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RESEARCH HIGHLIGHT

What a difference a day makes! The contribution of intrinsic FGF9 signalling to germline masculinisation

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Although the key discovery of sex-determining region Y (*SRY*) 20 years ago as the essential sex-determining gene emerged from studies of sex-reversed humans,[1,2] most of our knowledge of how male and female germ cells diverge after sex determination comes from studies of mice. This is demonstrated in a recent publication from Bowles and colleagues in *Developmental Cell*, which documents the functional importance of fibroblast growth factor 9 (FGF9) signalling in murine fetal germ cells.[3] The study reveals events immediately following sex determination which influence how germline cells in the testis respond to FGF9 in a manner that reinforces the process of masculinisation. The picture that is coming into focus is of a signalling network which mediates the correct timing of progressive changes, thereby ensuring that germ cells and the somatic cells which create their niche develop together.

Retinoic acid (RA) produced by the mesonephros adjacent to the fetal gonad has recently been identified as the key stimulant for fetal germ cell entry into meiosis: its degradation by the Cyp26b1 enzyme within the testis ensures that most male germ cells continue mitosis and do not enter cell cycle arrest until postnatal processes trigger the first wave of spermatogenesis.[4,5] The additional and antagonistic involvement of FGF9 in driving male germline development was discerned by Bowles and colleagues from several experimental observations of germ cells in *Cyp26b1*-null testes. These XY germ cells are exposed to high levels of RA but do not fully adopt the phenotype of their female counterparts. Transcripts encoding meiotic markers that are usually upregulated in XX germ cells are only partially increased in XY *Cyp26b1*-null germ cells, and quite remarkably, the levels of the pluripotency markers Oct4 and Sox2 persist as usual for male germline cells. This provided a clue to the existence of another factor which sustained male pathway development, and FGF9 was an obvious candidate considering that previous studies had shown how its genetic deletion from mice caused male to female sex reversal.[6]

A remarkable observation reported in this manuscript was the identification in isolated embryonic day (E) 11.5 germ cells of a transcript encoding a fibroblast growth factor receptor isoform that binds FGF9 with high affinity. This FGFR2-IIIc transcript was characterized through reverse transcription-PCR. The authors have deduced, by extrapolation from previous publications, that this highlights a switch in receptor isoform synthesis between E10.5 germ cells[7] and E11.5, the time frame identified for germline gender commitment in the testis.[8] This isoform switch, which is expected to render the cells highly responsive to FGF9, is mediated by alternative mRNA splicing which alters the structure of the receptors' extracellular domain. Isoform switching in FGF receptors is a well-documented phenomenon that is known to trigger changes over a similar, short time span in other developmental processes, is linked with oncogenic changes and can be induced by exposure to other FGF ligands (e.g., Ref. 9). Identification of the local factor(s) which mediate this change in transcript processing within the developing testis will enhance our understanding of the processes of germline masculinisation.

The results of this study have reinforced the knowledge that male and female germline developments are mutually exclusive. The authors have shown that FGF9 actions are antagonistic to, and independent of, RA signalling. FGF9 is initially present in both male and female gonads but it is greatly elevated in the testis by E11.5. The addition of FGF9 to urogenital ridge cultures did not alter *Cyp26b1* transcript levels, nor did the addition of RA or absence of Cyp26b1 diminish FGF9 mRNA levels. However, the exposure of E11.5 XX urogenital ridges to FGF9 does diminish synthesis of the direct RA target, S*tra8*, and this appears to be by diminishing the responsiveness of XX germline cells to RA. As the field of *in vitro* germline genesis progresses in parallel, information about the pathway by which FGF9 drives male fate may offer the potential for efficiencies and gender selectivity in gamete derivation from embryonic and induced pluripotent stem cells. Similarly, the importance of learning how pluripotency is sustained is of clear importance to testicular cancer and may even be applicable to harnessing spermatogonial stem cells as a potential source of therapeutic materials.

The study by Bowles, Koopman and colleagues[8] represents another piece in the puzzle of sex determination which has recently progressed from being focussed on somatic cell events to one in which germline events are now being delineated. The use of mice to understand the fundamental events which shape our genetic material is essential, due to the ethical concerns and practical restrictions that limit work on relevant human samples. What do we know about FGF9 in human gonad biology? The study presented here suggests clear starting points for learning how diseases arising from intrinsic germline defects may emerge due to disregulation of FGF signalling.

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