Abstract

The substances secreted by Sertoli cells (SCs) are crucial to determine male sex characteristics in embryos and regulate spermatogenesis in adulthood. The failure in SC maturation can cause sterility in men. Before puberty, SCs keep the ability to proliferate and have been considered as immature cells. They differ remarkably from mature cells in connection with their morphology and biochemical activity and thus they probably play a part in maintaining spermatogonia stem cells in an undifferentiated stage. The transient presence of cytokeratin in immature SCs has been reported in many species, but not in *Xenopus* yet. We investigated which molecules are expressing only in immature Sertoli cells of *X. tropicalis* testes. The regulation of cytokeratin and β-catenin was revealed by fluorescent immunostaining. Cytokeratin and membrane β-catenin co-expressed in *X.tropicalis* juvenile testes and in cultured SC progenitors, called XtiSCs, but they were absent in adulthood. There was no signal of cytokeratin in migrating SCs (pre-SCs) located outside the seminiferous tubules. The suppression of cytokeratin along with the breakdown of β-catenin-based cell contacts have been observed in XtiSCs after the treatment with a small molecule drug, CHIR99021 and led to their dedifferentiation back to stem cell-like state. These findings confirm the expression of cytokeratin and a novel molecule, β-catenin along with Sox9 in SCs as markers indicating their immature state.

However, the function of CK in SC development is poorly understood. We examined interconnection between CK and β-catenin-based cell junctions in immature SCs. Reversible dissociation of CK by acrylamide in XtiSCs induced breakdown of membrane-bound β-catenin but had no effect on F-actin and β-tubulin or cell adhesion proteins (focal adhesion kinase and integrin β1). On the contrary, disruption of membrane β-catenin via uncoupling of cadherins with Ca2+ by chelator EGTA didn't show any influence on the cytokeratin stability. The results suggest a new role of CK in the retention of β-catenin-based junctions in immature Sertoli cells, and thus serving as structural support for arrested germ cells and for the formation of proper seminiferous tubules.

Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) are fundamental processes in embryonic development. In general, EMT is characterized by the conversion of sessile epithelial cells into mesenchymal cells with the migratory potential, whereas MET activates a reverse process. A mesenchymal phenotype of pre-Sertoli cells has been observed suggesting that pre-SCs must undergo a MET to differentiate into mature cells. Our laboratory has been successful in the establishment of *Xenopus tropicalis* somatic cell line from testes of juvenile frogs, called XtTSCs. The isolated cells possessed characteristics of Sertoli cells with expression of immature markers including Sox9, vimentin, cytokeratin and β-catenin, so latter called XtiSCs. The main aim of my Ph.D. project was the determination of factors responsible for the induction of EMT, a reverse differentiation process of SCs, and identification of a stemness window where cells possess the greatest differentiation potential in XtiSCs. GSK-3 inhibitor (CHIR99021), FGF2 and/or TGF-β1 ligands were employed in XtiSCs culture to induce EMT. Our results showed that XtiSCs underwent full EMT after 3-day treatment with GSK-3 inhibitor and partial EMT with FGF2, but not with TGF-beta 1. The morphology change of CHIR-treated XtiSCs to the typical spindle-like cell shape was associated with the upregulation of mesenchymal proteins (fibronectin, integrin α5β1, Snai1 and Zeb1) and low expression of the epithelial marker, cytokeratin. Moreover, this inhibitor also promoted stem cells markers (Sox2, *cd44*) and the efficient derivation of stem cells from *Xenopus* testes. CHIR-treated, but not FGF2-treated or vehicle XtiSCs can differentiate into chondrocytes *in vitro* and cardiomyocytes *in vivo* after their microinjection into the peritoneal cavity of *X. tropicalis* tadpoles. Interestingly, the EMT-shifted cells could migrate toward cervical cancer cells *in vitro* (HeLa cells) and to the injury site *in vivo*. In general, our results provide a better understanding of signaling pathways underlying the generation of testis-derived stem cells. Moreover, XtiSCs could represent a novel model for the study of the EMT process and SC maturation.