Neurogenin 3 is a Critical Downstream Effector for STAT3-Regulated Differentiation of Male Germline Stem Cells

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Abstract

Sperm are vehicles for male genetic contribution to the next generation, and their formation relies on a series of germ cell differentiation events. Germ cell differentiation initiates with development of progenitors from a germline stem cell pool and further differentiation of these cells set the foundation for sperm production. Currently, the understanding of molecular pathways regulating differentiation is poorly understood within stem and progenitor germ cells. The molecule Signal Transducer and Activator of Transcription 3 (STAT3) regulates differentiation of progenitor spermatogonia but down-stream effectors are unknown. Also, expression of a second transcription factor Neurogenin 3 (Ngn3) coincides with formation of progenitor germ cells but a functional role in differentiation has not been described. In this study, we hypothesized that expression of Ngn3 is regulated by STAT3 in stem and progenitor germ cells to control differentiation. We found that expression of Ngn3 colocalizes with the activated form of STAT3 in progenitor spermatogonia, and Ngn3 expression is reduced upon inhibition of STAT3 signaling. Also, exposure to growth factors preventing differentiation of stem and progenitor germ cells suppressed activation of STAT3 and Ngn3 gene expression. Furthermore, STAT3 was found to bind the distal Ngn3 promoter region in progenitor spermatogonia and regulate transcription. Moreover, inhibition of Ngn3 expression in germline stem cells blocked the development of progenitors leading to germ cell differentiation arrest. Collectively, these results establish a critical mechanism for sperm production in mammals via activation of STAT3 regulating the expression of Ngn3 to subsequently drive differentiation of stem and progenitor germ cells.

Background / Methods

- Germine stem cells are the tissue specific stem cell population of the testis
- Only stem cell population to contribute to the next generation
- NGN3 is expressed in male germ cells
- Unique fate decisions of male germline stem cells
- Self-renew to maintain stem cell pool
- Differentiate to allow for continuous production of mature sperm throughout adulthood
- NGN3 expression is regulated by STAT3
- NGN3 is needed for proper germ cell differentiation

Hypothesis

STAT3 regulates expression of NGN3, which in turn promotes differentiation of male germline stem cells, to maintain production of sperm in adult mice.

Results

Do Ngn3 & STAT3 colocalize in male germ cells?

Yes. When cultured germline stem and progenitor cells are stained by antibodies that specifically recognize Ngn3 and the activated form of STAT3 (pSTAT3), colocalization is observed in all Ngn3+ cells. Approximately 95% of the germline stem and progenitor cells express pSTAT3 and ~30% express Ngn3, indicating that an overlapping subpopulation of these cells express Ngn3 (in green) and pSTAT3 (in red) simultaneously.

Does presence of self-renewal growth factor GDNF change the expression of STAT3 & Ngn3?

Yes. When cultured germline stem and progenitor cells are exposed to the self-renewal-promoting growth factor GDNF, activation of STAT3 protein and transcription of Ngn3 are suppressed. However, when GDNF is removed from culture media, STAT3 activation and Ngn3 transcription are significantly increased (1.5-fold and 5-fold, respectively). This negative regulation by the self-renewal growth factor GDNF indicates that STAT3 and Ngn3 play a role in germine stem/progenitor cell differentiation.

Conclusion

Together, STAT3 and Ngn3 regulate differentiation of male germline stem cells, which maintains sperm production in adult mice.