## Properties of smooth muscle cells differentiated from human amniotic fluid stem cells

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Functional smooth muscle engineering requires isolation and expansion of smooth muscle cells (SMC) and this process is particularly challenging for visceral smooth muscle tissue where progenitor cells have not been clearly identified. Herein we showed for the first time that efficient SMC can be obtained from human amniotic fluid stem cells (hAFSC). Clonal lines were generated from c-kit+ hAFSC. Differentiation towards SM lineage (SMhAFSC) was obtained using a medium conditioned by PDGF-BB and TGF- $\beta$ 1. Molecular assays revealed higher level of  $\alpha$ -SMA, desmin, calponin, myosin heavy chain in SMhAFSC when compared to hAFSC. Ultrastructural analysis demonstrated that SMhAFSC also presented in the cytoplasm increased intermediate filaments, dense bodies and glycogen deposits like SMC. SMhAFSC metabolism evaluated via mass spectrometry showed higher glucose oxidation in comparison to hAFSC. Patch clamp of transduced hAFSC with lentiviral vectors encoding ZsGreen under the control of the alpha smooth muscle actin ( $\alpha$ -SMA) promoter was performed demonstrating that SMhAFSC retained an electrophysiological fingerprint similar to visceral SMC. Eventually SMhAFSC contractility was evident both at single cell level and on a collagen gel. In conclusion, we showed here that hAFSC under selective culture conditions are able to give rise to functional SMC.

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