Shedding light on PCSK9 function: a new role as a co-activator of platelet reactivity

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The proprotein convertase subtilisin/kexin 9 (PCSK9) plays an important role in low-density lipoprotein cholesterol (LDL-C) homeostasis, inducing hepatic degradation of LDL receptor, thus leading to an increase in plasma LDL-C levels. Monoclonal antibodies against PCSK9 have been developed to lower LDL-C; the first studies show that the therapy with PCSK9 inhibitors in combination with statins allows to achieve lower levels than statin therapy alone. Plasma levels of PCSK9 predict recurrent cardiovascular events in patients with stable angina (SA+), even in those with well controlled LDL-C levels. Experimental studies suggest that PCSK9 contribution to cardiovascular events might be mediated by mechanisms occurring both via LDL-dependent and via unknown LDL-independent pathways, but the understanding of these mechanisms is limited. Platelets (PLT) play a key role both in the progression and in the acute complications of atherosclerosis. In patients with type-2 diabetes mellitus (T2DM) an increased PLT activation is well described, but the mechanisms involved in this hypereactivity are not fully elucidated. Interestingly PLT count is positively associated with plasma PCSK9 in SA+ patients and increased serum PCSK9 level is directly associated with PLT reactivity in patients with acute coronary syndrome. No study has so far evaluated whether PCSK9 affects PLT function.

Aim

The general objective of this study was to understand if PCSK9 is involved in the mechanisms underlying the enhanced PLT reactivity of SA+ patients with T2DM.

To this aim we evaluated whether PCSK9 is expressed in PLT from healthy subjects (HS) and SA+ patients with or without T2DM and to assess whether PCSK9 modulates PLT activation and aggregation.

Materials and Methods

PCSK9 expression in PLT was assessed by whole blood flow cytometry (FC) and further evaluated by Western blot (WB) analysis in lysates of PLT and also of human megakaryocytes (MEG-01 cell line). PCSK9 levels in PLT lysates from 30 SA+ (15 T2DM+, 15 T2DM-), 10 T2DM+/SA- patients and 10 HS were measured by ELISA.

PCSK9 effect on PLT function was studied by 0.6μM epinephrine-induced PLT aggregation in PLT-rich plasma preincubated or not with PCSK9 (5μg/mL). The effect of PCSK9 on PLT activation was investigated by FC evaluation of P-selectin, activated glycoprotein IIb/IIIa (aGPIIb/IIIa) and Tissue Factor (TF) expression induced by epinephrine.

Results

WB data indicate that the protein is expressed in both PLT and MEG-01. Of interest, 20% of PLT stained positively for PCSK9 by FC. Quantitative assessment by ELISA assay showed that PLT from SA+/T2DM+ patients contained twice the amount of PCSK9 compared to the other groups (21.6±7.7 pg/µg protein, p<0,001) whereas no difference in plasma PCSK9 levels were found among the groups.

PCSK9 significantly potentiates PLT aggregation induced by sub-threshold concentration of epinephrine (+40%AUC; +78%Slope; -15%LagTime; +15%Maximum Aggregation) and this finding was further supported by an increased PAC-1 expression (+50%; p<0.05). The PLT activation markers P-selectin and TF (+40%,+25%) were also upregulated in PLT stimulated by epinephrine+PCSK9 compared to epinephrine alone.

Conclusions

These data show for the first time that PCSK9 is expressed in human PLT and significantly higher levels are found in SA+/T2DM+ patients. The presence of PCSK9 in MEG-01 suggests a direct transfer from the precursor cells to circulating PLT. Moreover we provided the evidence that PCSK9 plays a role in PLT activation and aggregation. Considering the relevance of PLT contribution to cardiovascular disease, these findings provide novel knowledge which may help to further shed light on the molecular basis of PLT hyperreactive phenotype in SA+/T2DM+ patients. The pharmacological control of PCSK9 by means of the recently developed antibodies, in addition to lower the plasma cholesterol level, might have the added value to also control platelet activation.