POSTER 21: The proliferative capacity and differentiation potential of mesenchymal stem and progenitor cells in relation to the expression of aldehyde dehydrogenase and oxidative status

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High expression of aldehyde dehydrogenase (ALDH) is considered a marker of cell primitivity. Previous work suggests that ALDH+ mesenchymal stromal cells (MSCs) are enriched in stem cells markers and appear to be less engaged than $ALDH^{+}$. The purpose of our work is to obtain functional data and study the proliferation capacities of MSC ALDH+ and $ALDH^{-}$ cells previously incubated at low 02 concentration existing in stem cell niches (0.1 % 02). Under the control condition, MSCs are incubated in a standard 0^2 concentration (21 % 02).

We studied clones from a single cell based on its ability to generate colonies in the primary and secondary culture. The division of the content of a clone present in primary culture into 3 in secondary culture with the stimulation of a differentiation pathway in each well allowed us to determine the potency of the cell (unipotent, bipotent or multipotent). The ALDH+ population contains twice as many cells capable of forming colonies (derived from progenitors CFU-F (- 50% vs. -20%) and 95% of these clones generated a colony in the secondary culture (75% of CFU-F from of the ALDH· fraction). Thus, nearly 40% of ALDH+ MSCs have the capacity to generate secondary vs. 21 % of MSC ALDH·. However, there is no significant difference between these two populations in differentiation potential (-50% multipotent -40% bipotent, -7-8% unipotent.). Therefore, multipotent cells with a high proliferative capacity are relatively more frequent in the ALDH+ population than in ALDH·. This corresponds with a stimulation of glycolytic activity and a decrease in mitochondrial respiration in ALDH+ cells. However, half of the ALDH+ population is multipotent, so ALDH positivity should not be viewed as an exclusive marker of stem cells. However, the capacity of MSCs to form secondary colonies was significantly greater in the condition resulting from the low 02 concentration than in the hyperoxia condition (21% 02), detected in the ALDH+ as well as ALDH· population. In order to metabolically explain this advantage of the low 0^2 concentration, we measured the production of mitochondrial superoxide anion (0^{2-}) (Mitosox marker). This revealed that mitochondrial ROS production is selectively observed only in the ALDH· highly apoptotic population under 0.1 % 0^2 condition. In contrast, the expression of ALDH does not correlate with the production of mitochondrial ROS because Mitosox was detected without difference in the ALDH+ population as well as in ALDH· population under the 21 % 02 condition. Paradoxically, overall production of 02· seems to be higher at 0, 1 % 0^2 in respect to MSC cultivated at 21 % 02. To elucidate this observation, we evaluated oxidative status in selected ALDH+ vs ALDH·population. This revealed that very low 02 concentration diminished oxidative stress according to significantly decrease of reduced glutathione content compacting to 21 % 0^2 no matter the expression of ALDH. Therefore, we suggest that observed increase of 0^{2}· production at 0. I% 0^2, play a role as a signaling molecule in hypoxia induced cellular responses.

In conclusion, our results reveal that the expression of the ALDH marker, especially in low 0^2 concentration, indicates a population of primitive but not exclusively stem mesenchymal cells and this low 0^2 concentration protects cells from oxidative stress as well.