SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: ____Borsa per Soggiorno all'estero_____

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): __Finale_____

TITOLO DELLA RELAZIONE: _ VEGF-A Regulates Cellular Localization of SR-BI as Well as Transendothelial Transport of HDL but Not LDL _____

RELAZIONE:

Low- and high-density lipoproteins (LDL and HDL) must pass the endothelial layer to exert pro- and antiatherogenic activities, respectively, within the vascular wall. However, the rate-limiting factors that mediate transendothelial transport of lipoproteins are yet little known. Therefore, we performed a high-throughput screen with kinase drug inhibitors to identify modulators of transendothelial LDL and HDL transport.

The scavenger receptor class B–I (SR-BI) has been shown to be involved in the protective effects of HDL on the endothelium, namely angiogenesis, migration, activation of endothelial nitric oxide synthase, and monocyte adhesion. Recent studies have shown the association of enhanced expression of SR-BI expression in endothelial cells with decreased atherosclerosis in mice.

Results: Microscopy-based high-content screening was performed by incubating human aortic endothelial cells with 141 kinase-inhibiting drugs and fluorescent-labeled LDL or HDL. Inhibitors of vascular endothelial growth factor (VEGF) receptors (VEGFR) significantly decreased the uptake of HDL but not LDL. Silencing of VEGF receptor 2 significantly decreased cellular binding, association, and transendothelial transport of ^{125}I-HDL but not ^{125}I-LDL. RNA

interference with VEGF receptor 1 or VEGF receptor 3 had no effect. Binding, uptake, and transport of HDL but not LDL were strongly reduced in the absence of VEGF-A from the cell culture medium and were restored by the addition of VEGF-A. The restoring effect of VEGF-A on endothelial binding, uptake, and transport of HDL was abrogated by pharmacological inhibition of phosphatidyl-inositol 3 kinase/protein kinase B or p38 mitogen-activated protein kinase, as well as silencing of scavenger receptor BI. Moreover, the presence of VEGF-A was found to be a prerequisite for the localization of scavenger receptor BI in the plasma membrane of endothelial cells.

Discussion:

HAECs also bind, internalize, and transport HDL, as well as LDL. Using a high-content drug screening approach, we identified VEGF-A/VEGFR2 signaling as a rate-limiting factor for the cell surface abundance of

SR-BI and, as a consequence, regulator of uptake and transport of HDL by HAECs. Interestingly, VEGF-A and VEGFR2 had no effect on endothelial binding, uptake, and transport of LDL. VEGFs are important regulators of both vasculogenesis and angiogenesis in the adult.

In mammals, the VEGF family encompasses 5 different isoforms namely, VEGF-A, - B, -C, and –D, as well as placenta growth factor. These ligands bind to 3 VEGF receptors—VEGFR1, VEGFR2, and VEGFR3—in an overlapping pattern.

In accordance with this, our results show that RNA interference with VEGFR2 but not with VEGFR1 decreases HDL. Our data show some analogy with those reported in literature, that observed an improved lymphatic function and transport of HDL after VEGF-C treatment in mice. Binding of VEGF-A induces conformational changes and dimerization of VEGFR2, which in turn triggers kinase activation, tyrosine phosphorylation of the dimerized VEGFR2, and subsequent phosphorylation of SH2-containing intra-cellular signaling proteins, including phospholipase C-γ1, Src family tyrosine kinases, and PI3K and Ras GTPase- activating protein residues of Ras-Raf-MEK-MAPK pathway. VEGF was also shown to induce actin remodeling through activation of CDC42 and p38 MAPK. Both by pharmacological inhibition and RNA interference, we revealed the involvement of PI3K/Akt, p38 MAPK, and the Ras-Raf-MEK pathway in binding and uptake of HDL. However, the inhibition of the Ras-Raf-MEK pathway but not the inhibition of PI3K/Akt and p38 MAPK could be overcome by VEGF stimulation. Thus, VEGF regulates the interaction of HDL with endothelial cells by activating PI3K/Akt and p38 MAPK but not the Ras-Raf-MEK pathway.

PI3K is known to be involved in endosomal membrane trafficking and its inhibition by wortmannin in polarized cells affects early trafficking in the endocytic pathway, as well as inward vesicularization for the formation of multi-vesicular bodies in the later stages. Thus, it will be interesting to identify agonists beyond VEGF that regulate the endothelial binding, uptake, and transport of HDL by activating the MEK pathway. In this regard, it is also important to note our finding that RNA interference with MEK but not with Akt inhibited the binding and association of LDL with endothelial cells. We found that in HAECs, VEGF-A is required for the translocation of SR-BI from intracellular compartments to the cell surface, which in turn facilitates the binding, uptake, and transport of HDL. Consequently, it seems that in endothelial cells, HDL and SR-BI are both upstream regulators and downstream targets of the VEGF/VEGFR2 system.

These lipoprotein- specific effects of VEGF on the processing of HDL and LDL by HAECs indicate the existence of additional regulators and routes of transendothelial transport, for example, activin-like kinase 1, which was recently identified by a genome-wide RNAi-screen as an endothelial LDL-binding protein mediating uptake and transcytosis of LDL. From a more general perspective, our findings provide further evidence that transendothelial lipoprotein transport occurs by regulated processes rather than passive filtration. Dysregulated transendothelial lipoprotein transport may influence the pathogenesis of atherosclerosis beyond plasma levels of LDL and HDL.

In conclusion, we here showed that the VEGF-A/VEGFR2 regulates endothelial binding, uptake, and transport of HDL through PI3K/Akt and p38 MAPK and, as the final result, cell surface expression of SR-BI. Thereby, VEGF-A may play an important regulatory role for the vascular protective effects of HDL, as well as reverse cholesterol transport.

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