AMOTL2 role in pluripotency and human stem cell differentiation

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The transcriptional regulation of developmental processes was extensively studied over the last years, yet there is still a considerable gap of knowledge concerning the mechanistic and metabolic cues, and underlying morphological events that accompany human pluripotent stem cell (hPSC) differentiation. In search of such signals, I first identified the *Amotl2* gene in the single-cell RNA-sequencing (scRNA-seq) data of the developing murine pancreata from embryonic days 14.5 (e14.5) and 16.5 (e16.5). Interestingly, despite conserved canonical markers, the endocrine progenitor (EP) populations from these timepoints differed significantly in their transcriptomes, with e16.5 EPs being more prone to form pancreatic β-cells, and e14.5 EPs preferentially forming α-cells. *Amotl2* was specifically expressed in the subtype of e16.5 EPs, which reflected EPs delamination from epithelial cords, a poorly understood event that might influence endocrine cell fate.

AMOTL2 is a member of the angiomotin family. It is a cytoplasmic protein that resides either in cytosol or at the cellular junctions, facilitating signal transduction, cytoskeleton organization, and modulation of signaling pathways. AMOTL2 is a known regulator of Hippo pathway and its downstream effector YAP. One of the aims of this thesis was identification of *AMOTL2* expression patterns in developing human pancreas *in vivo* and pancreatic differentiation *in vitro* using publicly available scRNA-seq datasets. *AMOTL2* was expressed at the different stages of pancreatic differentiation, including definitive endoderm, pancreatic progenitors, and EPs, *in vitro*, and in EPs, mesenchyme, and acinar cells *in vivo*. *AMOTL2* expression did not persist into mature endocrine cell. Importantly, here, for the first time, is shown the existence of the *NGN3*(+) / *AMOTL2*(+) EP population in human, in both *in vitro* and *in vivo*, which was previously identified only in mice. *AMOTL2* expression was also identified in the preimplantation embryo and hPSCs, suggesting that it might influence also earlier cell fate decisions.

In this thesis, I developed an AMOTL2 knockout (KO) hPSC line using CRISPR/Cas9 approach. During initial characterization, a strong phenotype concerning colony morphology emerged, with AMOTL2 KO hPSC colonies being more irregular and less tightly packed than in wild type (WT). Additionally, AMOTL2 KO hPSCs showed increased confluency compared to WT hPSCs, which was a result of increased proliferation and decreased apoptosis rates.

RNA-sequencing revealed substantial changes in terms connected with cytoskeleton, adhesion, and migration, development, cell metabolism, and signaling pathways. It also shown a potential developmental bias of AMOTL2 KO cells towards ectoderm, at the expense of endoderm and mesoderm, which was confirmed with spontaneous and directed differentiation. In search of the mechanism behind the differentiation bias, the F-actin depolymerization and increased YAP activity were identified as possible culprits. Surprisingly, spontaneous differentiation with F-actin depolymerizing agent, Y-27632, or YAP inhibitor, verteporfin, failed to repeat or rescue AMOTL2 KO developmental phenotype. This indicates that there are other, more potent players in the game, by which AMOTL2 controls cell fate, which, based on RNA-sequencing and preliminary functional data, might include energy production mechanism or cellular metabolism.