Characterization of the angiogenic potential of human monocytes and programmable cells of monocytic origin (PCMO) under hypoxic conditions

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Background: After reprogramming *in-vitro*, human blood monocytes can acquire a state of plasticity. These so called *programmable cells of monocytic origin* (PCMOs) hold great promise in diseases in which damaged or non-functional cells need to be replaced¹⁻⁴. A study of our group showed that PCMO cells release proteins involved in angiogenesis and that PCMO inoculation, but not the inoculation of naïve monocytes may present a treatment strategy for ischemia-associated illnesses¹.

Aim of the study: To characterize and compare the angiogenic potential of human monocytes and programmable cells of monocytic origin (PCMO) under hypoxic conditions *in-vitro*.

Materials and methods: Mononuclear cells were isolated from peripheral blood by density gradient centrifugation and PCMO cells were obtained as described earlier⁵. Enzymatic hypoxia^{6,7} was applied to monocytes or PCMO for 3h (pO₂<10 mmHg) and 24h after the end of hypoxia the respective conditioned culture medium (CCM) was collected. Assays for lactate dehydrogenase (LDH) release and angiogenesis proteome profiling arrays were used to characterize the secretory profile of the cells. The experimental setting is depicted in Fig.1.

Results: 3h of hypoxia reduced the release of LDH in cultures of human naïve monocytes (n=11; p<0.001), while no significant differences in LDH release were observed in PCMO cultures (n=5; p>0.05; Fig. 2). 24h after the end of hypoxia period, 28 of 55 (51%) angiogenic proteins were detectable in monocyte-CCM. Of these, 17/28 (61%) were up regulated, 3/28 (11%) were down-regulated and 8/28 (28%) did not show any regulation. In PCMO-CCM, 26 of 55 angiogenesis proteins (47%) were detectable. 12/26 (46%) proteins were up regulated, 6/26 (23%) were down-regulated, while 8/26 (31%) proteins did not reveal regulation by hypoxia (Fig. 3A and B). Proteins with the strongest increase in secretion by hypoxia were: MCP-1 (92-fold in monocyte-CCM and 53-fold in PCMO-CCM), MIP-1α (35-fold in monocyte-CCM and 16-fold in PCMO-CCM) and GM-CSF (40-fold in monocyte-CCM and 15-fold in PCMO-CCM, while IL-8 and Thrombospondin 2 showed differential secretion in monocytes or PCMO (Fig. 3C).

Fig. 1 Experimental setting. Human mononuclear cells were retrieved from peripheral blood of healthy donors (n=13) and were allowed to attach for 1h in culture plates. After washing, monocytes-enriched monolayers were maintained in culture with the addition of IL-3 (1ng/ml) and M-CSF (5ng/ml) for 5 days to obtain PCMOs. Monocytes and PCMOs were subjected to hypoxia or normoxia for 3h and their conditioned culture medium (CCM) was collected 24h after the end of hypoxia. Scale bars: 100µm

Fig. 2 Cell damage. Conditioned culture media of monocytes and PCMO that were subjected to 3h hypoxia/normoxia in culture, were collected after 24h and assayed for cell damage using lactate dehydrogenase (LDH) assays. Columns denote the mean, bar show SEM; ***: p<0.001

Fig. 3 Secretion of human angiogenesis-associated proteins in culture media of monocytes and PCMO. Conditioned culture media from monocytes and PCMO were used in human angiogenesis proteins arrays. **A-** Proportion of proteins whose secretion is regulated by hypoxia in monocytes and PCMO. **B-** A list of all secreted proteins and their respective positions on the array membranes are shown in the left panel, and at the right, photomicrographs of the respective proteome profiling arrays show the duplicated spots of each protein. Most regulated and differentially secreted proteins (compared to normoxia), in monocytes (left arrays) and PCMO (right arrays), are marked with rectangles and are written in red. **C-** Differentially secreted protein between monocytes and PCMO are marked with rectangles and written in green.

Conclusion:

Naïve monocytes and PCMO show similarities in the release of several hypoxia-induced angiogenic factors but also cell type specific responses to the hypoxic stimulus. This might influence the potential of both cell types in therapies aiming at the reduction of ischemia/reperfusion injury and/or induction of angiogenesis in different organs.

Bibliography: ¹Berndt et al. *Stem Cell Res Ther* 2018; ²Ungefroren et al. *Stem Cell Int* 2016; ³Zachos et al. *J Orthopaedic Res* 2014; ⁴Ungefroren and Fandrich. *Adv Exp Med Biol* 2010; ⁵Ungefroren et al. *Stem Cells Dev* 2010; ⁶Zitta et al. *Eur J Pharmacol* 2016; ⁷Hummitzsch et al. *Exp Cell Res* 2014.