-7/AZ breast cancer cells [Steelant et al., 2001] and was used as a molecular probe in the presented study.

2. TLC patterns of GSLs from cell extracts of MCF-7AZ and MCF-7/6 cells

The major GSLs identified in the two human breast cancer cell lines, MCF-7/AZ and MCF-7/6, were characterized as: globo-series Gb3, Gb4, MsGb5 and ganglio-series structure GM2, by TLC developed in a solvent system of chloroform/methanol/aqueous $CaCl_2$ and visualized with orcinol staining (Figure 1) [Saito et al., 1971]. Lacto-series structures were presumably below the detection limit.
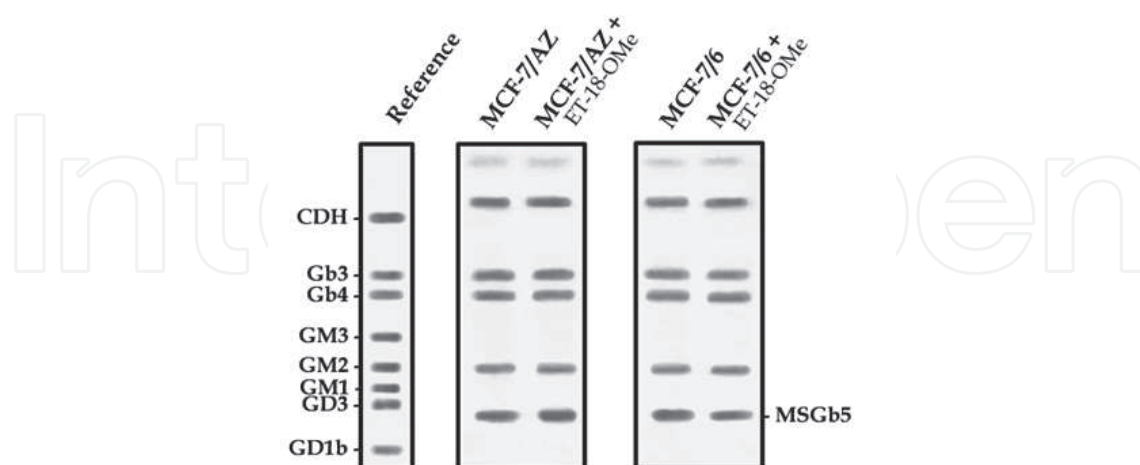

Fig. 1. TLC pattern of GSLs from whole cell extracts of MCF-7/AZ and MCF-7/6 cells with or without ET-18-OMe. GSLs were extracted from cells of the same protein weight, spotted onto TLC plates, developed in a solvent system of chloroform/methanol/0.2% aqueous $CaCl_2$, visualized by spraying with 0.5% orcinol in 2M sulfuric acid. CDH (lactosylceramide), Gb3, Gb4, MSGb5, GM3, GM1, GD3 and GD1b were used as reference markers.

All identified GSLs were present in the insoluble low–density fractions, as prepared after elimination of sucrose by dialysis and detected by TLC immunostaining with specific monoclonal antibodies (mouse IgM 1A4 to Gb3, mouse IgM 9G7 to Gb4, mouse IgM SSEA-3 to Gb5, mouse IgM RM1 to MSGb5, mouse IgM MBr1 to globo-H ($\alpha1{\rightarrow}2$ fucosyl-Gb5), mouse IgM MK1-8 to GM2, mouse IgG$_3$ DH2 to GM3 and mouse IgM 5F3 to disialyl-Gb5) [Kannagi & Hakomori, 2001; Ito et al., 2001] (Figure 2). The cell variants in this study lack the abundant presence of GM3 and GM1 as was reported by Nohara et al. (1998) in MCF-7 cells, but show a comparable expression pattern for Gb3 and other globo-series structures similar to Gb5 and globo-H. Furthermore, we found that treatment with the ether lipid ET-18-OMe did not alter the expression levels of GSLs present in these cell lines.
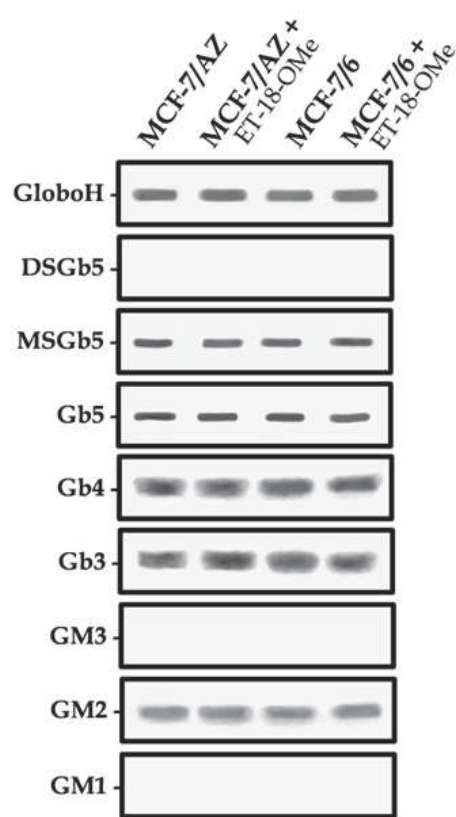

Fig. 2. TLC pattern of GSLs in low-density fractions of MCF-7/AZ and MCF-7/6 cells with or without ET-18-OMe. GSL fractions were prepared after elimination of sucrose by dialysis using C$_{18}$ columns. Immunostaining of various GLS structures present in the low-density fractions were detected by using nine antibodies directed against the respective structures.

3. RM1 and ET-18-OMe stimulate motility and invasion through clustering of MsGb5 in MCF-7/AZ cells

The two human breast cancer cell variants, MCF-7/AZ and MCF-7/6, are non-invasive into a collagen type I gel-layer as determined by a method using specific assembly [Bracke et al., 1999] and the motility of the two variants as revealed by wound migration assay is basically the same. When MCF-7/AZ cells were treated with monoclonal anti-MSGb5 antibody RM1 or with ET-18-OMe, their invasiveness into collagen I layer was greatly enhanced, while no such effect could be observed on MCF-7/6 cells. Control IgM antibodies from normal mouse serum

and antibodies to other GSLs (anti-globo-H MBr-1, anti-Gb3 1A4, anti-Gb5 SSEA-3, anti-GM2 MK1-8) [Steelant et al., 2002], did not increase invasiveness of either cell variant (Figure 3).
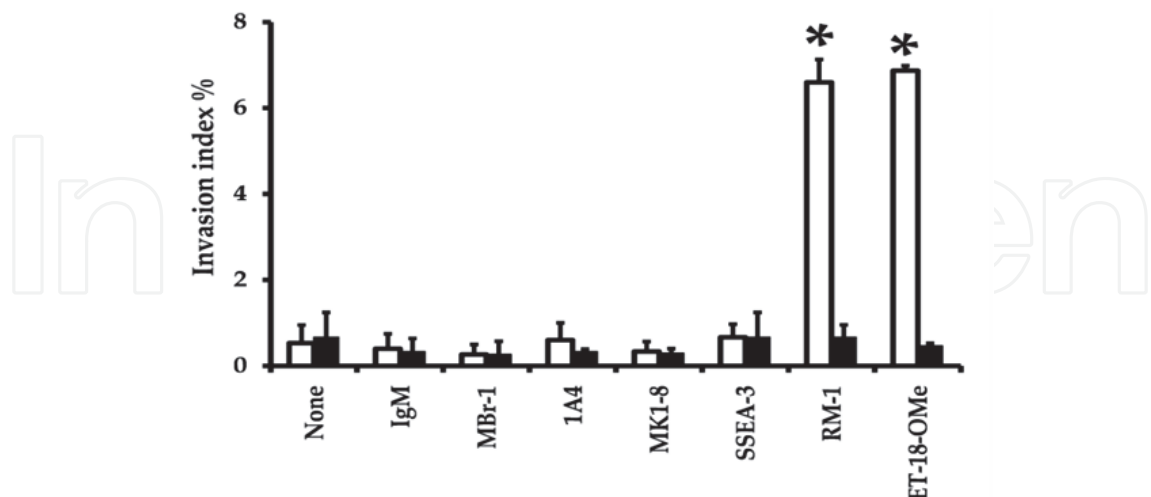

Fig. 3. Invasiveness of MCF-7/AZ cells and MCF-7/6 cells. Effect of mouse IgM or antibodies against Globo-H (MBr-1), Gb3 (1A4), Gb5 (SSEA-3), GM2 (MK1-8), MSGb5 (RM1) and ET-18-OMe on invasion into collagen type I of MCF-7/AZ cells (open columns) and MCF-7/6 cells (black columns). The invasion index expresses the percentage of cells invading into collagen type I over the total number of cells after 24 h. Results are means + S.D., * indicate statistical difference from control conditions, untreated MCF-7/AZ and MCF-7/6 cells, p< 0.05.

The motility of MCF-7/AZ cells was also enhanced upon treatment with anti-MSGb5 monoclonal antibody RM1, and again similar treatment did not influence the migratory capacity of the other variant cell line, MCF-7/6 (Figure 4).
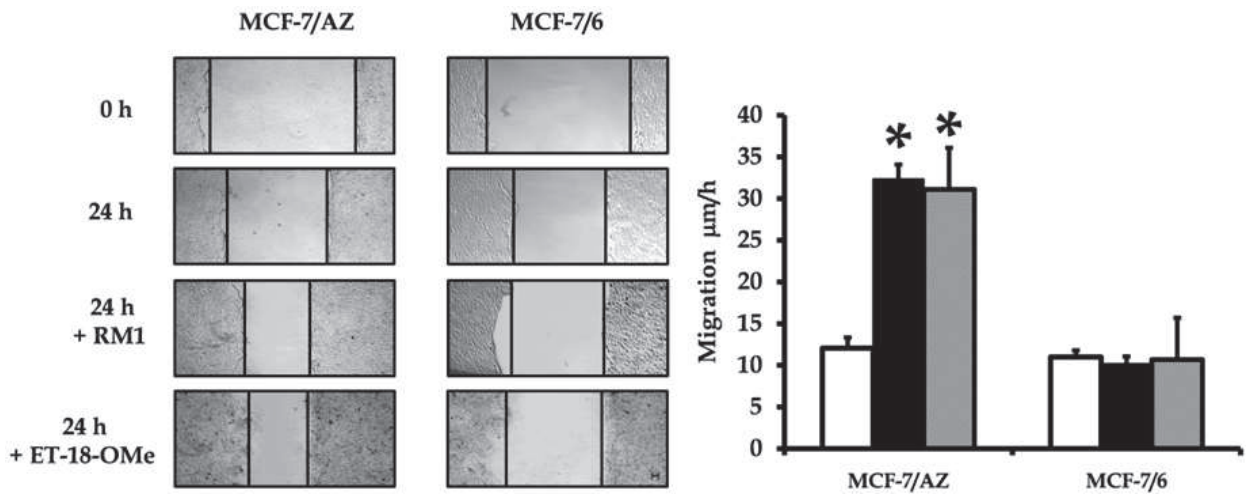

Fig. 4. Migration of MCF-7/AZ and MCF-7/6 cells. Effect of MSGb5 antibody RM1 and ET-18-OMe on MCF-7/AZ and MCF-7/6 cells in wound migration assay. Confluent cells were wounded, measured and allowed to grow in the presence of RM1 and ET-18-OMe. Scale bar = 250 μm. After 24 h, the distances over which the cells migrated were measured and results are expressed as migratory velocity (μm/h). *, indicate statistical difference from untreated MCF-7/AZ and MCF-7/6 cells (p<0.05).

Although both cell lines displayed no differences in their GSL composition upon treatment with ET-18-OMe, the possibility arose that changes in organization and clustering of GSLs could be responsible for the observed biological activity and this presumably through assembly with and activation of associated signal transducers. Such an activation model was reported for disialylgalactosylgloboside, DSGG, in the renal cell carcinoma cell line TOS-1 [Satoh et al., 2000] and for GM3 in B16 melanoma cells [Iwabuchi et al., 1998; Iwabuchi et al., 1998]. Alterations in the organization pattern of MSGb5 were observed by immunofluoresescence and confocal microscopy (Figure 5). MSGb5 was detected in both variant cell lines MCF-7/AZ and MCF-7/6, which was consistent with the TLC data. Fluorescence examination of MCF-7/AZ cells treated with ET-18-OMe revealed clustering of MSGb5 at the membrane within minutes. Clustering of MSGb5 in MCF-7/6 cells was not observed (data not shown), nor clustering of other GSLs, for example Gb3 and Gb5 in MCF-7/AZ cells after ET-18-OMe treatment.
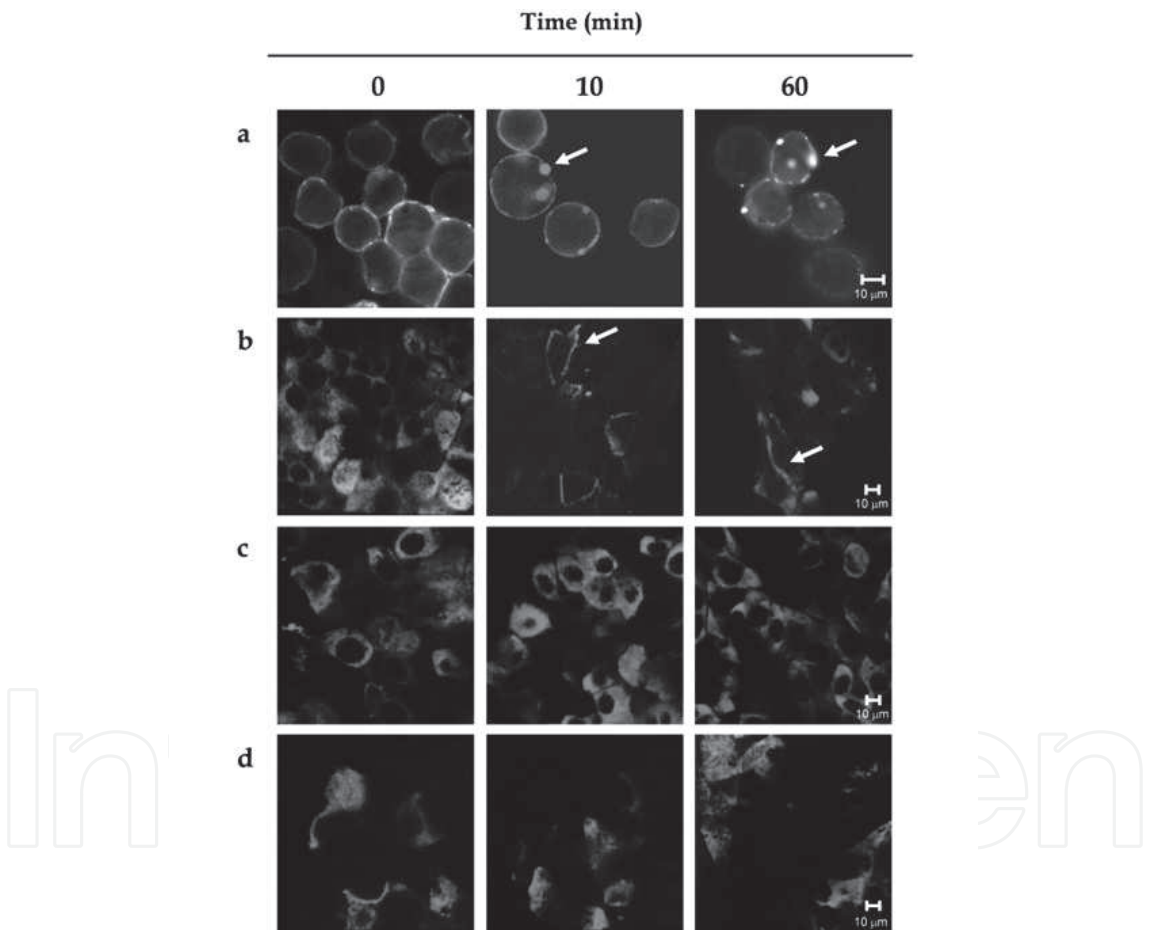

Fig. 5. Clustering of MSGb5 in MCF-7/AZ cells. The organizational pattern of MSGb5 by fluorescence(a) and confocal microscopy(b). Cells in suspension (a) or grown on glass coverslips (b-d), and analysed using antibodies against MSGb5, Gb3 and Gb5 and detected with FITC-labeled anti-mouse antibody. (a) and (b) show the homogenous organization of MSGb5, in untreated MCF-7/AZ cells; arrows indicate clustering of MSGb5 on the membrane of MCF-7/AZ cells after 10 and 60 min ET-18-OMe treatment. Organizational pattern of Gb3 (c) and Gb5 (d) by confocal microscopy, by using antibodies 1A4 against Gb3 and SSEA-3 against Gb5, followed by FITC-labeled mouse antibodies. Scale bar = 10 μm.

4. Src, the FAK-src signalling complex and the activation of the downstream pathway mediating invasion and motility in MCF-7/AZ cells

Several studies provide evidence that the non-receptor tyrosine kinase, src, is implicated in cancer progression of several cancer types. Changes in its expression and tyrosine phosphorylation correlate with the acquisition of an invasive cell phenotype [Guarino, 2010]. The motility and invasion promoting effects of RM1 and ET-18-OMe on MCF-7/AZ cells in our studies suggested that RM1 and ET-18-OMe initiate signalling pathways in MCF-7/AZ cells but not in MCF-7/6 cells. The possible involvement of src kinase in the signalling pathway leading to enhanced migration and invasion could be demonstrated by pretreating MCF-7/AZ cells with PP1, a pharmacological inhibitor of src kinase activity in the collagen I and wound migration assays (Figure 6).
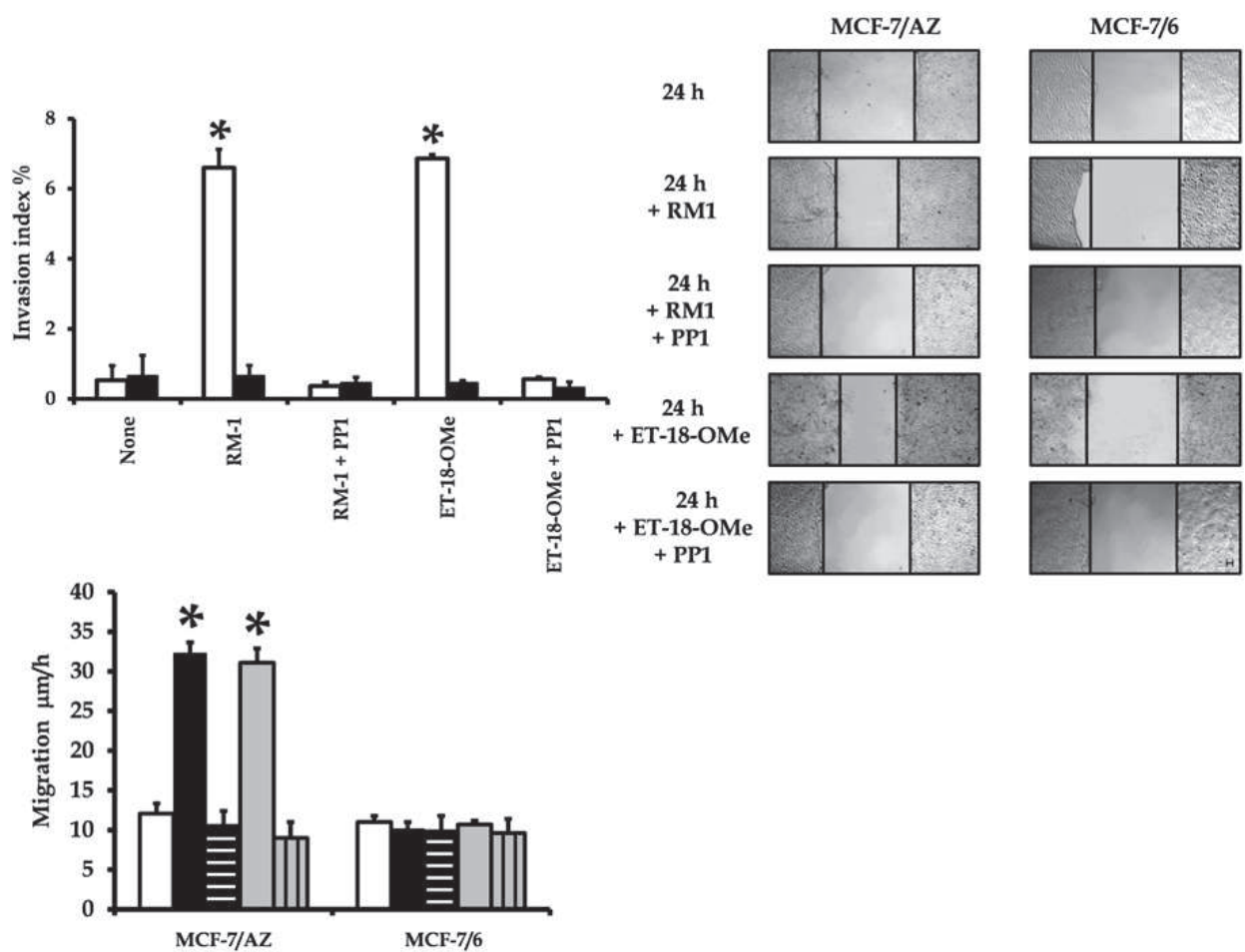

Fig. 6. Invasion and migration of MCF-7/AZ and MCF-7/6 cells. Effect of pharmacological inhibitor PP1 on RM1 or ET-18-OMe-mediated enhanced invasiveness (left panel) and migration of MCF-7/AZ cells (Right panel). Left: Invasion into collagen type I of MCF-7/AZ (open columns) and MCF-7/6 cells (black columns). The invasion index expresses the percentage of cells invading into collagen type I over the total number of cells. Right: Wound migration assay of MCF-7/AZ and MCF-7/6 cells. Scale bar = 250 μm. After 24 h, the distances over which the cells migrated were measured and results are expressed as migratory velocity (μm/h). *, indicate statistical difference from untreated MCF-7/AZ and MCF-7/6 cells ($p < 0.05$).

Accordingly, western blotting results revealed that src kinase activity in MCF-7/AZ cells was greatly increased within minutes of RM1 or ET-18-OMe treatment, and abolished by pretreatment with PP1, while there was no such activation of src by both treatments in MCF-7/6 cells. In addition, expression levels of src were left unaltered (Figure 7).
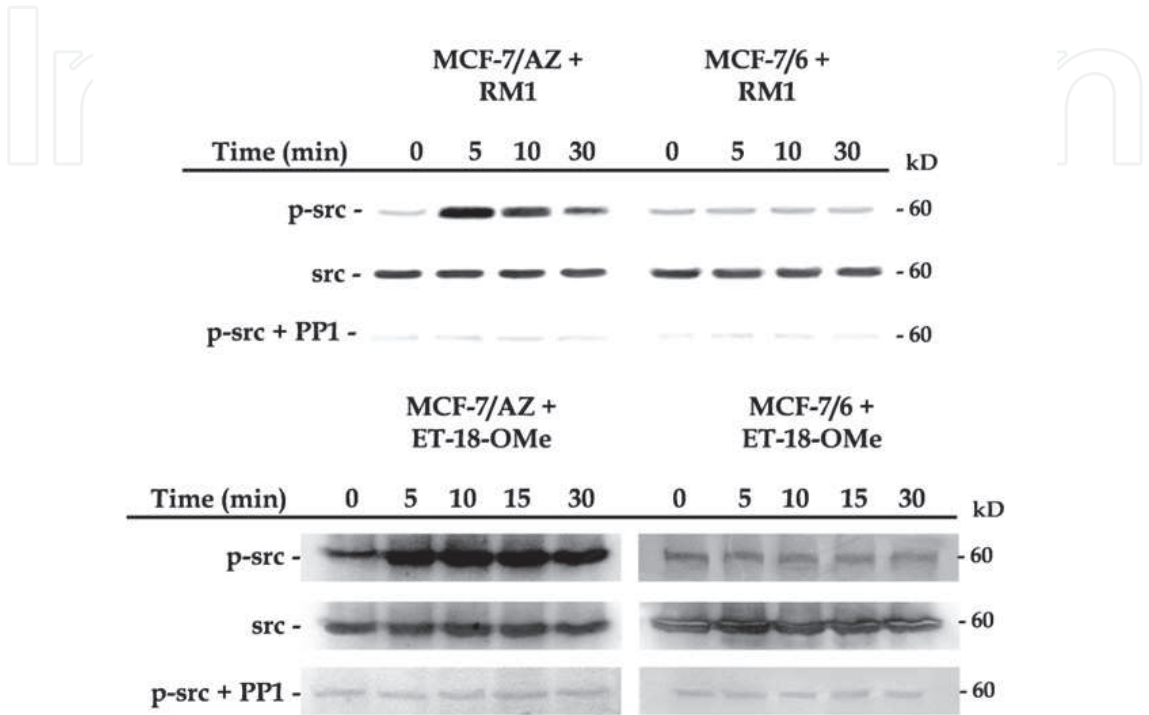

Fig. 7. Expression levels of phosphorylated src (Tyr416) upon RM1, RM1 + PP1, ET-18-OMe and ET-18-OMe + PP1 treatment in MCF-7/AZ and MCF-7/6 cells. Cells of 70% confluency were treated for indicated times, and lysed. Cell lysates, containing 30 µg of proteins, were analysed by SDS-PAGE (7.5% gels) and immunoblotted with antibody against src (Tyr416). The membrane was stripped at 50°C for 30 min in stripping buffer (100 mM 2-mercapto-ethanol, 2% (w/v) SDS, 62.5 mM Tris-HCl (pH 6.8)) and reblotted with anti-src antibody, for src total expression levels and equal loading.

These results clearly demonstrated a prominent role for src in motility and invasion induced by RM1 and ET-18-OMe. A possible mechanism by which src further promotes invasive behavior is through formation of a transient complex with focal adhesion kinase (FAK) [Hauck et al., 2002; Hsia et al., 2003]. FAK is another non-receptor tyrosine kinase recognized in cancer progression since it is found upregulated in malignant human tumour samples [Chatzizacharias et al., 2008]. Correspondingly, in our western blotting studies, RM1 and ET-18-OMe treatment increased the activity of FAK at tyrosine residue 397 (FAK Tyr397), the major autophosphorylation site [Parsons, 2003], in MCF-7/AZ cells and this also within minutes of treatment, while chemical levels of FAK remained unchanged (Figure 8). Similar treatment of MCF-7/6 cells did not change the activation of FAK Tyr397.
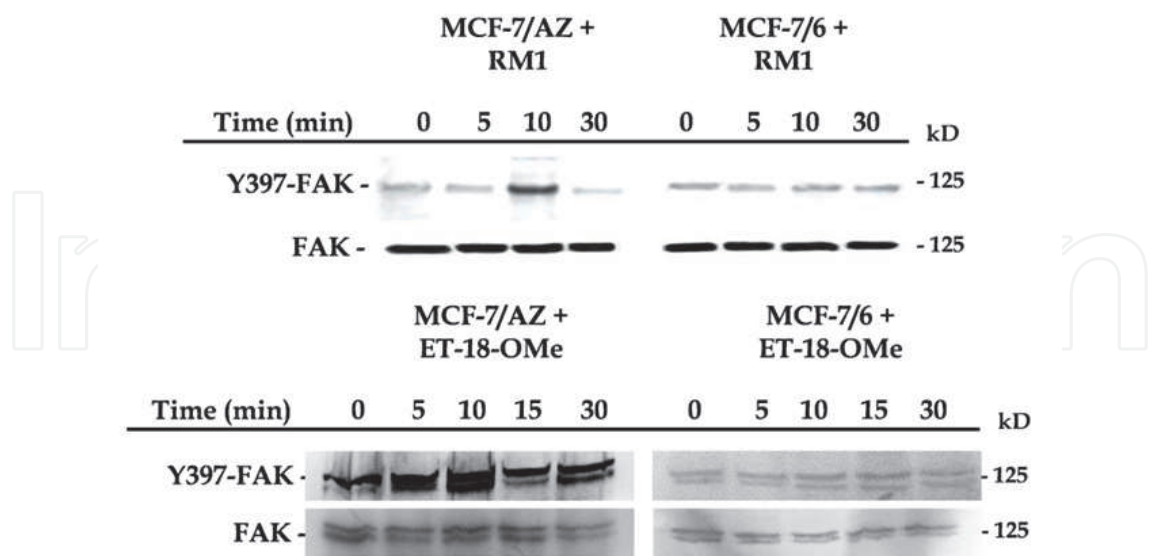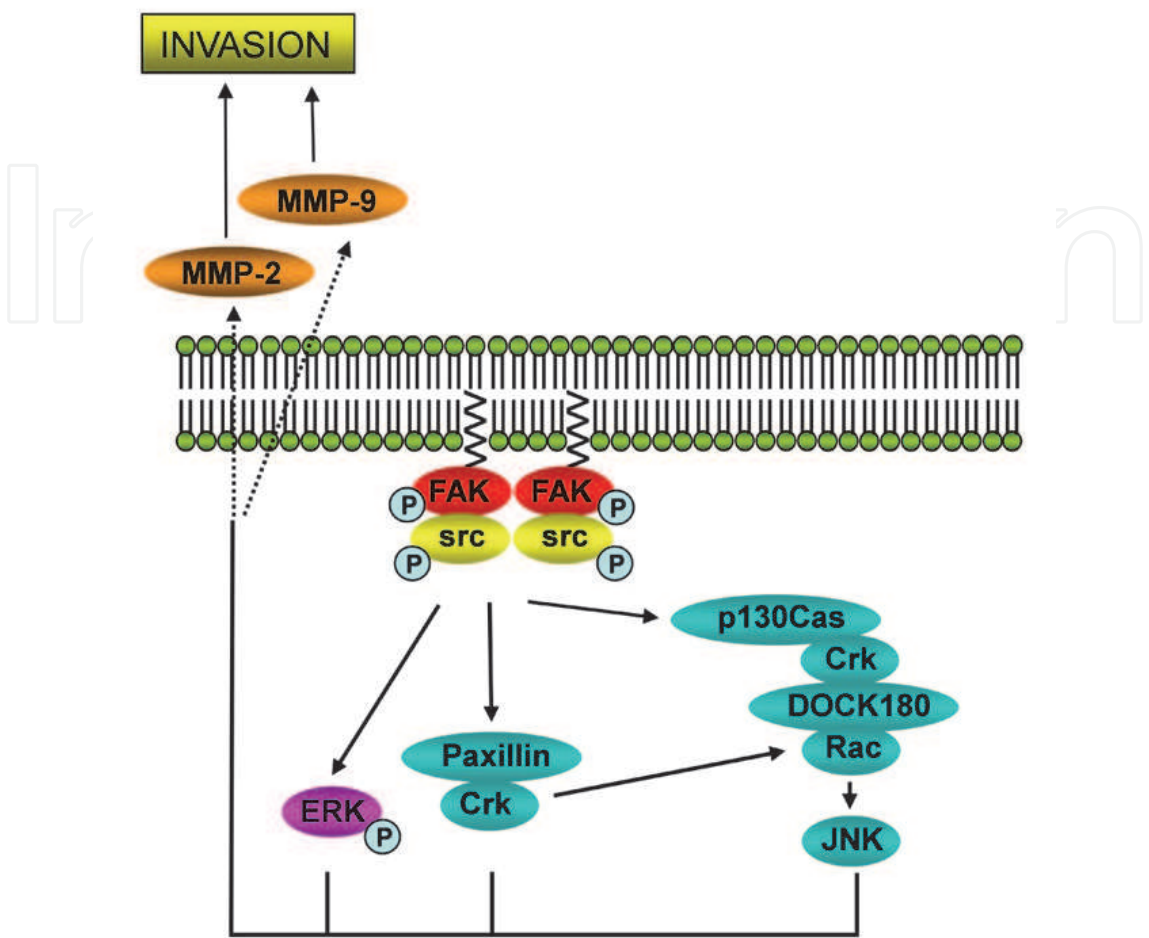
Fig. 8. Expression levels of phosphorylated FAK (Tyr397) upon RM1 and ET-18- OMe treatment in MCF-7/AZ and MCF-7/6 cells. Cells of 70% confluency were treated for indicated times, and lysed. Cell lysates, containing 30 µg of proteins, were analysed by SDS-PAGE (7.5% gels) and immunoblotted with antibody against FAK (Tyr397). The membrane was stripped and reblotted with anti-FAK, for FAK total expression levels and equal loading.

FAK plays an important role in relaying signals to intracellular targets, such as src, generated by cellular adhesion molecules and other cell surface molecules when they interact with the surrounding extracellular matrix [Parsons, 2003; Mitra et al., 2005]. The activation of FAK results in increased phosphorylation at Tyr397, the major autophosphorylation site, and subsequently in the recruitment and binding of src through its SH2 domain and further stabilization of the src-FAK interaction by src's SH3 domain [Thomas et al., 1998]. The formation of this transient bipartite kinase complex disrupts an inhibitory intramolecular interaction, resulting in increased activity of src [Schaller et al., 1994; Xing et al., 1994]. The specific organization plays a crucial role in src-dependent and mediated phosphorylation of other tyrosine residues on FAK within the kinase domain activation loop (Tyr576 and Tyr577) and at the C-terminal domain residues, Tyr861 and Tyr925 [Calalb et al., 1995; Calalb et al., 1996; Schlaepfer et al., 1996]. Interesting is that the activated FAK (Tyr397)-src signalling complex allows the activation of multiple different downstream pathways depending on which specific tyrosine residue on FAK is activated [Brunton et al., 2005]. As shown in scheme 1, the activated FAK-src complex can result in the activation of the mitogen-activated protein kinase (MAPK)-cascade through FAK Tyr925 [Schlaepfer et al. 1998], p130Cas via FAK Tyr861 [Lim et al., 2004] or paxillin by FAK Tyr576 [Calalb et al., 1995]. These three signalling pathways downstream of the FAK-src signalling complex, all lead to increased release of matrix metalloproteinase-2 (MMP-2) and MMP-9, the major proteinases responsible for the degradation of collagen type I [Kurata et al., 2000; Liu et al., 2000; Hsia et al., 2003; Brabek et al., 2004; Brabek et al., 2005; Bjorklund & Koivunen, 2005].

Scheme 1. Possible pathways downstream of the transient FAK-src complex leading to increased expression of MMPs, facilitating invasion into collagen type I layer.

Our results support that ET-18-OMe treatment results in the formation of a temporary FAK-src complex through FAK (Tyr397) activation and the subsequent src-dependent phosphorylation of FAK on Tyr925, since pretreatment of MCF-7/AZ cells with PP1, blocked the activation of src (Figure 7) and FAK Tyr925, while the activation of FAK at Tyr397 was only partially reduced (Figure 9). These results also point out that ET-18-OMe-mediated activation and autophosphorylation of FAK at Tyr397 is upstream and required for the activation of src and that src activity is responsible for the activation of the additional tyrosine residue 925 of FAK but not of FAK Tyr576 and 861 (Figure 9). Furthermore, we provide evidence linking FAK Tyr925 phosphorylation to the activation of the MAPK-pathway, since treatment of MCF-7/AZ cells with ET-18-OMe resulted in the downstream activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) and PP1 blocked the enhanced activation. In addition, we demonstrated that p130Cas and paxillin, which are known substrates of src associated with phosphorylation of FAK on Tyr861 and 576 respectively [Lim et al., 2004; Calalb et al., 1995], are not involved in the ET-18-OMe-induced effect and confirmed the lack of phosphorylation at FAK Tyr861 and 576 (Figure 10).
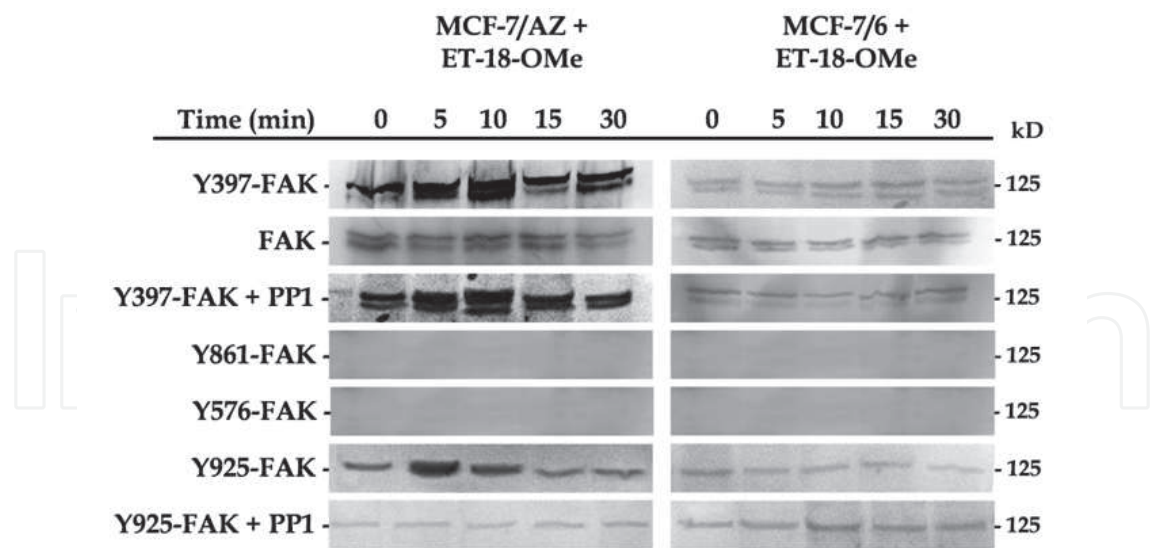
Fig. 9. Expression levels of FAK (Tyr397, Tyr925) upon ET-18-OMe and ET-18-OMe + PP1 treatment in MCF-7/AZ and MCF-7/6 cells. Cells of 70% confluency were treated for indicated times, and lysed. Cell lysates, containing 30 μg of proteins, were analysed by SDS-PAGE (7.5% gels) and immunoblotted with antibodies against FAK (Tyr397, Tyr576, Tyr861 and Tyr925). The membranes were stripped and reblotted with anti-FAK, for FAK total expression levels and equal loading.
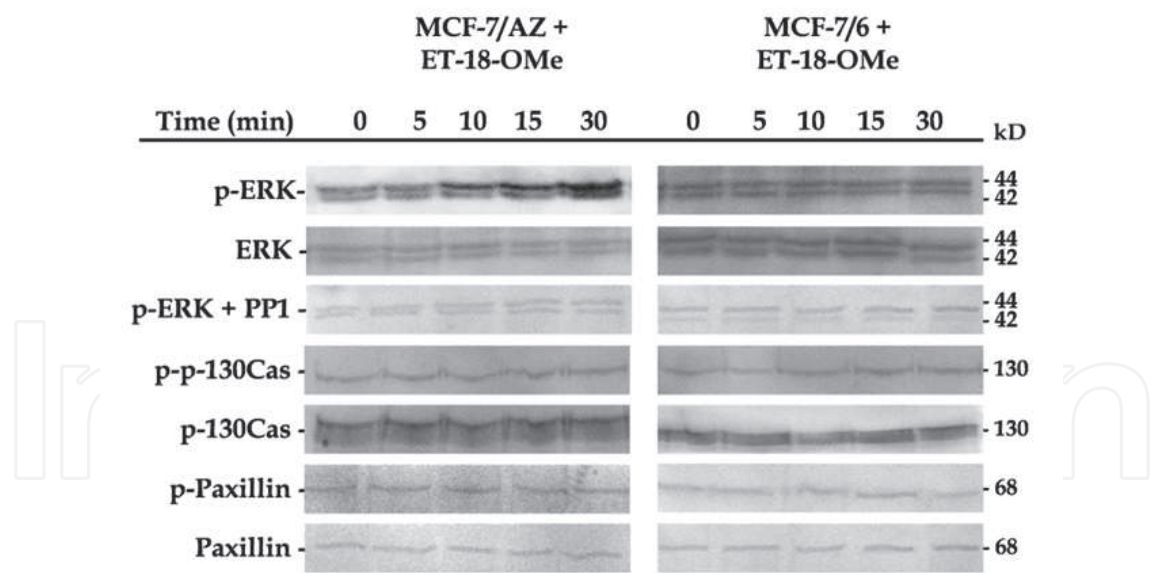

Fig. 10. Expression levels of ERK (Thr202/Tyr204) upon ET-18-OMe and ET-18-OMe + PP1 treatment in MCF-7/AZ and MCF-7/6 cells. Cells of 70% confluency were treated for indicated times, and lysed. Cell lysates, containing 30 μg of proteins, were analysed by SDS-PAGE (7.5% gels) and immunoblotted with antibodies against ERK (Thr202/Tyr204), p130Cas (Tyr410) and paxillin (Ser178). The membranes were stripped and reblotted with anti-ERK, anti-p130Cas and paxillin for total ERK, p130Cas and paxillin expression levels and equal loading.

5. MSGb5 activates the FAK-src signalling complex

Tumour cell invasion includes alterations of expression levels of integrin receptors. Integrin receptors are important upstream regulators of intracellular and downstream signalling events associated with cancer cell invasion. This family of heterodimeric α/β subunit receptors, expressed on every cell type, is capable of interacting with specific ligands in the surrounding extracellular matrix. Binding to these components results in clustering of integrin receptors in the plasmamembrane and recruitment and association of signalling proteins with integrin cytoplasmic domains to initiate downstream signalling events [Schwartz, 2001]. Since src and FAK are recognized as two critical mediators of integrin signalling, we addressed the question whether particular integrins were implicated in ET-18-OMe-induced activation of FAK and src and invasiveness of MCF-7/AZ cells. The integrin receptors α1β1 and α2β1 are the major receptors binding to collagen type I in the extracellular matrix [Gullberg et al., 1992]. Given that RM1 and ET-18-OMe induce invasiveness of MCF-7/AZ cells in collagen type I layer, we examined the expression levels of integrin subunits α1, β1 and α2 upon treatment with ET-18-OMe by Western blotting. Expression of the α1 and β1 subunits were found decreased over time in MCF-7/AZ cells, and no changes of either integrin subunit was observed in the variant cell line (Figure 11), whereas the integrin α2 subunit was not detectable in both cell lines (data not shown).
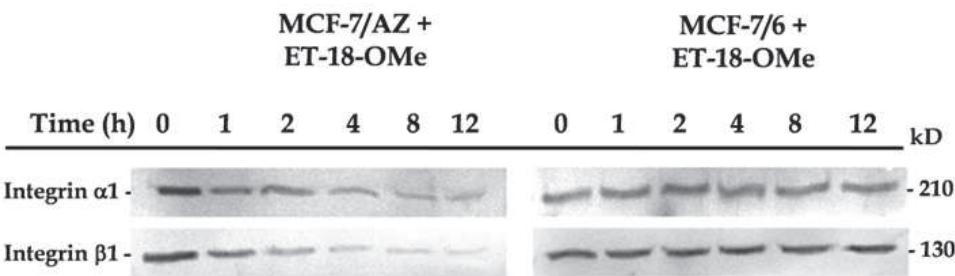

Fig. 11. Expression of integrin subunits α1 and β1 in MCF-7/AZ and MCF-7/6 cells upon ET-18-OMe treatment. Cells of 70% confluency were treated for indicated times, and lysed. Cell lysates, containing 30 μg of proteins, were analysed by SDS-PAGE (7.5% gels) and immunoblotted with antibodies against the integrin α1 and β1 subunit.

Since activation of integrin-dependent signalling occurs via clustering of integrin receptors and association with FAK signalling elements to the cytoplasmic tails of the receptor [Miyamoto et al., 1995], organizational patterns of the α1 and β1 subunits and co-clustering with signalling molecules FAK and src, upon ET-18-OMe treatment were examined by fluorescence microscopy and co-immunoprecipitation experiments. The microscopy data revealed that ET-18-OMe did not change the organization of the respective integrin subunits and the fact that signalling molecules FAK and src could not be detected in the immunoprecipitates of either integrin subunits confirmed that integrin receptors α1β1 and α2β1 were not of crucial importance in the activation of downstream signalling events in MCF-7/AZ cells mediated by ET-18-OMe or RM1 (data not shown). This was in sharp contrast with data reported by other investigators [Schaller et al., 1995]. However, the recognition that glycosphingolipids may influence cellular phenotype by clustering and assembly with signal transducing molecules, let us to explore the possible involvement of the glycosphingolipid MSGb5 in initiating the signalling events in MCF-7/AZ cells upon

RM1 or ET-18-OMe treatment. This idea was supported by two of our observations namely that RM1 and ET-18-OMe treatment resulted in clustering of MSGb5 in MCF-7/AZ cells and that similar treatment profoundly affected the cellular activities associated with the migratory and invasive capacity of MCF-7/AZ cells. A close connection between MSGb5 and signalling molecules was shown by co-immunoprecipitation experiments, in which aliquots of cell lysates were immunoprecipitated by incubation with anti-MSGb5 antibody and captured with protein G-Sepharose beads. The signalling molecules, FAK and src as well as their activated forms, src (Tyr416) and FAK (Tyr397), were detected in MSGb5 immunoprecipitates prepared from MCF-7/AZ cells treated with ET-18-OMe for indicated time points, and the obtained results were in line with the earlier performed kinase experiments. In MCF-7/6 cells, FAK and src were found associated with MSGb5, and as expected no kinase activity was observed upon ET-18-OMe treatment (Figure 12). The involvement of other glycosphingolipids could be excluded since no clustering and no association between FAK and src with Gb3, Gb5 or GM2 (data not shown) could be found in MCF-7/AZ cells. Equal levels of MSGb5 were detected in the immunoprecipitates of both cell lines.
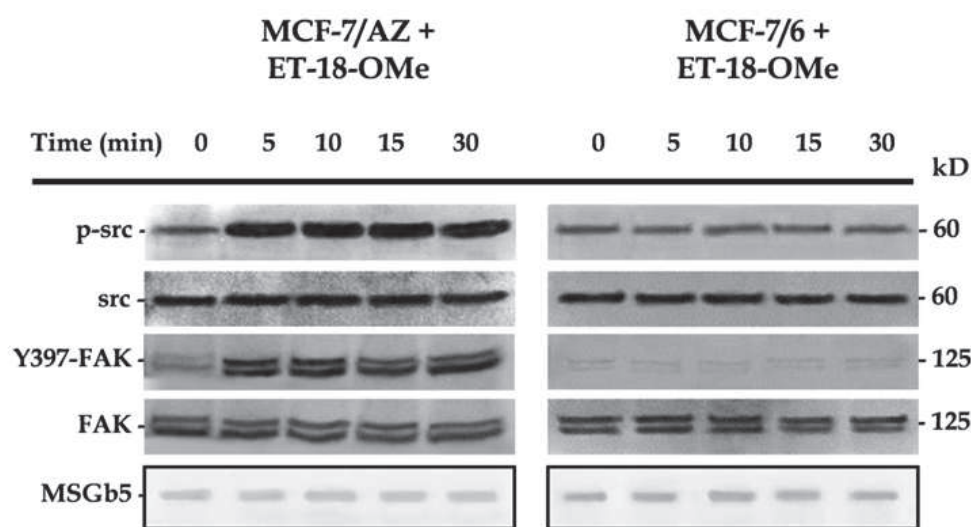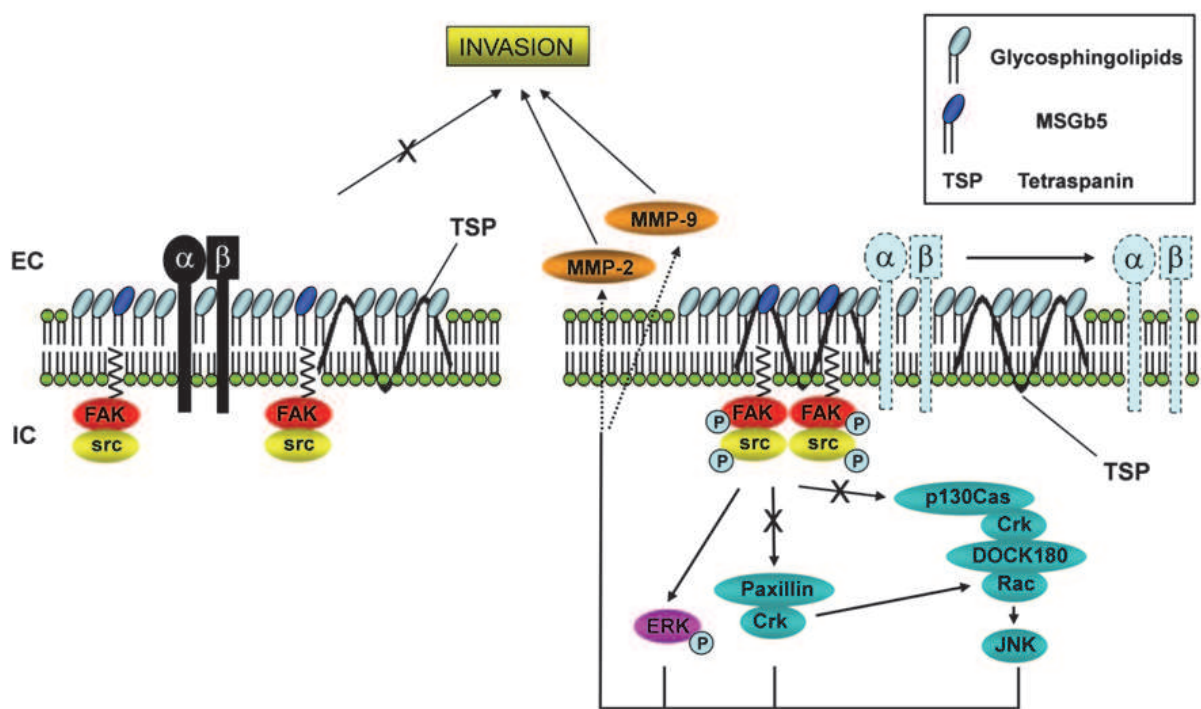

Fig. 12. MSGb5 associates with src (Tyr416) and FAK (Tyr397) in MCF-7/AZ cells after ET-18-OMe treatment. MSGb5 was immunoprecipitated from whole cell lysates with mouse IgG$_3$ anti-MSGb5 antibody. Aliquots of immunoprecipitates were electrophoresed, transferred and immunoblotted, with anti-src (Tyr416), anti-src, anti-FAK (Tyr397) and anti-FAK. MSGb5 content in the different immunoprecipitates was determined by TLC.

6. Concept of "Glycosynapse"

Glycosphingolipids are highly expressed during defined stages of development and after oncogenic transformation and are referred to as stage-specific embryonic antigen and tumour-associated antigens respectively [Hakomori, 1998]. Most studies, however, use the presence of GSLs as markers of low-density membrane fractions and are neither focused on their structural variety nor their possible functional roles. In this chapter, we present data supporting a functional role for MSGb5 in migration and invasion of MCF-7/AZ cells upon stimulation with RM1 or treatment with ET-18-OMe, resulting in MSGb5 clustering and

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activation of associated signalling molecules. These observations can be placed in the concept of the 'glycosynapse' [Hakomori, 2002]. This term defines glycosylation-dependent adhesion and signalling, mediated via glycosylepitopes of GSLs in microenvironments where tumour cells interface with other tumour cells, host cells or the surrounding matrix, in analogy to the 'immune synapse' which on its turn controls functional adhesion and signalling between immunocytes [Ilangumaran et al., 2000]. In addition to glycosylation-dependent adhesion of GSLs between interfacing glycosynapses, conversion of phenotypes is highly controlled by the presence, interactions and organization of other crucial molecules in the glycosynapse, such as growth factor receptors, integrin receptors, tetraspanins, mucins and gangliosides [Hakomori & Handa, 2002]. Furthermore, this new concept has been extended to phenotypic conversion induced through the deletion or addition of a single component, resulting in a disorganized glycosynapse framework and initiating altered signalling events [Mitsuzuka et al., 2005]. We can relate our observations to the latter revised glycosynapse concept and more specifically to the formation of a disorganized glycosynapse framework. We conclude that phenotypic conversion from non-invasive to invasive MCF-7/AZ breast cancer cells is induced by: (i) an aberrant MSGb5 pattern; (ii) loss of integrin receptor subunits α1 and β1; and (iii) high tetraspanin CD9 expression levels [Steelant et al., 2002], all of which are responsible for the formation of disorganized glycosynapse framework interfaces, thereby inducing activation of FAK, src and downstream ERK, with consequent enhanced secretion and activity of MMP-2 and MMP-9, and thus leading to invasion.

Scheme 2. Cancer cell invasion revised. In non-invasive MCF-7/AZ cells integrin subunits α1 and β1 form a stable complex with tetraspanin CD9 and GLSs, associated with non-active signalling molecules, FAK and src. In invasive MCF-7/AZ cells clustering of MSGb5 and loss of integrin subunits α1 and β1 disorganize the glycosynapse framework, resulting in activation of downstream signalling to invasion. EC, extracellular; IC, intracellular; TSP, tetraspanin.

7. Conclusion

In conclusion, our studies are an extension of previous work on the glycosynapse [Hakomori, 2002], re-formulating the classic concept of integrin-dependent invasion of tumour cells and providing evidence that phenotypic conversion can be explained by differences in composition and organization of crucial molecules in the glycosynapse. At present, only a few studies have appeared that focus, in particular, on GM3 [Mitsuzuka et al., 2005, Toledo et al., 2005]. The present study reveals a novel insight into the composition and organization of the glycosynapses in MCF-7/AZ breast cancer cells, which explain phenotypic changes. Further studies along this line are necessary to understand the complex interplay of distinct molecules in invasion, as well as other basic cellular mechanisms, and their implications on disease processes, which will be expected to lead to novel therapeutic approaches.

8. Acknowledgements

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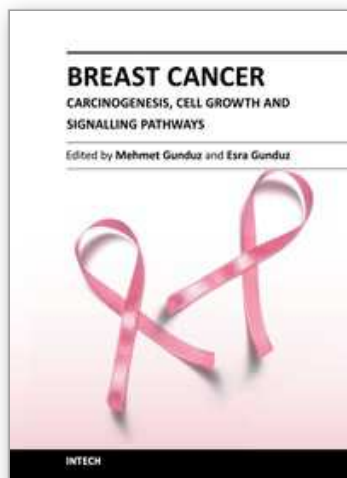
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Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways

Edited by Prof. Mehmet Gunduz

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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