Germ Cell Nuclear Factor Regulates Craniofacial, Axial and Reproductive Development

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Germ cell nuclear factor (GCNF/NR6A1/RTR) is an orphan nuclear receptor essential for reproduction and embryonic development. Loss of GCNF in mouse results in neural tube closure defects, axial truncation, and failure of chorioallantoic fusion, culminating in embryonic lethality by E10.5. Expressed in the germ cells, placenta and developing nervous system, GCNF functions as a transcriptional regulator. During embryogenesis, GCNF is known to repress the pluripotency-associated factors *Oct4* and *Nanog*. However, little is understood about the network of genes regulated by GCNF beyond these factors that make the orphan nuclear receptor so crucial to development. Here, we describe an additional role for GCNF in embryonic development as a regulator of neural crest cell formation, epithelial-mesenchymal transition and survival. Neural crest cells are a transient migratory cell population that generate the craniofacial skeleton and peripheral nervous system among other tissues during embryogenesis. Specified along the dorsal axis of the embryo in the neural plate, neural crest cells undergo an epithelial-mesenchymal transition, delaminate and migrate to target destinations throughout the embryo for differentiation. *In situ* hybridization and transcriptomic analysis showed GCNF is expressed at the right time and location to regulate neural crest cell development during embryogenesis. Downregulation of neural crest cell-specifier and epithelial-mesenchymal genes revealed these processes were perturbed in GCNF null embryos. Lineage tracing confirmed GCNF null embryos have a deficiency in migratory neural crest cells, and immunostaining determined this was due to a disruption in the specification of neural crest cells within the neural plate. Neuroepithelial cells of the neural plate were maintained in a proliferative, undifferentiated state, aligning with GCNF's critical activity as a repressor of *Oct4* and *Nanog*. Competition binding, EMSA and targeted ChIP assays demonstrated GCNF directly binds to putative DR0 binding sites located in the promoter regions of neural crest cell-specifier and epithelial-mesenchymal genes. These results suggest GCNF acts as a regulator of neural crest cell formation and epithelial-mesenchymal transition through the repression of pluripotency-associated genes and activation of neural crest cell-specifier and epithelial-mesenchymal transition genes. Altogether, we have identified an important role for GCNF during embryogenesis as a regulator of neural crest cell development. Overall, our findings have expanded our understanding of the transcriptional network governed by GCNF and thereby have the potential to inform other processes affected by GCNF regulation such as axial elongation, germ cell development and reproduction.