Smooth muscle cells on the move: the battle for actin

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See article by Blindt et al. [2] (pages 630–639) in this issue.

1. Introduction

Restenosis has been troubling patients and cardiologists, following percutaneous transluminal coronary angioplasty (PTCA), since the technique was introduced [1]. De-differentiation, migration and proliferation of smooth muscle cell (SMC) can cause a new obstructive lesion, at the site of the mechanical intravascular balloon intervention. Gradually our knowledge about SMC mobility is increasing. This mobility is the consequence of SMC (de)differentiation. The ability of these cells to switch from a contractile to a synthetic/proliferative phenotype has been a prime target of research in the cardiovascular field. Although the different phenotypes of SMC have been known for years, the underlying signalling, changes in gene expression and protein activation and the mechanism of cell mobility adaptation, still remain to be resolved. Also the keys to the maintenance of SMC (de)differentiation are currently unknown. Many clues indicate a crucial role for actin stabilisation and assembly regulating proteins.

2. Moesin expression and function

In this issue important work is reported on the role of moesin, one of the actin binding proteins on SMC mobility by Blindt et al. [2]. The moesin gene is positioned on the X-chromosome and part of a protein family indicated by ERM (ezrin, radixin and moesin). All three proteins are composed of a N-terminal FERM domain (F: four point one protein), a central \( \alpha \)-domain and a C-terminal tail domain. Inactivated moesin is folded such that the FERM domain bites and holds the tail. Upon phosphorylation of moesin the protein unfolds, whereafter the FERM domain binds to specific sites in the membrane (like for instance CD44, CD43, ICAM-1, ICAM-2 and \( \lambda \)-selectin in lymphocytes), while the tail interacts with F-actin [3,4]. The activated protein therefore connects F-actin to the plasma membrane. Blindt et al. [2] showed the increased expression of moesin following PTCA in pigs. Furthermore, in cell culture experiments they showed increased invasive potential in human SMC. Mel Im (melenoma metastatic cell line) and HepG2 cells upon moesin overexpression. The migration potential is reduced by blocking moesin in Mel Im cells, using antisense molecules [2]. In this paper, there is no information provided on the phosphorylation state of moesin, neither are we informed about the behaviour of ezrin and radixin. The marked effects of interventions on moesin expression levels seem surprising since in moesin-deficient mice no phenotype could be detected. This is probably due to redundancy of the ERM proteins although under physiologic conditions the various ERM proteins are expressed in different tissues [5]. In these mice the behaviour and function of platelets, fibroblasts and mast cells was measured, but no changes were observed. Even fibroblast migration was comparable in wild-type and moesin-deficient mice. The lack of phenotypical abnormalities could be related to the fact that these moesin-deficient mice (and isolated cells) were never challenged.

Several lines of evidence suggest that moesin could be the family member responding to cell stress. For instance, in lymphocytes the interaction of moesin with the membrane bound \( \lambda \)-selectin protein is mediated by PKC activation, whereas the interaction of ezrin with \( \lambda \)-selectin is not depending on cell activation [3]. In addition, moesin is not randomly distributed throughout the cortical cytoskeleton,
but concentrated in specialized microdomains. Moesin expression has been visualized in the intracellular core of microextensions known as filopodia, microvilli and retraction fibers. The distribution pattern follows closely the dynamic changes in cell shape in relation to spreading and moving of cells either spontaneously or in response to extracellular stimuli [6]. These characteristics would make moesin a likely candidate to contribute to or effectuate changes in phenotype of SMC following stress.

3. SMC signalling

The signalling events involved in SMC (de)differentiation are numerous including cytokines and growth factor-dependent pathways. Blocking SMC dedifferentiation and thus neointima formation after PTCA in healthy animal vessels was performed successfully with platelet-derived growth factor (PDGF) antibodies and also antisense approaches blocking the receptor. The results are indicative of important role for this growth factor [7,8]. In addition to interventions in the PDGF pathway, other growth factors were demonstrated to contribute, including TGF-β and IGF-1, both during angiogenesis and under pathologic circumstances. Unfortunately, many promising approaches were designed for and tested in healthy animal vessels, and the obtained results could not be (fully) reproduced in human atherosclerotic vessels. The only exception thus far is based on a total block of protein synthesis by the local application of the immunosupressor rapamycin, now called sirolimus [9,10].

For the recruitment and differentiation of SMC, but also during the dedifferentiation an important role for rho-GTPase and associated proteins like the rho-GDP dissociation inhibitor has been suggested. RhoA activates two groups of kinases protein kinase N and Rho kinases (ROK and p160). Blocking rhoA or rhoA kinase p160 interferes with the organisation of actin into stress fibers and coronary SMC differentiation during embryonic development as well as vascular remodeling during atherosclerosis [11,12]. The crucial role of rhoA for rat SMC-specific gene expression was elucidated by looking at the promoter activity of the SMC-specific promoters, SM22 and SM alpha-actin. Expression of a dominant positive rhoA increased promoter activity, while inhibition of stress fiber formation with the rho-kinase inhibitor Y-27632 significantly decreased the activity of SM22 and SM alpha-actin promoters. The direct interaction between rho-kinase activation and moesin has been demonstrated in COS7 cells [13]. In addition to interactions with moesin, rho-kinase activation results in myosin light chain phosphorylation and LIM kinase activation [14]. LIM kinase can inactivate cofillin, a protein that promotes actin disassembly. Yet another study in NIH3T3 and Hela cells indicates a dominant role for phosphatidyl-inositol 4-phosphate 5-kinase in ERM activation [15]. Obviously studies report variation in the most relevant pathway from rhoA to ERM activation. Both stabilising and destabilising proteins are downstream of rhoA activation, which is suggestive for a delicate balance between stabilising and destabilising proteins competing for actin binding. In addition, as suggested by Mack et al. [16] RhoA signaling may function as a convergence point for the different pathways involved. Further interactions between rhoA and actin have been suggested and links between the level of rhoA activity and G- and F-actin ratios have been demonstrated [17]. In fact, actin appears to be not only the target but also a modulator of activity of actin associated proteins such as moesin, smoothelin and tropomyosin (Fig. 1).

4. The battle for actin

F-actin assembly and disassembly is the driving process behind phenotypic changes of SMC. Assembly is needed to provide the cells with a contractile apparatus or prepare them for migration and proliferation. As indicated above, several signaling molecules target actin directly or indirectly. In addition, there is cross talk between actin and the associated proteins. Such proteins can be specific for the phenotype of the SMC or for the status of actin. At present, a number of such marker proteins are available to monitor SMC differentiation processes (Table 1) [18–21]. Calponin and SM22 are specific for SMC, but their binding to actin is controversial. Proteins as smoothelin, caldesmon and tropomyosin are expressed in contractile SMC and appear to play a role in contraction, but only the expression of smoothelin is limited to and therefore specific for the contractile phenotype of SMC [22,23]. The protein is not detected in any other cell type. Moesin and the other ERM proteins interact with actin in migrating undifferentiated SMC, but these proteins are also expressed in non-muscle cells. Proteins such as gelsolin, profilin, cofillin and actinin regulate the polymeric state and length of the actin filaments. All these proteins compete for a position on or near actin from which they can influence actin assembly and, therefore, the SMC differentiation state. An influence they exert by tilting the ratio of filamentous versus globular actin, which again interferes with signaling pathways. Understanding the complicated interplay between actin, actin-associated proteins and signaling proteins will provide new ways to approach pathological developments such as restenosis and but also atherosclerosis. As these processes are extremely important for oncology, it seems likely that insight will be gained faster in the molecular analysis of metastasis and cell division. The scientific interest in restenosis could be markedly reduced if the drug eluting stents hold their promise [10].
Fig. 1. The relation between SMC phenotype and the physical structure of actin is mediated by actin-interacting proteins. One group (gelsolin etc.) facilitates polymerization and break-down of actin. Another group (calponin etc.) stabilizes F-actin. Moesin and smoothelin belong to this group but stand apart from the other members since they specifically associate with the higher structures which the F-actin fibers are embedded in. Smoothelin and moesin can be classified as phenotype-specific. Rho-kinase is (one of) the central player in this scheme but only the most relevant relationships have been depicted.

5. Differentiated vs. undifferentiated SMC

The crucial role of RhoA in actin regulation determining cell mobility has been established. Moesin could play an important role as a RhoA downstream effector.

An important issue is the inflammatory response (TNF-α and TGF-β) evoked by the damage to the vascular wall either through the disease atherosclerosis or the treatment remains. After PTCA macrophages will infiltrate and factors such as NF-κB will be activated [24]. NF-κB, a ubiquitous transcription factor involved in acute phase responses, mediates changes in gene expression. This sequence of events results in dedifferentiation, migration towards the lumen of the blood vessels, and finally proliferation of SMC. Information on the interaction between inflammation dependent activators and the other signaling pathways described above remains an exciting field to explore.

One would expect that knowledge of expression patterns of genes, albeit similar (moesin and tropomodulin) or mutual exclusive (smoothelin vs. moesin), could provide a route to analyse the mechanism of SMC (de)differentiation by comparison of promoters or other regulatory elements. Identification of common enhancers or repressors could lead to the identification of factors governing SMC differentiation. Although promoters of actin and associating proteins contain many conserved cis-elements, no common building plan has been revealed thus far. Currently, it is also unclear whether one transcription factor or a whole cluster of transcription regulating genes is required for a phenotypic change. Despite all the difficulties, improved bioinformatics combined with promoter analysis appears to hold little promise for the elucidation of processes like SMC differentiation.

An alternative approach in search for the key players in SMC (de)differentiation appears to be proteomics, gene array or differential display analysis of growth factor and cytokine influences on SMC [25].

Although our knowledge of interacting genes is growing exponentially, functional studies remain the cornerstone of molecular studies. The suggested importance of molecules like moesin for SMC migration should be confirmed by genetic interventions in vivo.

References


