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

(WES) datasets. By using the full set of whole-genome sequenced (WGS) TCGTs from the Genomics England platform, we intend to fully characterise TCGTs, thereby contributing substantially to the knowledge underpinning effective genomic testing for this disease. This work will validate and facilitate the identification of genomic changes at the time of TGCT diagnosis, which may ultimately assist and influence effective clinical management.

Methods: We increase the discovery power for novel SNV, indel, copy number, and structural variant drivers of TGCTs by using a set of ~50 fresh-frozen, WGS tumours. After applying a rigorous quality control process to the provided variants, we use multiple tools separately and in combination to elucidate the various genomic aberrations present in TGCTs. This includes: copy number variants, structural variants, coding, noncoding, germline, and somatic drivers, the presence of selection, the variety of mutational signatures, the heterogeneity (subclonality) present, and the ordering of mutational events. In addition, we separate the sample set in multiple directions (seminomatous:nonseminomatous, primary:metastasis, early:late onset, etc) to explore clinical stratifiers.

Results: Early analyses have identified novel mutational drivers, copy number aberrations, and structural variants. We are exploring the subclonality present, categorising drivers, copy number aberrations, and structural variants as predominantly clonal or subclonal, alongside timing these various aberrational events. Based on this, we will categorise TGCTs into genomic groups, which may prove useful for clinical management.

GCT-22 Targeting the germline-specific gene regulatory network in testicular germ cell tumours

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Background: Germ cell neoplasia *in situ* (GCNIS), the common precursor of seminomas and non-seminomas, is thought to originate from arrested foetal germ cells. Previous studies have established that GCNIS cells shared similar morphology and gene expression pattern with human primordial germ cells (hPGCs) in the embryo. We hypothesize that hPGCs and GCNIS share a germline-specific gene regulatory network and such a common pathway could be used as a therapeutic target for testicular germ cell tumours (TGCTs).

Material and methods: We performed RNA-sequencing analysis on hPGCs, GCNIS, and the TCam-2 seminoma cell line to identify the gene regulatory networks in germ cell development and cancer. We used ex vivo foetal and adult testis culture systems to investigate the function of the common molecular pathway in hPGCs and GCNIS.

Preliminary results: We found that hPGCs, GCNIS, and TCam-2 share the expression of critical germ cell transcription factors SOX17 and OCT4. SOX17 physically interacts with OCT4 to establish and maintain the germ cell transcriptional network. Inhibition of OCT4-SOX17 interaction by a small molecular inhibitor abrogated hPGC development. Strikingly, application of the inhibitor to ex vivo cultured testicular cancer tissues completely eliminated the GCNIS cells within a week. We demonstrate that mechanistic insights into human germ cell development could lead to a new therapeutic strategy for TGCTs.

Biology – Models To Understand GCT Pathogenesis

GCT-23 DNA damage response mechanisms in the origins and therapeutic sensitivity of testicular germ cell tumours

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Background: Testicular-germ-cell-tumours (TGCTs) differ from other solid cancers in terms of their DNA damage response (DDR) features as well as their striking hypersensitivity to conventional genotoxic chemotherapy. Whereas solid cancers of somatic origins typically activate the DDR early in tumorigenesis and subsequently accumulate mutations in DDR genes, TGCTs appear to lack early-stage DDR activation and rarely acquire mutations in DDR genes like *p53* and *ATM*.

Methods: To elucidate the underlying mechanisms, we developed a mouse TGCT model featuring germ cell-specific oncogenic *Kras* activation and tumour suppressor *Pten* deletion.

Results: The resulting mice rapidly developed metastatic testicular cancers composed of both teratoma and embryonal carcinoma (EC), the latter of which exhibited stem cell characteristics, including expression of the pluripotency factor OCT4. Treatment of tumour-bearing mice with genotoxic chemotherapy prolonged survival, reduced tumour size, and selectively eliminated the OCT4-positive EC cells. Consistent with studies of human TGCTs, the murine cancers lacked early-stage DDR activation but mounted a robust DDR after chemotherapy treatment. EC cell lines were created from the murine tumours and, upon transplantation, generated teratocarcinomas that were indistinguishable from primary TGCTs. *In vitro* differentiation of EC cultures resulted in loss of tumour propagating activity and reduced genotoxin sensitivity, consistent with the findings that EC cells function as chemosensitive cancer stem cells *in vivo*. On-going studies aim to identify molecular mechanisms responsible for the differences in chemoresponsiveness between EC cells and their differentiated derivatives, which we hypothesize may be due in part to differential regulation of DNA damage repair and tolerance pathways.

GCT-24 Integrated genomic analysis reveals aberrations in WNT signaling in germ cell tumours of childhood and adolescence

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