Î±-Melanocyte stimulating hormone inhibits fetal vascular smooth muscle cell phenotypic switching

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Abstract

The alpha-Melanocyte Stimulating Hormone (Î±-MSH) is a potent endogenous anti-inflammatory melanocortin peptide, which plays key roles in regulating cardiovascular functions [1]. Î±-MSH mediates these actions in part through central melanocortin receptors (MCRs), in part by local effects on the vasculature [2]. It has been recently shown that treatment with Î±-MSH attenuated vascular dysfunction and ameliorated plaque inflammation in a mouse model of pre-established atherosclerosis [3]. Vascular smooth muscle cells (VSMCs) are a key cellular population involved in plaque formation and progression and in vessel remodeling, by switching from a contractile to a synthetic phenotype in response to endothelial dysfunction and inflammation [4]. We investigated whether human VSMCs express a functional melanocortin system and whether Î±-MSH could modulate their phenotype.

Detection of melanocortin system components was performed by real-time PCR, Western-Blot, and immunocytochemistry in six different primary human aortic SMCs (HAoSMCs). Given that MCRs are highly polymorphic genes, we sequenced by the Sanger method the MCR open reading frames in all cell lines. We selected for subsequent experiments a fetal cell line bearing wild type genes. Phenotypic markers were detected by immunofluorescence. Fetal VSMC differentiation and migration was analyzed using gap-closure and transwell assays by time-lapse confocal microscopy imaging, comparing Î±-MSH-treated and untreated cells (at 3, 6, 9, 12, and 24h). VSMC proliferation was evaluated by a modified BrDu fluorescent assay at 24h.

We showed that all primary HAoSMCs analyzed (adult and fetal) express two MCR types, MC1R and MC4R, but not the Î±-MSH precursor proopiomelanocortin (POMC). *In vitro* stimulation with Î±-MSH promoted fetal VSMC differentiation from a synthetic, proliferative, and migratory phenotype with an epithelioid morphology, to a contractile, quiescent phenotype with an elongated, spindle-shaped conformation. Indeed, treatment with Î±-MSH significantly promotes fetal HAoSMC elongation with peak effects at 6-12 hours (mean fold differences of the number of elongated cells with respect to control: 2.0-2.6, $p<0.01$). Î±-MSH enhanced the expression of smooth muscle Î±-actin, which is a marker of the contractile phenotype (mean fold difference 2.1, $p<0.001$), while reducing vimentin, marker of the synthetic phenotype (mean fold difference -1.5, $p<0.001$). MCR activation by Î±-MSH slowed down VSMC migration both in gap-closure assay, with a peak effect at 9h (mean fold difference 4.9, $p<0.001$), and in transwell migration assay, using PDGF-BB as chemoattractant (mean fold difference 1.95, $p<0.01$). Î±-MSH has a moderate, but significant effect also on fetal HAoSMC proliferation (mean fold difference 1.2, $p<0.05$).

In conclusion, our results provide new knowledge about the involvement of the melanocortin system in vessel homeostasis and, possibly, on modulation of vascular inflammation. VSMC MCRs may be a pahrmacological target for managing pathological remodeling and related diseases. The use of antiinflammatory anolagos of Î±-MSH could represent a strategy to maintain VSMCs in the differentiated state or to revert their phenotype from a proliferative state.

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