

Wharton's jelly mesenchymal stromal cells immunomodulatory molecules: their journey from umbilical cord to differentiated cells

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Wharton's jelly mesenchymal stromal cells (WJ-MSCs) have a unique ability to cross lineage borders. Their immunomodulatory and anti-inflammatory features, further render these cells promising for regenerative medicine applications. Few data are present in literature on the expression of immunomodulatory molecules in umbilical cord (UC) tissue and their maintenance in paired cultured WJ-MSCs, an important aspect in cellular therapy applications. In addition, few data exist on the maintenance of expression of immunomodulatory molecules in mature cell types differentiated from MSCs. Therefore we investigated, *in vivo* (in UC at full term) and *in vitro* (in either undifferentiated or differentiated WJ-MSCs), the expression of different markers and their maintenance alongside cell culture, *ex vivo* expansion and differentiation.

IHC, ICC, RT-PCR and flow cytometry were used to detect expression of markers in both paired UC sections and WJ-MSCs. Differentiation was performed towards the standard mesenchymal lineages as well as hepatocyte-like cells.

Paired ICC and IHC analyses showed that for most of the analyzed molecules the expression at the protein level is maintained in both UC tissue and WJ-MSCs. Structural molecules were expressed in both WJ and umbilical epithelium (UE), as well as in WJ-MSCs. We showed for the first time that UE and WJ were positive for both HLA-ABC and HLA-E, while HLA-DR was not detectable. The same data were confirmed on WJ-MSCs. Both B7-1 and B7-2 were absent in UC and WJ-MSCs, while we showed for the first time that B7-H3 was highly expressed in both WJ and WJ-MSCs. Differentiation experiments showed that immunomodulatory molecules were expressed upon application of complex differentiation protocols, in parallel to the acquisition of mature markers or functions.

Some important conclusions may be drawn from the current experiments: i) WJ-MSCs mostly maintain the expression of molecules just present in their "niche", under standard culture conditions; ii) the parallel expression of immunomodulatory molecules sheds new light on the ability of WJ-MSCs to modulate host immune responses; iii) the *in vivo* expression of molecules such as HLA-E and B7-H3 opens new questions on the role of WJ during pregnancy; iv) the expression of these molecules in differentiated cells provides key features for *in vivo* applications, in particular for hepatocyte-like cells.

This work was supported by grants from University of Palermo (FFR 2012) and IEMEST to GLR.