irreversibly committed to only produce gametes. The process leading to this restriction of developmental potential of the germ-line, in any vertebrate, remains unknown.
Methods: Through genome-wide analyses of embryonic germ-line in humans and mice, we have identified a conserved developmental program, activated in PGCs after gonadal colonization, which demarcates definitive germ-cells from other somatic lineages. Through genetic studies in mice, we demonstrate that DAZL, is necessary *in vivo* for restriction of developmental potential. Germ-line cells deficient in *Dazl* migrate to nascent gonads but maintain prolonged expression of pluripotency factors (e.g. *Nanog*), and extended capacity for derivation of pluripotent cell-lines. This leads to spontaneous teratomas in both sexes of mice and in *DAZL*-deficient pigs. Further, germ-line cells failing to restrict their developmental potential usually undertake cell-death. By genetically attenuating apoptosis, *Dazl*-deficient male mice develop bilateral teratomas.
Results: We propose a revised model for germ-line lifecycle of mammals where migratory PGCs are developmentally uncommitted, and germ-line undertakes a restriction of developmental potency only after PGC colonization of gonad. Through comparative analyses, we infer that the germ-cell commitment program is likely operated in the common ancestor of all vertebrates. Finally, failure to complete this process of germ-cell commitment in the embryo, together with cell-death evasion, may account for the origin of mammalian germ-cell-tumours.

GCT-19 Generation of human primordial germ cell-like cell culture models reflecting genetic characteristics of human testicular Type II germ cell tumours for studying molecular events during early pathogenesis

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Background: The familial risk of testicular Type II germ cell tumours is among the highest in human malignancies, suggesting strong contributions of genetic factors to their pathogenesis. These tumours typically harbour iso-chromosome 12p while they have few gDNA mutations. Gain-of-function *KIT* mutations are detected in ~30% of seminomas while it is rare in non-seminomas. Whole-genome association studies identified a few linked loci, including *KITLG* and *BAK1*.
Methods: Human iPSC clones expressing gain-of-function KIT mutants (imatinib-resistant D816V and sensitive N822K) were generated using lentiviral vectors. Knockout clones lacking *BAK1*, *BAX*, or both were generated by CRISPR/Cas9. Clones harbouring trisomy 12p were generated by prolonged maintenance in the naïve pluripotency (5i/LAF) condition [1]. PGC-Like Cells (PGCLCs) mimicking migrating PGCs were generated from human iPSCs as we previously described [2].
Results: iPSCs expressing gain-of-function KIT mutants showed significant growth advantage in the primed-pluripotency culture condition. *BAK1/BAX* double-knockout hiPSCs were significantly resistant to the intrinsic-pathway apoptosis whereas single knockout showed minimal effects. Trisomy 12p did not affect growth or apoptosis of iPSCs. The KIT mutants, *BAK1/BAX* KO, or trisomy 12p – without combinations – did not cause apparent transformation of PGCLCs in embryoid bodies or cell culture conditions. Effects of combinations of these genetic manipulations are being examined.

References

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GCT-20 The molecular and (epi)genetic mechanisms driving microenvironment-triggered reprogramming of seminomas into an embryonal carcinoma cell fate

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Background: Testicular germ-cell-tumours (GCTs) are very common in young men and are stratified into seminomas and nonseminomas. While seminomas share a similar gene expression and epigenetic profile with primordial germ cells, the stem cell population of the nonseminomas, the embryonal carcinoma (EC), resembles malignant embryonic stem cells. Thus, ECs are able to differentiate into cells of all three germ layers (teratomas) and even extra-embryonic-tissue-like cells (yolk-sac-tumour, choriocarcinoma).
Methods: We demonstrated that cellular microenvironment considerably influences the plasticity of seminomas (TCam-2 cells). Upon microenvironment-triggered inhibition of BMP signalling pathway *in vivo* (murine flank/brain), seminomatous TCam-2 cells reprogram to an EC-like cell fate. We identified SOX2 as a key factor activated upon BMP inhibition mediating the reprogramming process by regulating pluripotency, reprogramming and epigenetic factors. Indeed, CRISPR/Cas9 SOX2-deleted TCam-2 cells were able to maintain a seminoma-cell fate *in vivo* for about six weeks, but small sub-populations still initiated differentiation – potentially driven by FOXA2, since many FOXA2-interacting genes and differentiation factors like AFP/EOMES/CDX1/ALB/HAND1/DKK/DLK1/MSX1/PITX2 were upregulated. We generated TCam-2 cells double-deficient for SOX2+FOXA2 using the CRISPR/Cas9 technique and xenografted those cells into the flank of nude mice.
Results: Upon loss of SOX2 and FOXA2, TCam-2 maintained a seminoma cell fate for at least twelve weeks, demonstrating that both factors are key players in the reprogramming to an EC-like cell fate. Therefore, our studies add important pieces to the puzzle of GCT development and plasticity, providing interesting insights in what can be expected in a patient, when GCT cells are confronted with different microenvironments.

GCT-21 Testicular cancer genomics England Clinical Interpretation Partnership (GECIP): A genomic exploration of testicular germ cell tumours

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Background: The characterisation of testicular germ cell tumours (TCGTs) has to-date been limited to panel or whole-exome sequenced