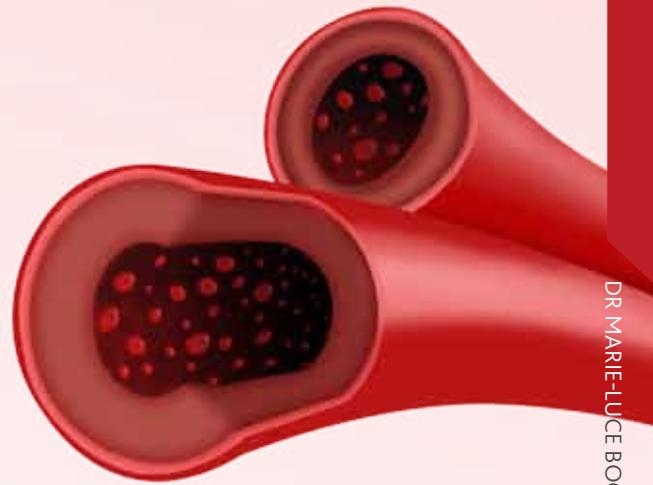


Bridging gaps in cardiovascular knowledge



Dr Marie-Luce Bochaton-Piallat discusses how close alliances have been critical to her successful contribution in the field of arterial smooth muscle heterogeneity research



Could you offer an outline of the circumstances from which this project arose?

I started my thesis at the Faculty of Medicine of the University of Geneva at the end of the 1980s. At that time the role of smooth muscle cells (SMCs) in atherosclerosis (the major cause of cardiovascular diseases) was gaining interest. SMCs are the main component of the arterial middle layer (media) where they are responsible for vessel contraction/relaxation. In response to noxious stimuli, SMCs migrate towards the innermost layer of the vessel (intima) where they accumulate and undergo phenotypic changes, ie. they acquire a synthetic phenotype accounting for extracellular matrix protein production. These processes are crucial for atherosclerotic plaque formation. Similar phenomena are the main cause of failure after revascularisation procedures (eg. restenosis following angioplasty or stent implantation).

It was not known, and indeed the question remains open, as to whether any SMCs in the media can undergo such phenotypic changes or whether a pre-existing SMC subpopulation is prone to accumulate within the intima. The second possibility is based on the observation that the atheromatous plaque has a monoclonal or oligoclonal origin. My first contribution to the field of SMC heterogeneity

was the identification of a distinct SMC population in the rat model with biological features compatible with their accumulation into the intima (ie. the existence of an atheroma-prone phenotype). Subsequently, we began to look for biomarkers of this SMC population.

How will your collaborative studies with clinicians help your research work?

Our experimental approach would have been much less successful without the close collaboration of the interventional cardiologists at the University Hospitals of Geneva, who have offered their skills to develop a porcine model of atherosclerosis. We believe that the relevance of our findings obtained with animal models awaits urgent confirmation in humans. For this purpose, we have developed a network of collaboration with expert cardiovascular pathologists, who make their unique vascular tissue collection available for testing the biomarkers identified as our quest goes along. More recently, thanks to a collaboration with vascular surgeons we could isolate human SMCs from fresh arterial specimens, an extremely challenging goal. The choice of working with large animals as experimental models, as well as with tissues derived from humans, implies that our research, albeit fundamental, has to appeal to a plurality of clinicians with very different backgrounds. It is therefore of the utmost importance to have them actively involved in our projects. Although it can sometimes be demanding to create a long-lasting bridge between basic scientists and clinicians (translational research), it is certainly a rewarding and fruitful experience for all these partners.

Have you been pursuing partnerships with industry that may help to bridge the gap between basic research and novel therapeutics?

We have already set up a number of collaborations with industries interested in using our porcine cell model to test their own products. Now, we plan to contact them

to develop tools to inhibit S100A4, a newly identified biomarker of the atheroma-prone phenotype, which can be a novel target to prevent atherosclerosis development and restenosis. For our latest work we have applied various interdisciplinary approaches by using techniques ranging from primary cell culture (human and porcine tissues), biochemistry (western blotting, immunoprecipitation, ELISA, 2D-gel electrophoresis), immunochemistry (immunofluorescence and immunohistochemical staining) to molecular biology (realtime PCR, silencing RNA and plasmid transfection). For these approaches we have developed a homemade S100A4 antibody. We have always started with an experimental approach in animals and then we test the relevance of our findings on human specimens.

With the second phase of your project now complete, what is the current status of your work on SMC heterogeneity and is there more that you wish to discover?

In the human model, we have shown that plaque macrophages play a pivotal role in the selective migration from the media of an atheroma-prone SMC population. Identification of factor(s) released by macrophages could be used to design new strategies aimed at blocking accumulation/migration of SMC in the plaque. Moreover, a thorough study of the different SMC populations could be instrumental in understanding atherosclerosis pathogenesis and in planning therapeutic strategies. In the pig, the unexpected finding that S100A4 in its extracellular form is a key modulator of SMC phenotypic changes gave rise to new directions in our research. We are currently setting up several collaborations with clinicians worldwide to test the potential of S100A4 plasma levels as a biomarker of cardiovascular diseases (including stroke, myocardial infarction, peripheral vascular diseases and aneurysmal degeneration). The quest for a drug inhibiting an extracellular and/or circulating S100A4 applicable to the human situation is very attractive for us and a pathway we are keen to follow.

Unlocking the secrets of arterial diseases

Researchers at the **University of Geneva** are developing tools that will influence the evolution of atherosclerotic and restenotic lesions, thereby providing new insights into how diseases of the human arteries advance

DURING ATHEROSCLEROSIS AND restenosis, smooth muscle cells (SMCs) migrate from the media toward the intima where they proliferate and undergo phenotypic changes. It has been proposed that SMCs from the arterial wall are phenotypically heterogeneous, and hence that a subset of medial SMCs is prone to accumulate into the intima. A group of scientists based at the Department of Pathology and Immunology within the Faculty of Medicine at the University of Geneva is working on identifying biomarkers typical of the atheroma-prone phenotype. Through this work, they hope to explore their role in the phenotypic modulation of intimal SMCs. Dr Marie-Luce Bochaton-Piallat is heading up a research project called 'Arterial smooth muscle cell heterogeneity: implications for atherosclerosis and restenosis'. She explains that her group has managed to isolate two distinct SMC populations, spindle-shaped (S) and rhomboid (R) SMCs, from the porcine coronary artery. "We have successfully identified S100A4, a Ca²⁺-binding protein, as being a marker of the R-SMC population *in vitro* and of intimal SMCs, both in pig and human coronary arteries".

This accomplishment has allowed them to further investigate whether S100A4 plays a role in SMCs accumulating in the intima and thus test the possibility that modulation of S100A4 expression induces phenotypic and biological changes in porcine coronary artery S- and R-SMC populations. Using silencing RNA and cDNA transfections in R- and S-SMCs, respectively, the study has shown that S100A4 is responsible for a switch from S- to R-phenotype associated with enhanced proliferation. The project is now set to continue into a new phase, investigating the role of S100A4 in the migratory and contractile SMC activities and exploring the role of extracellular S100A4 on SMC phenotypic changes using tools such as recombinant S100A4, specific peptides and neutralising S100A4 antibodies.

BUILDING AN SMC TOOLBOX

To date, investigating SMC heterogeneity in humans as opposed to animal models has been difficult due to the limited availability of

human arterial SMCs. As part of the project, a collaborative study has been set up with F Mascoli, from the Vascular Surgery Unit at the University Hospital of Ferrara, Italy, and one of his MD fellows, M Coen, who has collected fresh specimens from the human common carotid artery after endarterectomy. Bochaton-Piallat points out that thanks to this collaboration, they have been able to establish the culture conditions to reproducibly isolate distinct SMC



FIGURE 1. HUMAN ATHEROSCLEROTIC VESSEL (MASSON'S TRICHROME STAINING)

populations, one of them exhibiting biological and functional features compatible with an atheroma-prone phenotype. It is noteworthy, she believes, that atherosclerotic plaque macrophages are essential to promote the selective growth of the atheroma-prone SMC population. This breakthrough has allowed the group to overcome the challenges associated with the limited availability of human arterial SMCs.

THE APPROACH

Bochaton-Piallat is harnessing a wide range of techniques in order to investigate the mechanisms of atherosclerosis: "In the framework of this grant, we aim to compare the two populations by a proteomic approach in order to identify novel biomarkers of the atheroma-prone phenotype". The project will test whether the newly identified molecules play a role in SMC phenotypic changes and whether they are expressed in human lesions. In addition, her team aims to identify factors involved in the selective growth of the atheroma-prone SMC population. "We plan to use cytokine-antibody arrays that allow the profiling of cytokines released from plaque tissues," explains Bochaton-Piallat. "Once identified, the role of different factor(s) on SMC phenotypic modulation will be investigated." The ultimate goal of this research is to develop tools that affect how atherosclerotic and restenotic lesions progress. As one example, it is thought that S100A4 inhibition could result in a remodelling of the plaque leading to plaque stability (not prone to rupture and thrombosis that occlude the vessel) and/or plaque size decrease: "We are convinced that the identification of differentially expressed proteins by the atheroma-prone SMCs will almost certainly provide fresh understanding of atherosclerosis and restenosis".

THE BENEFIT OF PORCINE MODELS

In 1995 cellular retinol binding protein-1 was identified as a marker of atheroma-prone phenotype in the rat, but this was not relevant to human atherosclerotic lesions. For this reason, the research collaboration decided to move to the porcine model, it being physiologically and anatomically close to the human. Devices such as stent, balloon catheter or valve prosthesis are commonly tested in pigs before they are used in humans. Indeed, the project has identified S100A4 in pigs and they have further demonstrated that it is relevant to humans. Interestingly, molecules discovered in the rodent models with promising effects to inhibit atheromatous plaque formation were not efficient in the human model.

Bochaton-Piallat's studies have revealed different effects of S100A4 on the mechanisms of SMC accumulation in porcine and human arteries. They have identified S100A4, a Ca²⁺-binding protein, as being a marker of the atheroma-prone phenotype *in vitro* and of intimal SMC in experimentally-induced intimal thickening after stent implantation in the

The project will test whether the newly identified molecules play a role in SMC phenotypic changes and whether they are expressed in human lesions

porcine coronary artery. In the human coronary artery, S100A4 is strongly expressed in atherosclerotic and restenotic lesions. S100A4 is barely detectable in the media, both in pigs and humans. Therefore, S100A4 is a marker of the intimal SMCs in both species. Bochaton-Piallat is confident that her group's ongoing *in vitro* studies, whilst still under revision for publication, demonstrate a key role of S100A4 in porcine coronary artery SMC phenotypic changes. "In *vivo*, we plan to test the relevance of our finding in a model of experimentally-induced intimal thickening in the porcine coronary artery," she explains. Encouragingly, the project findings to date suggest that

the development of drug inhibiting S100A4 could be useful to influence the evolution of atherosclerotic and restenotic lesions.

IDENTIFYING S100A4 AS A BIOMARKER

This project has also involved the analysis of the expression of S100A4 in porcine coronary artery intimal thickening induced after stent implantation. Analysis showed that whichever model was used to induce the intimal thickening, S100A4 was present. Through this research, the group have analysed experimental intimal thickening after percutaneous transluminal coronary angioplasty and stent implantation. In addition, they have investigated mainly coronary and carotid arteries, which are particularly prone to atherosclerosis development, the major cause that underlies myocardial infarction and stroke. As a result of this work they have been able to confirm that S100A4 is a marker of R-SMCs *in vitro* and of SMCs responsible for experimentally-induced intimal thickening. These findings also apply to human lesions. In Bochaton-Piallat's opinion they are demonstrating that S100A4, in addition to representing a marker of SMC activation during intimal thickening formation, plays a role in SMC phenotypic changes and she hopes to further clarify the function of extracellular S100A4 on intimal SMC accumulation. "Taken together," she concludes, "our results suggest that a better understanding of S100A4 expression, secretion and regulation in SMCs will help to shed light on the mechanisms of SMC accumulation in the intima."

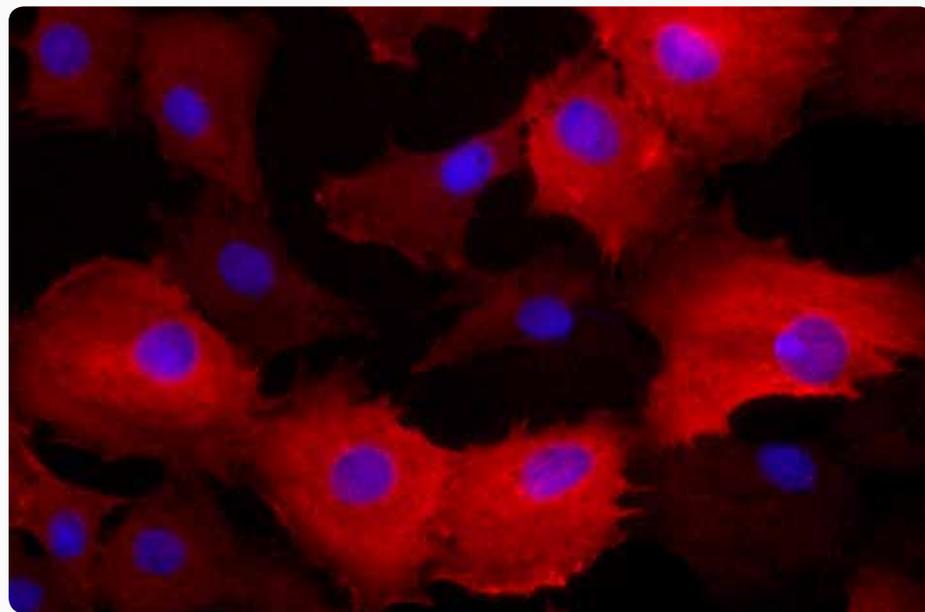


FIGURE 2. S100A4-expressing porcine rhomboid smooth muscle cell (Immunofluorescence staining using S100A4 specific antibody revealed in red; nuclei appear in blue).

INTELLIGENCE

ARTERIAL SMOOTH MUSCLE HETEROGENEITY: IMPLICATIONS FOR ATHEROSCLEROSIS AND RESTENOSIS

OBJECTIVES

- To isolate and characterise the atheroma-prone smooth muscle cell (SMC) phenotype: a cell population involved in atherosclerosis, responsible for most of cardiovascular diseases, and restenosis, the main cause of failure of revascularisation procedures
- To identify biomarkers typical of the atheroma-prone phenotype and to investigate their potential role in the accumulation of SMCs into the atherosclerotic and restenotic lesions

KEY COLLABORATORS

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Edoardo Camenzind, Francesco Mascoli, Renu Virmani, Hiroyuki Hao, Claus Heizmann

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completed her PhD in Biochemistry at the University of Geneva in 1993. During her postdoctoral period she was involved as scientific coordinator in the Bioimaging Core Facility at the Faculty of Medicine of the University of Geneva until 2002, when she started as an independent group leader. She teaches cardiovascular pathophysiology at the Faculty.



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