Background and Results

Background: Granulosa cell tumors (GCTs) are rare tumors in ovaries accounting for 2% of all ovarian cancers. GCT malignancies are often low-grade with a five-year survival rate up to 90%, however a clinical characteristic of these tumors is a tendency for late recurrence and a high recurrent rate is the most critical factor for GCT death. As GCTs are rare tumors and tissue availability is very limited, we used a dual multiplexing approach in order to maximize the data output from a total of 14 FFPE rare GCT samples. Gene expression levels in these 14 GCT samples were compared to levels in 5 high-grade serous ovarian cancer (HGSOC) FFPE samples.

Methods: For protein multiplexing we have used MultiOmyx™, an immunofluorescence (IF) multiplexing assay utilizing a pair of directly conjugated Cy3, Cy5, and Cy7 antibodies per round of staining. Each round of staining is imaged and followed by dye inactivation, and dense-learning based cell classification algorithms identify positive cells for each gene. Expression analysis was done using the Nanostring PanCancer immune gene 770 gene panel assay. RNA was extracted from the adjacent 10 μm section and then processed with hybridization, purification and immobilization and quantified on manufacturer’s protocol.

Results: On protein level we confirmed previous findings that ovaries are so-called “cold” tumors, with a very low density of T cell infiltration which is even reduced further in GCT samples compared to HGSOC samples. This is also reflected by a significant decrease in the gene score for cytotoxicity. When analyzing tumor markers (S100, viimentin, and pan-cytokeratin in GCT samples we observed an almost complete loss of cytokeratin, but increases in vimentin and S100 protein level), and a highly significant mRNA decrease in MUC1 which codes for a negative marker, for adult GCT. On both mRNA and protein level we found a reduction in macrophages in GCT samples, and on protein level we also observed a reduction in proliferation marker Ki67. Density of angiogenic vessels in the GCT microenvironment was increased, possibly linked to a high significant increase in NSCL A mRNA which codes for the protein nitric oxide synthase (NOS2), a known modulator of angiogenesis.

NanoString nCounter data – Cell Type Profiling & Differential Expression

Reduced expression of genes for cytotoxic cells and TAMs in GCT samples

MultiOmyx Overlay Images – Tumor Markers, TILs, TAMs, & Vessels

Decrease in cytokeratin, CTLs, TAMs, Ki67 in GCT samples, but increase in S100, vimentin & CD34.

MultiOmyx Data – Protein Density Quantification

Key Findings

- We have used a dual multiplexing approach to immunoprofile the tumor microenvironment of rare ovarian Granulosa Cell Tumor FFPE samples on both mRNA level (Nanostring nCounter assay), and protein level (MultiOmyx analysis).
- Gene signatures and protein levels for T cytotoxic lymphocytes and tumor-associated macrophages are reduced in GCTs compared to control HGSOC tumors.
- Angiogenic vessel protein density is increased in GCTs, possibly linked to a highly significant over-expression of the gene for NOS2 which is a modulator of angiogenesis.