M1055

In Situ Detection of Mesenchymal Stromal Cells in Human Placenta by Chipcytometry

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PURPOSE

Mesenchymal stromal (stem) cells (MSCs) present promising opportunities for tissue engineering and regenerative medicine. They can be isolated from various tissues, e.g. bone marrow, adipose tissue, placenta etc. Here, we report for the first time in situ detection of cells expressing this marker combination in human placenta using high-parameter immunohistology.

METHODS

Placenta samples were embedded in Tissue-Tek® and cryopreserved. All chips were shipped and stored at 4 degree C. The applied antibodies were CD105-PE (clone 43A3, Biolegend), CD73-PE (AD2, Biolegend), CD90-PE (5E10, Biolegend), CD34-PE (561, Biolegend), CD19-PE (HIB19, eBiosciences), CD14-PE (RMO52, Beckman Coulter), CD45-Alexa Fluor 488 (HI30, Biolegend) and HLA-DR-BUV395 (G46-6, BD Biosciences). Antibodies were tested with purified BM-MSCs. Cell segmentation, fluorescence value calculation and data analysis of all chips were performed with Chipcytometry software.

RESULTS I

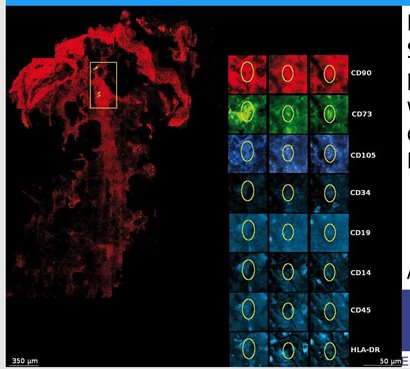

Fig. 1
Staining of the whole human placenta cryosection (5 µm) with CD90 (left) and expression of the entire marker set by three MSC areas (right).

Acknowledgement:

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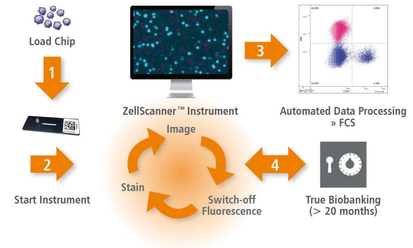
PRINCIPLE



INSTRUMENT

CHIPCYTOMETRY 95-PLEX ASSAY

CD3	CD21	CD40	CD73	CD152 (CTLA-4)	CD206	Granz. B	IL17A
CD4	CD24	CD45	CD80	CD161	CD244	Helios	IL17F
CD5	CD25	CD45RA	CD81	CD163	CD257 (BAFF)	HLA-DR	Ki-67
CD8	CD27	CD45RO	CD86	CD172a/b	CD273 (PD-L2)	IFNγ	LC (κ)
CD10	CD28	CD54	CD90	CD183 (CXCR3)*	CD274 (PD-L1)	IgA	LC (λ)
CD11b	CD29	CD56	CD95	CD184 (CXCR4)*	CD278 (ICOS)	IgD	pan Cytok.
CD11c	CD30	CD57	CD105	CD185 (CXCR5)*	CD279 (PD-1)	IgG	RORγ(t)
CD14	CD31	CD64	CD115	CD193 (CCR3)*	CD319 (CRACC)	IgM	T-bet
CD15	CD32*	CD66b	CD123	CD194 (CCR4)*	CD326 (EpCAM)	IL1b	TNFα
CD16	CD34	CD68	CD127	CD195 (CCR5)*	AIOLOS (IKZF3)	IL8	Vimentin
CD19	CD38	CD69*	CD138	CD196 (CCR6)*	FoxP3	IL10	Zap-70
CD20	CD39	CD71	CD141	CD197 (CCR7)*	GM-CSF	IL12	*live stain

RESULTS II

Human placenta cryosections deriving from early terminations (up to 12th week of pregnancy) as well as from full term pregnancies were analysed. According to the classical ISCT definition, we found six MSCs on tissue section M126694, three MSCs on M126688 and no MSCs on M126696 (Tab 1). One representative staining of section M126688 is depicted in Figure 1. Since MSCs might not express all typical surface molecules CD105, CD73 and CD90 in vivo, we also searched for single- and double-positive cells (Tab 1). Between one and seven CD73+ CD90+ regions could be detected on the different sections, while none or up to two CD73+ CD90+ areas, could be localized, respectively.

RESULTS III

Chip ID	Week of gestation	CD73+ CD105+ CD90+ lineage -	CD73+ CD90+ lineage -	CD73+ CD105+ lineage -	CD73+ lineage -	CD105+ lineage -
M126688	11th	3	3	2	3	5
M126694	39+0	6	7	0	0	0
M126696	39+6	0	1	1	3	0

Table 1: Number of regions on the scanned human placenta cryosections positive for MSC-typical marker combinations. All regions listed are CD34- CD45- CD19- CD14- HLA-DR-. One region corresponds to a single cell or to a few cells in very close proximity of each other.

CONCLUSIONS

- To date, it is not known if MSCs express the same set of surface markers in vivo and after in vitro expansion. Here, we present for the first time that CD73+ CD90+ CD105+ CD45- CD34- CD14- CD19- MSCs could be found in human placenta cryosections.
- However, the numbers of triple-positive areas are rather low, suggesting that most MSCs do not express all characteristic markers in vivo but up-regulate their expression during in vitro culture. In line with this, we also detected some additional CD73+ CD90+ and very few CD73+ CD105+ double-positive lineage marker-negative regions, which might be precursors or subsets of MSCs.
- Noteworthy, our method allows different applications to further understand the role and function of MSCs in vivo. For example, it offers the opportunity to track injected MSCs in patient biopsies and thereby understand their fate and interaction with recipient immune cells after application.
- Chipcytometry is useful tool in translational pathology. Further research is needed to validate Chipcytometry for use in clinical trial support.