Mutational Landscape of Adult Granulosa Cell Tumors of the Ovary from Whole Exome Sequencing

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Background

- Granulosa cell tumors (GCT) are the most common type of malignant ovarian sex-cord stromal tumor (1).
- Adult GCT are defined by the presence of the C134W somatic mutation in the FOXL2 gene which was identified using RNA-Seq of 4 GCT (2).
- GCT are generally regarded as having a better prognosis than epithelial tumors, however they have a propensity for late recurrence.
- As all GCT have the FOXL2 mutation, subsequent genetic events must determine recurrence and/or aggressive behaviour.
- Transcriptomic analysis comparing stage 1 with stage 3 GCT shows remarkable homogeneity (3) .
- At present, there are no reliable methods of predicting relapse, nor are the molecular mechanisms of relapse or aggressive behaviour understood.
- **Whole exome sequencing (WES)** provides a powerful strategy to identify potential somatic driver mutations in malignancy (4).

Aims

We sought to identify the additional somatic mutations responsible for recurrence and/or aggressive behaviour in adult GCT by:

1) WES of stage 1 and stage 3 GCT to identify driver somatic mutations;
2) Copy number analysis of the WES data to identify large scale; chromosomal loss or duplication; and
3) Targeted PCR of the *TERT* gene promoter to seek the known mutations *C228T* and *C250T*.

Methods

Tissues

Tumoral DNA from 22 fresh frozen, FOXL2 C134W mutation positive GCT (14 x stage 1 and 8 x stage 3) were subjected to WES. Two of the stage 3 GCT also had paired peripheral blood samples.

Whole exome sequencing (WES)

WES was performed using the SureSelect XT2 Human All Exon V5 capture system (Agilent) and sequencing was performed on a HiSeq 1500 (Illumina). The initial variant predictions were filtered to require that the variant was present in ≥33 bidirectional reads, with the variant allele frequency ≤ 0.70. Remaining germline SNPs or common sequence artefacts were eliminated by comparing against a database of 147 in-house germline exome sequences, as well as the 1000 Genome, Exome Variant Server and Exome Aggregation Consortium databases.
A subset of these variants has been independently validated by Sanger sequencing.

Copy Number Analysis

Copy Number Analysis from the WES data was performed using the program ADTEx (Aberration Detection in Tumor Exome) (5).

TERT Mutation Status

The *TERT* promoter is not targeted in the Agilent exome capture system so the *TERT* gene promoter region was amplified from the tumor DNA and additional samples (3) using nested PCR: 1st round primers: Forward 5'-ACGAACGTGGCCAGCGGCAG-3'; Reverse 5'-CTGGCGTCCCTGCACCCTGG-3' with an annealing temperature of 62C. 2nd round primers: Forward 5'–CAGCGCTGCCTGAAACTC-3'; Reverse 5'-GTCCTGCCCCTTCACCTT-3' with an annealing temperature of 55C. The amplicon was subjected to Sanger sequencing to identify the known hot-spot mutations in the telomerase promoter, *C228T* and *C250T*.

Results

WES

The FOXL2 mutation was confirmed in all cases, a de facto positive control. Initial analysis identified on average **64 coding and essential splice-site variants** (SNV and/or indels) in each GCT (4). The matched germline data was used as reference for the 2 paired samples in a separate analysis: this identified **15 coding SNVs** only across the 2 paired samples (4).

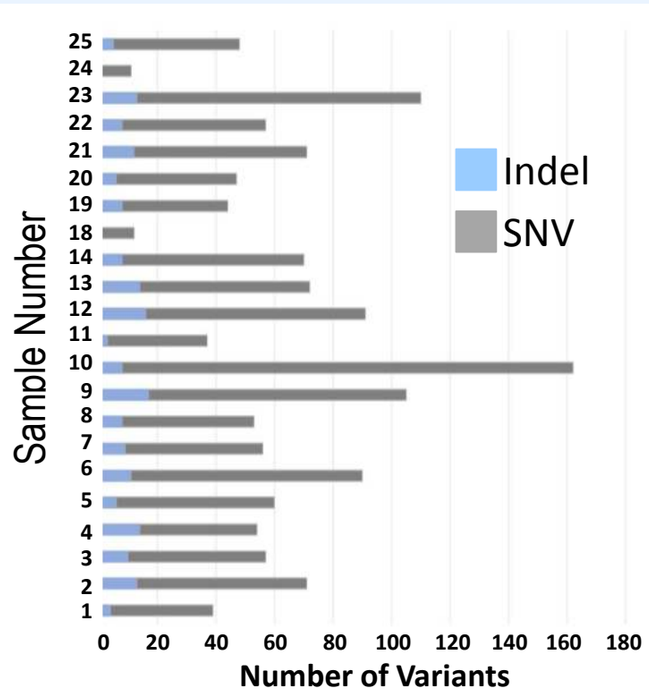

Figure 1: *Distribution of SNV and indel mutations across each sample*

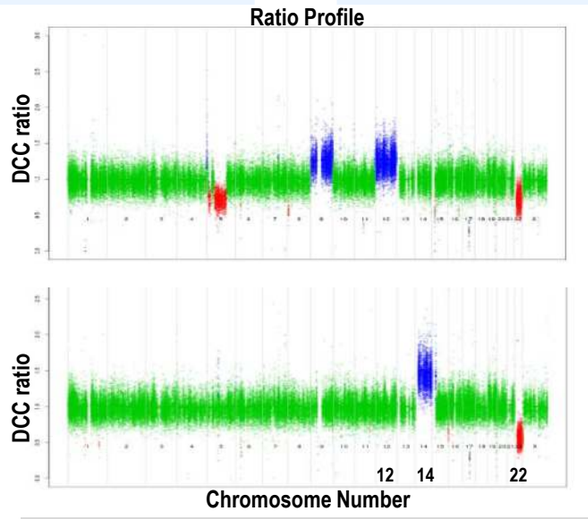

Figure 2: *Representative copy number profiles for 2 tumors demonstrating the three common changes: trisomy at chromosomes 12 (Panel A) & 14 (Panel B) and monosomy at 22 (Panels A and B). Other changes are also seen.*

Recurrent mutations were not identified in specific genes or in a pathway analysis. Potential driver mutations in genes of known oncogenic or granulosa cell function were verified in individual tumors including: **XIAP** (X-linked inhibitor of apoptosis) which is overexpressed in GCT and a potential therapeutic targetand **SMAD3** which mediates TGFβ/activin signaling which plays a central role in granulosa cell biology (4).

Copy number analysis

Copy number analysis achieved adequate resolution in 15 of the 22 GCT. This confirmed previous cytogenetic observations that trisomy at chromosomes 12 and 14 occurs in ~30% (our results 4/15 = ~27%) of GCT and monosomy at 22 in ~40% (Our results show 8/15 = ~53%); other large scale changes are more random and less frequent (4).

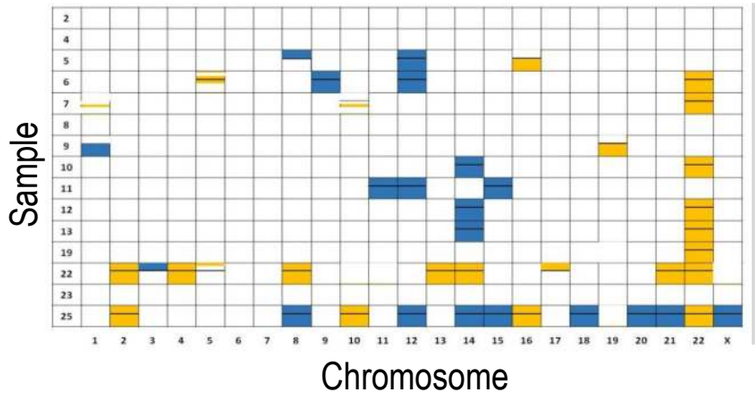

Figure 3: *Schematic representation of the changes observed in those samples with adequate copy number resolution. In each box the short arm is at the top. Yellow represents monosomy and blue trisomy.*

TERT Promoter Mutation Status

The *TERT* gene encodes the catalytic subunit of telomerase. Elongation/preservation of telomere length is regarded as a hallmark of cancer. Two hot-spot mutations in the telomerase promoter, *C228T* and *C250T*, are commonly found in cancer.
11 of 26 i.e. 42% of the GCT in our analysis were heterozygous for the *C228T TERT* promoter mutation; a frequency that is at least as high as that seen in papillary thyroid cancer. 29% of stage 1 GCT (5/17) versus 67% of stage 3 GCT (6/9) were heterozygous for the mutation; the *C250T* mutation was not found (4).The mutation is also present in the human GCT-derived FOXL2 C134W mutation positive KGN cell line (1).

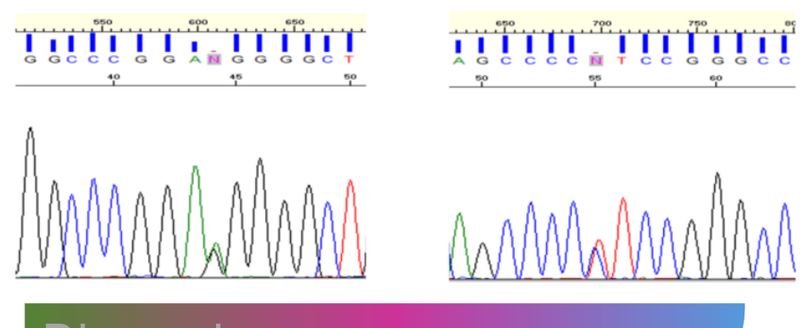

Figure 4: *Representative sequencing chromatograms of both strands showing the C228T TERT promoter mutation in GCT.*

Discussion

- This first comprehensive exome-wide analysis of the mutational landscape of GCT does not demonstrate recurrent mutations that define tumor recurrence and/or aggressive behaviour. This suggests that "second-hit" mutations are totally random events or that, as in other cancer types, translocation events should be sought.
- The functional significance of the copy number changes needs further characterization, although the observed changes do not correlate with tumor stage or behaviour.
- The high incidence of the TERT promoter mutation, which is twice as frequent in the advanced disease, raises the possibility that it may be of prognostic significance as is seen in thyroid cancer. Our results are consisted with a recently published report showing similar findings (6)

References

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