Upregulation of the cytoskeletal-associated protein Moesin in the neointima of coronary arteries after balloon angioplasty: a new marker of smooth muscle cell migration?

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Abstract

Migrating cells like coronary smooth muscle cells in restenosis change their cell shape and form cellular protrusions called filopodia. A prerequisite for filopodia formation is the rearrangement of the actin cytoskeleton. An essential role of the 78-kDa protein Moesin is described for Rho- and Rac-dependent assembly of actin filaments. In vivo Moesin is not observed in mature smooth muscle cells. The objective of this study was to demonstrate that Moesin is upregulated in migrating coronary smooth muscle cells during restenosis development. In vivo expression of Moesin was upregulated in neointimal coronary smooth muscle cells of dilated porcine coronary arteries compared to the undilated left circumflex coronary artery of the same swine. Concordant to these results Moesin expression was upregulated in migrating and invading human arterial smooth muscle cells in vitro analyzed by FACS, Western blotting and RT-PCR. In addition, the invasive potential of Moesin-positive Mel Im cells transfected with Moesin sense DNA increased by 28% as compared to mock-transfected control, whereas antisense transfected cells had a decreased invasive potential of 32%. Transfection of Moesin-negative HepG2 with Moesin sense cDNA increased the invasive potential by 43%. Finally, transfection of human arterial smooth muscle cells with Moesin sense cDNA caused an increased invasive potential of 30%. Transfection of hSMCs with antisense cDNA decreased the invasive potential by 37% in comparison to mock-transfected control. These results demonstrate for the first time an upregulation of Moesin expression in coronary smooth muscle cells of the neointima after arterial injury. The increased migrative and invasive potential of cells transfected with Moesin confirmed the functional role of Moesin in cell migration. This indicates an important role of Moesin during restenosis development.

Keywords: Restenosis; Angioplasty; Smooth muscle; Extracellular matrix; Cell culture/isolation

This article is referred to in the Editorial by P.A. Doevendans and G. van Eys (pages 499–502) in this issue.

1. Introduction

Moesin, Ezrin and Radixin are part of a closely related gene family, called ERM [1]. Although identified and characterized independently of each other, these proteins share amino acid sequence homology of 70–80% [2]. ERM proteins consist of eight highly conserved regions denoted A–H. The NH2-terminal domain is characterized by a 320-residue globular secondary structure and functions as binding domain for membrane proteins. The 50-residue highly charged C-terminal domain contains a high affinity actin binding site in the region H [3]. Due to the co-existence of a membrane- and an actin-binding domain
it was suggested that ERM-proteins serve as cross-linker between the plasma membrane and actin-filaments [4–9]. The cross-linking activity is controlled by the Rho-dependent signaling pathway through binding to Rho-GDP dissociation inhibitor and / or Rho-dependent phosphorylation [10–14].

The proteins of the ERM family are required for the formation of membrane microextensions like filopodia and lamellipodia which are involved in cell movement and cell attachment. Yet, it has not finally been clarified whether the ERM proteins are functionally redundant, but recent data from Moesin knock-out mice revealed no pathological abnormalities of the Moesin-deficient animals [15].

Since its introduction in 1977, percutaneous coronary transluminal angioplasty (PTCA) has become the most common interventional treatment of coronary artery disease with more than 1.5 million procedures per year worldwide. Despite continuing improvement in technical equipment and medical therapy restenosis is still the major limitation of the procedure with a restenosis rate of 30–40% within the first 6 months after PTCA. Coronary stenting has been proposed to reduce the rate of restenosis but, nevertheless, in-stent restenosis occurs currently in approximately 30% within 6 months in an average patient population. Data from porcine restenosis models suggest that restenosis after vascular interventions is caused by a combination of early thrombus formation, migration and proliferation of coronary smooth muscle cells (SMC) and elastic remodeling of the arterial wall [16]. Furthermore, the arterial response seems to be determined by atherosclerotic plaque morphology and the extent of vessel injury.

Cell migration, as a key event in the process of restenosis development after PTCA and particularly after stent implantation, is a complex process which is characterized through dynamic reorganization of the actin cytoskeleton consisting of protrusion formation at the leading edge of the cell and retraction at the rear. In addition, secretion of new membrane components, controlled cell–matrix interaction and changes in gene transcription play an important role [17,18]. Polymerized actin in migrating cells is organized in three major subtypes: stress fibers, lamellipodia and filopodia. These contractile structures are thought to generate driving forces forwarding movement of the cell [19]. The molecular control of actin filament assembly is likely controlled by the Rho family of small GTPases, particularly Rho, Rac and Cdc42 [20]. For Cdc42 the regulation of filopodia formation was described during this process [21,22].

The goal of this study was to examine whether the cross-linking protein Moesin is differentially expressed in migrating coronary SMCs during restenosis development. Moesin expression was analysed in tissue sections from a porcine restenosis model in vivo. Differential Moesin expression was further examined in human arterial smooth muscle cells (hASMCs) in vitro. The influence of Moesin on the migratory and invasive potential of hASMCs, Moesin-negative HepG2 cells and Moesin-positive Mel-Im cells was tested with a modified Boyden chamber system.

2. Methods

2.1. Animal model

A porcine model of coronary arterial injury was used. The animal study was approved by the Institutional Animal Care and Use Committee and conformed to the tenets of the American Heart Association on research animal use. Twelve Domestic Troll pigs (25–40 kg) on a normal chow diet were medicated with aspirin (500 mg) the evening before vascular intervention. For general anesthesia pigs were sedated with intramuscular ketamine (20 mg/kg) and azaperone (5 mg/kg). After endotracheal intubation, they were ventilated with 1 l/min oxygen, 3 l/min nitrous oxide and isoflurane (1%) to maintain anesthesia throughout the experiment. An 8F sheath was placed in the right carotid artery and an intraarterial bolus of heparin (7500 U) was administered. The left main coronary artery was engaged with a left Amplatz 3.0 guiding catheter. The left anterior descending (LAD) artery was injured with an oversized balloon (3.0–3.5 mm) which was inflated for 30 s (10–12 atm), whereas the noninstrumented left circumflex artery served as undilated control for the Moesin expression pattern. Animals were maintained on a normal laboratory diet until sacrifice. Always three pigs were sacrificed after 1, 7, 14 and 28 days, respectively, with a lethal dose of barbiturate.

2.2. Tissue preparation

Immediately after euthanasia the hearts were harvested and the epicardial coronary arteries were removed. The arteries were sectioned into 3–5-mm segments, tissue sections were performed as described previously [23]. The arteries from at least three animals were obtained at each time point.

2.3. Cell culture

Human mammary arteries were obtained from patients during coronary artery bypass operation after obtaining written informed consent. HaSMCs were isolated and cultured as described previously [24,25].

All cells were maintained in Dulbecco's modified Eagle's medium (DMEM/Nut.Mix F-12 with Glutamax, Gibco-BRL) supplemented with penicillin (100 U/ml), streptomycin (10 μg/ml) (both Sigma–Aldrich) and 10% fetal calf serum (Gibco-BRL) and split 1:2 at confluence. Cells were detached by incubation with 0.05% trypsin, 0.04% EDTA (Sigma–Aldrich) in PBS for 5 min at 37 °C. They were identified as vascular haSMCs through their characteristic hill-and-valley growth pattern. Immunostain-
ing confirmed the presence of smooth muscle actin (SMA) as positive marker for SMCs, von Willebrand factor VIII served as negative marker and confirmed the absence of endothelial cells. By this method cultures of >95% haSMCs purity were routinely obtained. The studies were performed with cells at passages 3–4. Cells were explanted from 20 different donors.

Briefly, haSMCs with different migratory and invasive behavior were used. One phenotype resembling the non-proliferating, quiescent haSMCs in the medial wall of undilated vessels was established. For this reason, a growth arrest was induced in haSMCs. Quiescent haSMC were obtained by plating 2×10^5 cells/ml in a 75-cm² culture plastic flask. Cells were grown to confluence for at least 5 days prior to the assay with medium change containing DMEM (10% FCS).

On the other hand, haSMCs mimicking the proliferating, migrating and invasive phenotype during restenosis development were gained by splitting subconfluent haSMCs 2 days prior to the assay. For proliferation haSMCs 2×10^5 cells/ml were plated in a 75-cm² flask, maintained in DMEM (10%FCS) and split 1:2 at subconfluency. Proliferating haSMCs generated by this method, were actively able to invade a matrix barrier in a Boyden chamber system, whereas the quiescent phenotype failed to invade the matrix barrier. This model is comparable to characteristic changes of haSMCs from the quiescent to the proliferating phenotype during restenosis development. All haSMCs were tested in the Boyden chamber and only cells with a clear distinguishable invasive or non-invasive phenotype were evaluated in further assays.

The following cell lines were used: the melanoma cell line Mel Im [26] and the hepatocarcinoma cell line HepG2 (ATCC HB 8065). The cells were split 1:4 at confluence. They were detached by incubation with 0.05% trypsin, 0.04% EDTA in PBS for 5 min at 37°C

2.4. Antibodies

To visualize Moesin, a Moesin polyclonal antibody was achieved by immunization of chicken with isolated Moesin from human placenta. This antibody showed a high specificity for Moesin, crossreactivity with Ezrin and Radixin could be excluded by Western blot analysis (data not shown). It was used for detection of Moesin by Western blotting. For staining of tissue sections, FACs analysis and co-immunoprecipitation, W. Lankes (Max-Delbrück Institute for Molecular Medicine, Berlin) provided the affinity-purified antibody 90-7m, which was previously shown to react specifically with Moesin [8]. A mouse monoclonal antibody against anti-SMA was purchased from Dako (Glostrup, Denmark), a monoclonal antibody against β-actin from Sigma–Aldrich and antibodies against ICAM 1, 2 and CD44 from Santa Cruz Biotechnology (Santa Cruz, USA). Anti-focal adhesion kinase (FAK) was purchased from Chemicon (Hofheim, Germany).

2.5. Immunofluorescence

Immunostaining was performed with 5-µm tissue sections as described previously [23]. Anti-Moesin antibody 90-7m (1:500) and anti-SMA (1:400) were used as primary antibodies, for detection TRITC (1:1000) and FITC (1:1000) labeled secondary antibody were used. Negative controls were carried out with nonimmune rabbit IgG. Slides were viewed in a Leitz microscope (Leitz, Wetzlar, Germany) equipped for immunofluorescence.

2.6. FACs analysis

FACS analysis was performed as described previously using the Moesin antibody 90-7m [24].

2.7. Chemotaxis and invasion assays

For chemotaxis experiments, Boyden-type blind well chambers were used (Costar, Cambridge, USA). Polycarbonate filters (13-mm diameter, 8-µm pore size; Nucleopore, Pleasanton, USA) were coated with gelatin (5 mg/ml) as described previously [24]. Invasion of haSMCs, Mel Im and HepG2 was measured in Boyden chambers as described previously [24]. Briefly, the filters were coated with a commercially available reconstituted basement membrane (Matrigel, Becton-Dickinson). The lower compartment was filled with conditioned medium as a chemoattractant. For haSMCs the conditioned medium was produced by proliferating HUVEC cells which were incubated for 24 h in serum-free medium. HUVEC conditioned medium had the same proliferation stimulus as PDGF AB (10 ng/ml) [24].

For Mel Im and HepG2 cells fibroblast-conditioned medium was obtained from skin biopsies. Briefly, fibroblasts were isolated from skin biopsies of healthy donors after obtaining written informed consent as described previously [27]. Epidermis and dermis were separated by dispase digestion. Fibroblasts in the dermis were isolated by trypsin digestion and cultivated in DMEM plus 10% FCS. Cells were used between passages 6 and 10 for the generation of fibroblast conditioned media. The medium was cleared from cell debris by centrifugation, stored at −20°C and used as chemoattractant without adding further supplements. Cells were harvested by trypsinization, resuspended in medium without FCS and placed in the upper compartment of the chambers. After incubation for 4 h at 37°C the filters were removed. The cells adhering to the lower surface were fixed, stained and counted. Each sample was assayed 6-fold.
2.8. Vector constructs and transient transfection

The coding region of the human Moesin gene was PCR amplified using the primers Moe for (5'-GAC GAA TTC ATG CCC AAA ACG ATC AGT) and Moe rev (5'-GAC GAA TTC AGA GGC TGG GTG CCC ATT AC). The cDNA was inserted into the vector pIRES-EGFP (Clontech, Palo Alto, USA) via EcoRI restriction sites both in sense and antisense direction.

For transfection, either 3×10⁵ cells were seeded into a T25 plate for functional assays or 5×10⁵ cells were seeded into each well of a six-well plate for mRNA and protein assays.

Lipofectamine plus (Gibco) was used for transfection of Mel Im and HepG2 cells. The cells were harvested 24 h after transfection and used in the functional assays for mRNA analysis by RT-PCR or for protein analysis by Western blotting or immunoprecipitation. For mock control empty pIRES-EGFP vector was used. The transfection efficiency of lipofectamine-transfected cell lines was 50%.

The amaxa Nucleofector™ technology was used for transfection of haSMCs. A total of 2×10⁴ to 5×10⁵ haSMCs was transfected with 5 µg cDNA according to the manufacturer’s protocol. This method allowed to reach a transfection efficacy of 50% (Amaca, Cologne, Germany).

Briefly, the cells were resuspended in 10 µl phosphate-buffered saline, pH 7.5 (PBS). The DNA and 90 µl amaxa’s Nucleofector™ solution (Amaca, Cologne, Germany) were added and the mixture was transferred into the electroporation cuvette (2 mm) (Invitrogen, Groningen, The Netherlands). Immediately after electroporation, the cells were suspended in 4.9 ml cell culture medium and transferred to T25 flasks. The cells were harvested 48 h after transfection and used for further assays [28].

2.9. Western blotting

Eight micrograms of radio immunoprecipitation (RIPA) cell lysate per lane was separated on a SDS–PAGE gradient gel (Novex) and subsequently blotted onto a PVDF membrane. After blocking for 1 h with 3% BSA–PBS the membrane was incubated for 2 h with the anti-Moesin-specific antibodies (1:500). The membrane was washed three times in PBS, incubated for 1 h with an alkaline phosphatase coupled secondary antibody (anti-chicken, 1:300) and then washed again. For detection nitro blue tetrazolium (NBT)–5-bromo-4-chloro-3-indolyl phosphate (BCIP) staining (Sigma) was used. Each sample was assayed in triplicate with three different pools of haSMCs from at least three to four donors, respectively.

2.10. Co-immunoprecipitation

After having adjusted the protein concentration of the haSMC lysates co-immunoprecipitations were carried out with 10 µg anti-Moesin antiserum coupled to protein A agarose beads following the manufacturer’s instruction (Roche, immunoprecipitation kit). Proteins bound to Agarose A were eluted by boiling in Laemmli’s buffer 3 min 95°C and were subjected to 10% SDS–PAGE.

2.11. Statistical analysis

For the chemotactic and invasion assays all results are expressed as mean±S.D. Statistical significance was evaluated using unpaired Student’s t-test for comparison between two means, or ANOVA followed by Dunnett’s post-hoc test for more than two means. A P value of <0.05 was considered to indicate statistical significance. Statistical analysis of FACS results was performed using Kolmogorow–Smirnov test.

3. Results

3.1. Analysis of Moesin expression in vitro

Differential Moesin expression was detected in quiescent, non-invasive and proliferating, invasive haSMCs in vitro using the model described above. By Western blot analysis, it was shown that Moesin was upregulated in invasive haSMCs (Fig. 1, lane 2) in comparison to non-invasive haSMCs (Fig. 1, lane 1), β-actin as internal standard was equally expressed in both phenotypes. An upregulation of Moesin in the invasive phenotype could also be confirmed by quantitative RT-PCR (data not shown). FACS analysis was performed to quantify Moesin

Fig. 1. Analysis of differential Moesin expression in invasive and non-invasive haSMCs. Western blot analysis of Moesin and β-actin expression in haSMC-lysate from invasive and non-invasive haSMCs. (1) Non-invasive haSMCs, (2) invasive haSMCs. Moesin was significantly upregulated in invasive haSMCs in comparison to non-invasive haSMCs. β-Actin as internal standard was equally expressed in both phenotypes.
expression on haSMCs and revealed a significant upregulation of Moesin in the invasive phenotype (90% in comparison to negative control, Fig. 2a) in comparison to the non-invasive phenotype (69% in comparison to negative control, Fig. 2b).

3.2. Analysis of Moesin expression in vivo

Tissue sections from the porcine restenosis model were double-stained with the Moesin-specific antibody 90-7m and anti-SMA. The undilated left circumflex artery always
served as comparison for the Moesin expression pattern. Nonimmune rabbit IgG was used as negative control. Tissue specimen were used from groups of three pigs, each after 1, 7, 14 and 28 days, respectively. In all dilated arteries, neointima formation was observable with a maximum between 14 and 28 days, whereas in non-dilated arteries no intimal proliferation was detectable. In dilated animals, after 14 days there was an upregulation of Moesin signals in the SMA-positive human coronary SMCs (hcSMCs) of the small neointima (Fig. 3a,b). After 28 days there was a clearly significant differential, upregulated Moesin expression of neointimal hcSMCs in comparison to the medial wall hcSMCs (Fig. 3c,d). The same sections stained with the nonimmune IgG showed only a modest unspecific background and no specific staining pattern (Fig. 3e). In the undilated control group without neointima formation there was only a weak Moesin expression which was equal in hcSMCs of the medial and intimal layer (Fig. 3f,g).

3.3. Moesin transfection of Mel Im cells and characterization of the migrative and invasive potential

To analyze the function of Moesin during cell migration, an expression vector encoding the full-length human Moesin sense and antisense cDNA downstream from the cytomegalovirus promoter was constructed. Transfection efficiency was controlled by fluorescence microscopy of EGFP-positive cells as well as by Western blotting of the transfected cells lysate and revealed an overall transfection efficiency of 50% for the liposome-mediated transfection of cell lines and for the electroporation of haSMCs.

Mel Im cells, which natively express Moesin, were transfected with sense and antisense cDNA constructs. The influence on the cells’ migrative and invasive potential was analyzed with the Boyden chamber model. Transfection with Moesin sense cDNA significantly increased Moesin expression (Fig. 4a, lane1) in comparison to mock transfected control (Fig. 4a, lane 2), whereas after transfection

Fig. 3. Analysis of differential Moesin expression in tissue sections from a porcine restenosis model. Tissue sections were double immunostained with antibodies against Moesin and SMA. The arrays always indicate the elastic internal lamina between the neointimal and medial wall. (a, TRITC-SMA; b, FITC-Moesin) Upregulated Moesin expression in SMA positive hcSMCs in the neointima of a dilated LAD 14 days after PTCA (20-fold magnification). (c, 20-fold magnification; d, 40-fold magnification) Significant Moesin upregulation in neointimal hcSMCs of a dilated LAD 28 days after PTCA. (e) A staining of the same dilated LAD with a nonimmune rabbit IgG as negative control. (f, TRITC-SMA; g, FITC-Moesin, 20-fold magnification) Equal but weak Moesin expression in medial and neointimal haSMCs of an undilated porcine left circumflex artery 28 days after PTCA which served as control for the expression pattern.
Fig. 4. Moesin expression (a) and invasive potential (b) of Moesin sense and antisense transient transfected Mel Im cells. Mel Im cells were transfected with a CMV promoter controlled Moesin sense and antisense expression vector. Moesin expression in the transfected cells was analyzed by Western blotting: (1) Mel Im sense; (2) mock control; (3) Mel Im antisense. The invasive potential of the transfected cells was tested in a Boyden chamber and revealed a significant increase of the invasive potential of Moesin-sense transfected cells, (+28±8%, P<0.05) in comparison to mock transfected control (2), whereas the invasive potential of the antisense transfected Mel Im cells (3) significantly decreased (−32±6%, P<0.05 for both). Each sample was assayed 6-fold.

with antisense cDNA Moesin expression was significantly decreased (Fig. 4a, lane 3). An analysis of transiently transfected Mel Im cells revealed a significant increase (+28±8%, P<0.05) of the invasive potential of Moesin sense cDNA transfected cells (Fig. 4b, 1) in comparison to the mock transfected control (Fig. 4b, 2). The invasive potential of Mel Im cells transfected with antisense Moesin cDNA (Fig. 4b, 3) showed a significant lower invasive potential in comparison to the mock transfected control (−32±6%, P<0.05).

3.4. Moesin transfection of Hep G2 cells and characterization of the migrative and invasive potential

To evaluate the influence of Moesin overexpression in cells which constitutively do not express significant amounts of Moesin (comparable to confluent, non-proliferating hSMCs in restenosis), Hep G2 cells were transfected with Moesin sense cDNA. Western blotting analysis confirmed a significant increase of Moesin expression in Moesin sense transfected cells (Fig. 5a, lane 1) in comparison to mock control (Fig. 5a, lane 2). The Hep G2 cells transfected with Moesin sense cDNA showed a significant increase of their invasive potential (+43±7%, P<0.05, Fig. 5b, 1) in comparison to mock transfected control (Fig. 5b, 2).

3.5. Moesin transfection of haSMCs and characterization of the migrative and invasive potential

Finally, haSMCs were transfected with Moesin sense and antisense cDNA and mock control. Western blotting confirmed increased and decreased Moesin expression in Moesin sense and antisense transfected cells (Fig. 6a, lanes 1 and 3) in comparison to mock control (Fig. 6a, lane 2). Significant up- and downregulation of Moesin could also be demonstrated by a more sensitive Moesin ELISA (data not shown).

Boyden chamber experiments revealed a significant increased invasive potential of the sense transfected haSMCs (+30±6%, P=0.03, Fig. 6b, 1) in comparison to mock-transfected control (Fig. 6b, 2), while antisense transfected cells had a decreased invasive potential of 37±2% (P=0.003, Fig. 6b, 3). These experiments confirm the important role of Moesin in the migrative and invasive process of haSMCs analogous to the results of Mel Im and HepG2 cells. Influences of Moesin on the proliferation potential of Mel Im and HepG2 cells as well as haSMCs
could be excluded (data not shown). There was no significant difference between the proliferation capacities of sense and antisense transfected cells compared to mock control.

3.6. Co-immunoprecipitation with Moesin antibody

If transformation of haSMCs from the quiescent to the migrative phenotype caused an upregulation of Moesin, detection of possible Moesin binding partners should clarify the function of Moesin during this process. Therefore co-immunoprecipitation of proliferating haSMCs with Moesin antibody 90-7m was performed. Silver staining of the co-immunoprecipitation fraction revealed, besides the Moesin band and the two bands of the denatured antibody at 30 and 60 kDa, two other unknown protein bands between 50 and 60 kDa (Fig. 7a). Immunoblotting of the same co-immunoprecipitation fraction with antibodies against known Moesin binding partners such as ICAM-1, ICAM-2 and CD44, as well as FAK could not detect these proteins in the co-immunoprecipitation fraction. Interestingly, also immunoblotting with anti-β-actin antibody did not detect actin in the co-immunoprecipitation fraction (Fig. 7b).

4. Discussion

Filopodia formation is a characteristic phenomenon of SMCs during proliferation and migration in restenosis development. A localized and transient actin-filament disassembly allows SMCs to extend filopodia towards platelet-derived growth factor (PDGF) enabled chemotaxis.
Subcellular Moesin concentration in filopodia and microspikes of several non-SMC cell types was formerly described [8]. Amieva and Furthmayr found that Moesin in cells with fibroblastic, epithelial and neuronal phenotype after attachment to their cell substrate was concentrated in filopodial extensions and in microspikes at the apical surface, in ruffles at the leading edge and in retraction fibers [8].

In this study, in vivo Moesin was only weakly expressed in quiescent coronary SMCs in undilated vessels of the restenosis model. In agreement with these observations, Amieva and Furthmayr also did not detect Moesin either in vascular and intestinal rat SMCs or in skeletal muscle cells and epithelial cells in vivo [8]. After onset of SMC invasion following balloon angioplasty stimulus, Moesin expression was upregulated in migrating neointimal SMCs in the dilated coronary arteries, whereas in the intimal and medial layer of non-dilated controls merely weak equal amounts of Moesin were detectable. Similar observations were made by Hugo et al. in tissue sections of experimental mesangio-proliferative nephritis where Moesin and Radixin was upregulated in the glomeruli of SMA-positive cells after day 5 until day 14 with a peak on day 7 [30]. Amieva and Furthmayr described that cultivation of Moesin-negative primary cells led to a de novo expression of Moesin [8]. It is well known that many primary cells pass a process of dedifferentiation under culture conditions, therefore Moesin upregulation during restenosis could be explained as a process of dedifferentiation of SMCs following the phenotypical change from the quiescent to the migrating and proliferating type.

Migration of SMCs in restenosis is stimulated by several chemokines, the most potent stimuli are PDGF and insulin-like growth factor (IGF) [31]. PDGF causes increased SMC proliferation via its receptor and directs SMC migration along the chemotactative stimulus with development of cell extensions and filopodia on the cells’ surface [32]. Furthermore, it was described that PDGF has a direct effect on the redistribution of the actin-cytoskeleton, probably through interaction with Cdc42.

To investigate whether the upregulation of Moesin causes a higher migrative and invasive potential, two different cell lines with constitutive high and low Moesin expression level and primary haSMCs were selected. In all cells Moesin was upregulated or blocked by sense and antisense strategies, respectively. In cell lines with an increased Moesin expression a significant increase of the invasive potential could be demonstrated, on the other hand the invasive potential was reduced in natively Moesin expressing cells transfected with Moesin antisense. Finally, the increased expression of Moesin in SMCs of the medial wall and neointima, which was demonstrated in vivo, could be functionally correlated with an increased and decreased migrative and invasive phenotype of primary haSMCs expressing more and less Moesin. These data demonstrate that increased cell migration is an effect of Moesin upregulation in addition to its function as a structural linker protein between the cell membrane and the actin-cytoskeleton. Increased haSMC proliferation through Moesin could be excluded in this study.

A possible explanation for the increase of the invasive potential is a cross talk between PDGF receptor-mediated downstream signal transduction pathways and Moesin regulation leading to a localized and transient Moesin activation and actin-filament disassembly enabling the cell to extend filopodia towards PDGF, thereby enabling chemotaxis to take place [29].

5. Conclusions

An upregulation of the cytoskeleton-associated protein Moesin during migration and invasion of haSMCs was demonstrated in vitro and in an in vivo porcine restenosis model. Functional relevance of Moesin upregulation in haSMC migration and invasion could be confirmed by transient transfection of different cell lines. An important role of Moesin was shown for cell SMC migration and invasion which is a crucial step of restenosis development after PTCA and stent implantation. This makes Moesin an interesting marker of SMC migration in restenosis development.

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