(miR-373-3p). These miRNAs were not predictive of pure teratoma. Serum miRNAs, particularly miR-371a-3p, can accurately differentiate small-volume viable GCT from benign processes or teratoma in patients undergoing primary RPLND, even with normal serum tumour markers. If validated in larger cohorts, these data suggest a basis to implement precision medicine strategies in treating patients with early-stage GCT.

GCT-47 Targeting oncogenic microRNAs in malignant germ cell tumours with locked nucleic acid-based inhibitors

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Background: MicroRNAs (miRNAs) are short, non-protein-coding RNAs that regulate gene expression, primarily through mRNA degradation. We have shown that all malignant germ-cell-tumours (mGCTs) overexpress two miRNA clusters, miR-371~373 and miR-302/367, resulting in downregulation of functionally significant mRNAs/pathways. Here, we inhibited these overexpressed miRNAs in mGCT cells *in vitro* to determine their functional significance and explore their potential role as therapeutic targets.
Methods: qRT-PCR confirmed that miR-371~373 and miR-302/367 levels in mGCT cell lines [seminoma (SEM), yolk sac tumour (YST) and embryonal carcinoma (EC)] corresponded to those in tissue samples of the relevant tumour subtype. MiRNA inhibitors composed primarily of locked nucleic acids (LNAs) were designed to target key mature miRNAs. Gene expression profiling of cells treated with inhibitor or control was performed using mRNA microarray. *Sylamer* analysis was used to identify direct mRNA targets of these miRNAs, and key pathways analysed using KEGG and Reactome for functional validation.
Results: Targeting mature miRNAs of the miR-302 family (miR-302a-d) using LNA-based inhibitors resulted in reproducible growth inhibition in SEM and YST cells, although EC cells were more resistant to treatment. In the mGCT cells with reduced growth, *Sylamer* revealed enrichment of upregulated mRNAs that contained the seed-complementary-region for miR-302a-d in their 3' untranslated regions. Pathway analysis of these upregulated mRNA targets primarily demonstrated involvement in cell cycle regulation. This was functionally validated through cell cycle analysis using flow cytometry. LNA-based miR-302 family inhibition results in cell cycle arrest in two main subtypes of mGCT and may represent a new therapeutic approach.

GCT-48 Analysis of circulating cell-free DNA identifies several gene mutations associated with germ cell tumours

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Background: Circulating cell-free DNA (cfDNA) has been described in various malignancies as a diagnostic and prognostic biomarker. We analyzed the significance of cell-free DNA in children with germ cell tumours (GCTs).
Methods: Cell-free DNA was isolated from the serum of 20 patients with GCTs, including 3 mature and 2 immature teratomas, and of 5 healthy individuals and then analyzed by next generation sequencing (NGS), using a custom cancer hot-spot panel covering hot-spot regions of 50 genes.
Results: In a total of 25 cfDNA samples analyzed, 8 had a pathogenic mutation in target genes, such as *TP53*, *IDH1*, *NOTCH1*, *PIK3CA*, *PTEN*, *SMAD4*, and *SMARCB1*. Seven tumours with pathogenic mutations in genes were advanced stages of mixed germ cell tumours, embryonal carcinoma, choriocarcinoma or yolk-sac tumour. One immature teratoma case showed a SMARCB1 mutation, which contained nephroblastoma component in this tissue. No hot-spot mutations were detected in the healthy individuals. Circulating cfDNA analysis using targeted NGS provided multi-gene detection that can be useful as a noninvasive and senstive tool for mutation profiling in GCT patients. This technology might provide both diagnostic and prognostic information in GCT. We should select target genes for practical and suitable cfDNA analysis in clinical settings for GCTs.

GCT-49 Mechanism and functional significance of downregulated microRNA expression in malignant germ cell tumours

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Background: Chemotherapy for malignant germ-cell-tumours (mGCTs) results in substantial long-term side-effects. Accordingly, identifying novel therapeutic targets is a priority. MicroRNAs are short, non-protein-coding RNAs that regulate gene expression. We previously showed that miR-99a-5p/miR-100-5p (which are functionally synonymous) and miR-125b-5p are universally under-expressed in mGCTs. Here, we investigate the causes and consequences of such under-expression.
Methods: qRT-PCR suggested that miR-99a-5p/miR-100-5p and miR-125b-5p levels were co-regulated in mGCT cell lines [seminoma (SEM), yolk-sac-tumour (YST), embryonal carcinoma (EC)], as well as corresponding tumour tissue subtypes. Cells were treated with the DNA-demethylation agent 5′-azacytidine and pyrosequencing done to elucidate upstream causes of microRNA under-expression. Mimics for miR-100-5p and miR-125b-5p were used in combination to treat mGCT cells *in vitro*, compared with control-treated cells. Gene expression profiling was performed using mRNA microarrays. The bioinformatics algorithm *Sylamer* was used to identify direct mRNA targets of the miRNAs and the cellular pathways affected.
Results: 5′-azacytidine treatment caused up-regulation of all three miRNAs (miR-99a-5p/miR-100-5p/miR-125b-5p). Pyrosequencing revealed DNA-hypermethylation of the miR-99a-5p/-100-5p and miR-125b-5p loci in mGCT cell lines. Combined miR-100-5p/miR-125b-5p mimic treatment resulted in reproducible growth inhibition in SEM and YST cells, although EC cells were more resistant. In mGCT cells with reduced growth, *Sylamer* revealed enrichment of downregulated mRNAs that contained the seed complementary region for miR-100-5p/miR-125b-5p in their 3′ untranslated regions. These downregulated mRNA targets were primarily involved in pro-proliferative pathways. miR-99a-5p/miR-100-5p and miR-125b-5p are silenced by DNA-hypermethylation and are potentially important in mGCT progression. Replenishment of these miRNAs may offer a novel therapeutic approach.