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Embryonic stem cells preserve pluripotency when injected in a foetal niche

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The stem cell niche is the microenvironment where stem cells reside. Different elements define the niche and regulate stem cell characteristics, like stromal support cells, gap junctions, soluble factors, extracellular matrix proteins, blood vessels, and neural inputs. Stem cells have been identified in human and murine amniotic fluid; both cell type are selected for the marker c-Kit and are called amniotic fluid stem (AFS) cells. AFS cells are characterized by the expression of pluripotency markers and differentiate in culture into all the three embryonic lineages. The aim of this study was to investigate the role of amnion (AM) and amniotic fluid (AF) as stem cell niche. Through in utero transplantation (IUT), we injected YFP+ mouse embryonic stem cells (YFP+ ESC) into the AF of E13.5 wild type fetuses (C57BL6/J), and 4 days after IUT we evaluated their pluripotency by immunofluorescence (IF), qRT-PCR, single cells multiplex PCR and teratoma assay. The proliferative and apoptotic status by EdU and TUNEL assay was assessed. The foetuses using haematoxylin and eosin staining, IF and PCR for detection of YFP was analysed. ELISA assay was performed for detection of stem cell factor (SCF), vascular-endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin growth factor (IGF-1) in the AF both before and after YFP+ ESC injection YFP+ ESC injected through IUT didn't integrate into foetuses. Only few YFP+ ESC resulted EdU+ in the AF and AM, whereas no apoptotic cells where identified. YFP+ ESC isolated from the AF and the AM at E17.5 maintained the expression of pluripotency markers (Oct4, Sox2, Nanog, c-Myc, Klf4) both at molecular and protein level. YFP+ ESC isolated from the AM were more similar to ESC in culture, in respect to YFP+ ESC isolated from the AF, and only YFP+ ESC isolated from AM were able to form teratoma. Moreover, cytokine analyses and oxygen concentration revealed the presence of fundamental niche characteristic factors in the foetal microenvironment constituted by AF and AM. This is the first indication that ESC may reside in the AM without differentiating, as it occurs in stem cell niches. The interplay between AM and ESC creates the dynamic system necessary for sustaining the undifferentiated state of the cells themselves.

Biography

Bertin E has graduated in Medical Biotechnology at the University of Padua in 2009 (BSc and MSc), in 2010 she started her PhD program at the Stem Cells and Regenerative Medicine Lab. During her PhD period she focused on the characterization of mouse amniotic fluid stem cells, performing studies both in Padua and in the Laboratory at the Samuel Lunenfeld Research Institute. She is co-author of many publications. She finished her PhD in December 2012, and is working as Post-doc in the same lab in Padua, where she is focusing her research on the study of amnion and amniotic fluid as a potential foetal stem cells niche.

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