The absence of PCSK9 impairs the response of Smooth Muscle Cells to PDGF-BB stimulation

S. Marchianò[1], A. Corsini[1,2], A.L. Catapano[1,2], and N. Ferri[3].

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy;
[2]Multimedica IRCCS, Milan, Italy;
[3] Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italy.

Background: Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is involved in the homeostasis of cholesterol, mainly by the degradation of the hepatic low-density lipoprotein receptor. We previously demonstrated that PCSK9 is expressed in cultured smooth muscle cells (SMCs) and it is detectable in human atherosclerotic plaques. In *in-vivo* studies, we found that $PCSK9^{-/-}$ mice develop less neointimal thickening than $PCSK9^{+/+}$ mice in response to perivascular manipulation of right carotid artery (collar model).

Objectives: In this study, we investigated the role of PCSK9 on SMC proliferation and migration capability in the presence of PDGF-BB.

Materials and Methods: The SMCs were isolated from aorta of $PCSK9^{-/-}$ mice and the expression of PCSK9 was restored by transduction with a retroviral plasmid encoding for human PCSK9. The proliferation rate was analyzed by cell counting and i-Celligence system; the cell-cycle analysis was performed by flow-cytometry. The expression of cell-cycle proteins was examined by qRT-PCR and western blotting analysis. Boyden's Chamber assay, G-LISA analysis and cytoskeletal staining were performed to determine cell migration and morphological changes. The PDGF-BB was used as growth factor and chemotactic agent in all of the previous experiments.

Results: The expression of exogenous PCSK9 in mSMCs $PCSK9^{-/-}$ induced a higher proliferation rate (doubling time 32.2±3.1 h vs. 41.2±1.9 h) and a pronounced transition to S phase. The induction of cell cycle progression by PCSK9 was associated with lower expression levels of $p21^{Cip1}$ and $p27^{Kip1}$, and higher expression of Cyclin E and D1; expression of PCSK9 induced also the migration of mSMCs under basal condition and in response to PDGF. This effect was associated with higher levels of Rac1-GTP levels and lamellipodia formation.

Conclusions: Smooth muscle cell proliferation and migration are key features of vascular restenosis, one of the major complication after endovascular surgeries. The results of this study indicate that the absence of PCSK9 could have a protective role in the vascular restenosis process, potentially through an impairment of PDGFR signaling.

The present work was supported by the grant 2012-0549 from Fondazione Cariplo, ITALY.