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A morpho-functional analysis of embryoid body-like structures from human amniotic fluid-derived stem cells (AFSCs) unselected for c-kit

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Human AFSCs, a novel class of stem cells sharing characteristics of both embryonic and adult stem cells, harbour high proliferative capacity and high differentiation potential and do not raise the ethical concerns associated with human embryonic stem cells (ESCs). The formation of three-dimensional aggregates known as embryoid bodies (EBs) is the main step in the differentiation of pluripotent embryonic stem cells. The purpose of this study was to investigate whether human AFSCs, unselected for c-kit, have features of pluripotency. With this aim, we evaluated both AFSC ability to form *in vitro* EB structures and transcriptional profiles of genes typically expressed in human ESCs. Total AFSCs were cultured in suspension in uncoated Petri dishes for EB formation, whose incidence was assessed in 5 independent experiments. EB-like structures were observed and morphometrically analysed under a LEICA phase contrast microscope equipped with a CoolSnap videocamera. A number of samples were processed for alkaline phosphatase (AP) or haematoxylin-eosin staining, immunofluorescence and transmission electron microscopy to follow-up morphology and markers of pluripotency. As to the expression studies, RNA was extracted from AFSCs at the 3th, 4th, 5th and 8th passage in culture and the presence of ESC and primordial germ cell (PGC) specific markers was assessed with RT-PCR. As early as after 5 days of culture we were able to observe the formation of EB-like solid structures of different size progressively increasing at later time intervals of incubation in cell culture medium (10-15 days). At these later time points EB aggregates showed the presence of an internal cavity and were surrounded by a wide cohort of bigger cells detaching from them. Both early and late time EBs were positive for alkaline phosphatase (AP) staining and specific markers of pluripotency (OCT4 and SOX2). The parallel analysis of AFSCs with RT-PCR demonstrated the presence of ESC and PGC specific gene transcripts and, moreover, the expression of alternatively spliced genes also detectable in EB cells. These findings demonstrate that AFSCs are a new and powerful biological system to recapitulate the three-dimensional and tissue level contexts of *in vivo* development.

Keywords: Embryoid bodies, human amniotic fluid stem cells, markers of pluripotency.