

Circulating active T-cells trigger HIF-1 activation in human carotid artery smooth muscle cells: a critical step in the etiology of vascular remodeling diseases

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Introduction: Vascular remodeling diseases (VRD) such as carotid artery stenosis is a proliferative remodeling disease characterized by enhanced carotid artery smooth muscle cells (CASMC) proliferation and resistance of apoptosis within the carotid artery vascular wall. We previously described that VRD is associated with the activation (i.e. nuclear translocation) of the Hypoxic inducible factor 1 (HIF-1) accounting for the downregulation of the voltage-gated K⁺ channel Kv1.5, increasing [Ca²⁺]_i (promotes proliferation), [K⁺]_i (decrease caspase activity), Bcl2 expression (anti-apoptotic factor) and hyperpolarizing the mitochondrial membrane potential ($\Delta\Psi_m$) in VRD-CASMC. The mechanism by which this normoxic activation of HIF-1 occurs in VRD-CASMC remains unknown. We previously published that the amount of active circulating T-cells are increased in patients with VRD (including pulmonary hypertension, carotid artery stenosis). Moreover, T-cells were also present within the carotid artery wall of VRD patients. We hypothesized that active T-cells trigger HIF-1 activation in human CASMC.

Methods: We used a Boyden chamber assay, in which the upper chamber was seeded with human T-cells isolated from 10 VRD patients and the bottom with healthy CASMC. $\Delta\Psi_m$ was measured by confocal microscopy using tetramethylrhodamine (TMRM) staining. CASMC proliferation and apoptosis were measured by PCNA/TUNEL staining. Fluo-3AM was used to measure the [Ca²⁺]_i. 3-[2-(4-adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid methyl ester was used as HIF-1 specific inhibitor. PI3K was inhibited by DHEA and TNF- α antibody was used to block the TNF pathway.

Results: We found that after 96h, VRD isolated T-cells induced HIF-1 translocation in 65% of healthy CASMC. As expected compared to healthy CASMC (inactive HIF-1), CASMC with active HIF-1 had 1.5-fold increase in [Ca²⁺]_i (n=25; p<0.05), resulting in 50% increase of proliferation (n=100; p<0.05). $\Delta\Psi_m$ was significantly hyperpolarized (n=100; p<0.05) in CASMC with active HIF-1 resulting in resistance to serum starvation (0.1% compared to the regular 10% serum concentration)-induced apoptosis. All these effects were reversed by the inhibition of HIF-1. Finally, because both TNF- α antibody and PI3K inhibitor, blocked T-cell induced HIF-1 nuclear translocation in CASMC suggested that HIF-1 activation was mediated by a TNF- α /PI3K axis, which remain to be described. Note that T-cells isolated from 5 healthy donors had no effect on healthy CASMC.

Discussion/Conclusion: We demonstrated for the first time that T-cells isolated from patients with vascular remodeling diseases, trigger HIF-1 activation in healthy CASMC through a novel TNF/PI3K axis, which changed their phenotype to VRD-CASMC. Therapies aimed to inhibit T-cells activation like cyclosporine or the TNF- α receptors like infliximab could be of great therapeutic interest for the treatment of vascular remodeling diseases like pulmonary hypertension and carotid artery stenosis.

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