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Matrix stiffness boosts pancreatic differentiation via the YAP/TAZ mechanotransduction mediated pathway.

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In the last years, many papers highlighted the possibility to use epigenetic modifiers to directly interact with the epigenetic signature of an adult mature cell (Pennarossa et al., 2013; Chandrakantan et al., 2016). In particular, the molecule 5-azacytidine (5-aza-CR), which is able to interfere with DNA methylation, through both a direct and an indirect effect (Manzoni et al., 2016), can be used to remove the epigenetic 'blocks' responsible for tissue specification and to facilitate cell transition to a different lineage. In parallel, recent evidence has also shown that epigenetic conversion is influenced by the 3D rearrangement and by the mechanical properties of the cellular microenvironment (Pennarossa et al., 2017). In the experiments here presented, we investigated the effect of a selected 3D culture system on the conversion process. We used INS-eGFP porcine fibroblasts, that express enhanced green fluorescent protein (eGFP) under the control of insulin gene promoter, as experimental model, and wild-type pig fibroblasts, as control. Both cell types, were plated either on plastic or on 1kPa polyacrylamide (PAA) gel, that mimics the stiffness of pancreatic tissue in vivo. Cells were erased with 5-aza-CR for 18h and exposed to specific differentiation stimuli for 36 days (Pennarossa et al., 2014). The use of INS-eGFP fibroblasts allowed real-time monitoring of cells progressing towards the pancreatic phenotype. Morphological analysis and pancreatic marker expression were checked for the entire length of the experiment. PAA gels encouraged the induction of islet-like structures, suggesting that the of tridimensional clusters may be a crucial aspect of pancreatic differentiation in vitro. Moreover, the use of an adequate substrate accelerated cell differentiation process and anticipated insulin secretion ability. The results obtained demonstrated the direct implication of the yes-associated protein/transcriptional co-activator with PDZ-binding motif (YAP/TAZ) mechanotransduction-mediated pathway (Figure 1), indicating that mechanical cues exert a key role in pancreatic phenotype definition.

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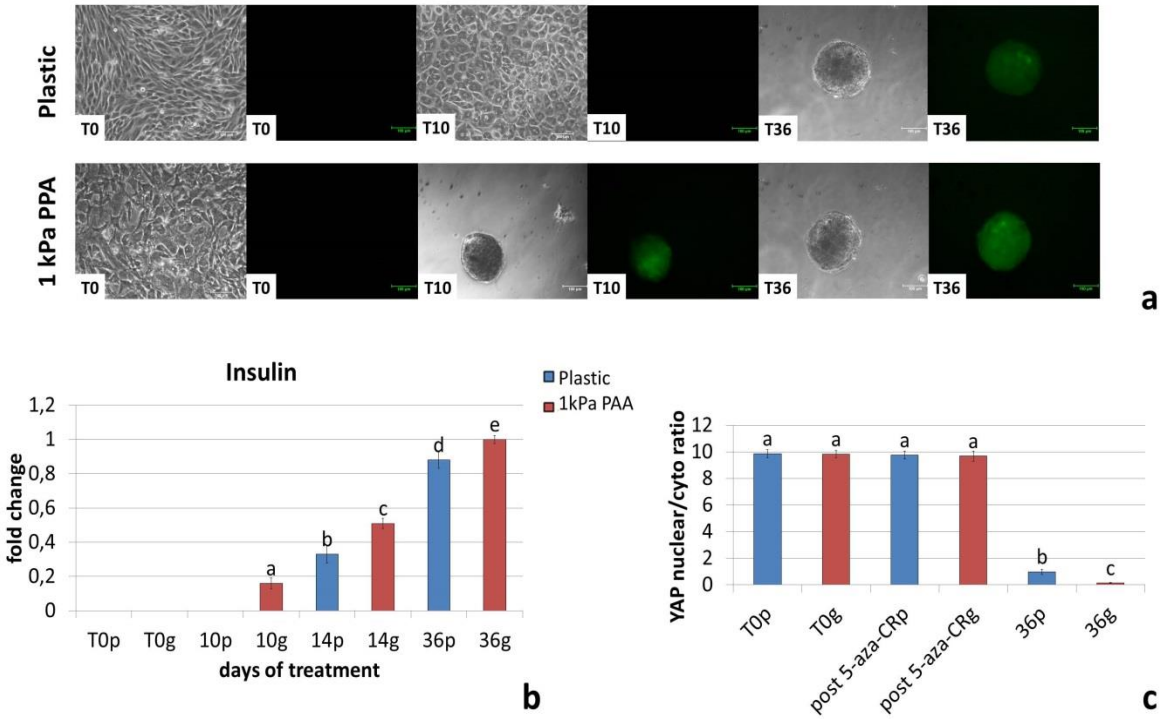
Figure 1: a Morphological changes in INS-eGFP porcine fibroblasts plated on standard plastic dishes (plastic) and PAA gels (1kPa PAA) at different time points of the endocrine pancreatic induction protocol. b Insulin expression changes in porcine skin fibroblasts plated on plastic dishes (Top, blue) and PAA gels (Tog, red) and subjected to endocrine pancreatic induction (days 10, 14, 36). Gene expression levels are reported with the highest expression set to 1 and all other times relative to this. Different superscripts denote significant differences between groups ($P < 0.05$). c Quantification of the nuclear/cytoplasmic ratio of YAP in untreated fibroblasts (Top, Tog), after 18-hour exposure to 5-aza-CR (Post 5-aza-CRp, Post 5-aza-CRg) and at the end of pancreatic induction (36p, 36g). Bars represent mean ± SD of three independent replicates. Different superscripts denote significant differences between groups ($P < 0.05$).



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