Translational Biology and MicroRNAs

GCT-44 An overview of circulating microRNAs for the management of germ cell tumours

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Background: The current biomarkers AFP/HCG have limited sensitivity/specificity for diagnosing malignant germ-cell-tumours (GCTs). MicroRNAs are non-protein-coding RNAs that regulate gene expression. We previously showed that microRNAs from the miR-371–373 and miR-302/367 clusters are universally overexpressed in all malignant GCT tissues, regardless of patient age, tumour site or histological subtype. These microRNA clusters are not co-ordinately over-expressed in any other cancer type or disease state. These microRNA characteristics lend themselves to being promising candidate biomarkers.
Material and methods: The Cambridge team developed a highly sensitive pre-amplified qRT-PCR technique for the robust detection of microRNAs from the miR-371-373 and miR-302/367 clusters in circulating biospecimens from patients with malignant GCTs, now adopted by multiple other groups. The pipeline includes quality control checks and the use of an exogenous spike-in control and the endogenous microRNA miR-30b-5p for normalisation purposes, prior to data analysis.
Results: Results from our and other groups show that a four-serum miRNA panel (miR-371a-3p, miR-372-3p, miR-373-3p and miR-367-3p) shows high sensitivity/specificity for diagnosing malignant GCTs. Of these, miR-371a-3p individually shows the most utility as it is most dynamic and most accurately reflects disease activity. These microRNA levels are useful for disease-monitoring and early detection of relapse. They should improve future clinical management of patients with malignant GCTs, in particular reducing CT scans in follow-up and identifying patients with apparent clinical stage I (CSI) seminoma who have persistently elevated serum microRNA levels post-orchidectomy (suggestive of micrometastatic disease), who may benefit from adjuvant chemotherapy to prevent subsequent recurrence.

GCT-45 Insights into the mechanisms of expression and (specific) secretion of microRNA (-371a-3p) in malignant paediatric and adult germ cell tumours: towards a clinical applicable protocol

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Background: An intriguing characteristic of all malignant germ-cell-tumours (GCT) is expression of microRNA cluster miR-371-3 on chromosome 19, normally only found during embryonic development. No explanatory mechanism has been identified yet, although the promotor shows consistent demethylation. The specific member of this cluster, miR-371a-3p, has been proven to be highly informative as a liquid biopsy-based biomarker (serum/plasma and cerebrospinal fluid) at diagnosis and during follow-up. However, relevant information is still lacking.
Methods: Haemolysis effects on serum/plasma results in >700 samples, obtained by magnetic bead-based miR-purification protocol, was studied. Detailed evaluation was done to elucidate mechanism(s) of miR(-371a-3p) expression/secretion. Therefore (GCT) cell lines (n = 5) with matched conditioned media, mouse xenografts and matched plasma, as well as primary GCT and matched serum were investigated using a high-throughput miR-profiling approach. In addition, cell lines were cultured under various *in vitro* conditions, to investigate the mechanism of miR secretion.
Results: Haemolysis only impacts in case of high severity. A significant difference between serum and plasma was identified on the normalization target miR-30b-5p (but not ath-miR-159 used for calibration). Therefore, serum and plasma samples should not be used in same evaluation. A highly selective, and stable, secretion pattern was identified compared with cellular expression. Secretion was highly robust and extremely fast (within minutes), temperature/proliferation-independent, and exosome-mediated. While all miR-371a-3p molecules were exosome-packaged, this was only partial for miR-30b-5p. No improvement of assay-sensitivity was obtained using exosome-purification instead of miR-bead-based isolation. These data are relevant in development of final standardized miR-371a-3p detection protocols for clinical implementation.

GCT-46 Serum microRNA-371a-3p levels predict viable germ cell tumour in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection

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Background: Serum microRNAs (miRNAs) are emerging as candidate biomarkers for diagnosing and monitoring germ cell tumours (GCTs). However, the ability of miRNA to inform treatment in low-stage chemotherapy-naïve patients has not been explored. We evaluated the performance of serum miRNA to predict viable GCT in chemotherapy-naïve patients undergoing primary retroperitoneal lymph node dissection (RPLND).
Methods: We prospectively collected presurgical serum samples from consecutive GCT patients undergoing primary RPLND at our institution from 2016–2019. Serum miRNAs (-367-3p/-371a-3p/-372-3p/-373-3p/-375) were isolated and quantified. RPLND histopathology was categorized as benign, viable GCT, or teratoma. miRNA levels were compared among groups. Receiver operating characteristic (ROC) curves were used to assess the discriminative ability of each miRNA signature to predict viable GCT.
Preliminary Results: 24 patients with stage I-II GCT underwent primary RPLND, revealing viable GCT in 11 (46%), teratoma in 3 (13%), and benign pathology in 10 (42%) patients. miR-371a-3p was the most discriminatory serum miRNA for viable GCT, exhibiting ~13,000-fold increase in expression over benign pathology. On ROC analysis, miR-371a-3p had AUC = 0.965, with sensitivity and specificity of 100% and 92%, respectively. AUCs for other serum miRNAs in predicting viable GCT were 0.874 (miR-367-3p), 0.846 (miR-372-3p), and 0.720