Epigenetically Acting Small Molecules Increase the Efficiency of iPSC Generation

Induced-pluripotent-stem cells (iPSCs) are de-differentiated somatic cells that possess the ability to transform into any of the three germ layers. iPSCs are important for the clinical development of patient-specific cells for transplants and to create organoids for drug discovery research. The first generation of iPSCs was accomplished by the Yamanaka method, which drives constitutive exogenous expression of four transcription factors (TFs; Oct4, Klf4, cMyc, Sox2) into the genome of somatic cells to induce pluripotency. Importantly, iPSCs generated via the Yamanaka method are reprogrammed inefficiently and may cause cancer. Recently, several small molecules that impact epigenetics have gained interest in cellular reprogramming due to their ability to activate the expression of endogenous reprogramming factors. We have identified five small molecules (Mocetinostat, Droxinostat, Tacedinaline, Entinostat, and Azacytidine) that enhance the activation of a silenced Oct4 locus (a phenotypic indicator of cell reprogramming) in CiA:Oct4 mouse embryonic fibroblast (MEF) cells by recruiting the transcriptional activator VP16 to that locus. Here, we combine the expression of the four Yamanaka method TFs using a polycistronic vector with treatment using the identified small molecules to test whether they increase the efficiency of induction of pluripotency. The treatment was assessed for a period of 30 days post-infection in an alternating manner. Azacytidine and Mocetinostat increased the efficiency of cellular reprogramming from 0.05%, as observed in the Yamanaka method, to 1.48% and 1.18%, respectively. Increasing the efficiency of cellular reprogramming using these small molecules may lead to advances in the translational and clinical uses of iPSCs as well as applications in other reprogramming methods such as neuronal transdifferentiation from somatic cells.