RAD51D-c.620C>T;p.S207L mutated cells. By co-immunoprecipitation and *in-vivo* single cell colocalization assays we show that this mutation impairs HR by disrupting the RAD51D-XRCC2 interaction. Sensitivity to PARP inhibitors (PARPi) was confirmed in RAD51D-c.620C>T; p.S207L mutant cells .

Conclusions: This work identifies *RAD51D*-c.620C>Tp. S207L as the first *bona fide* pathogenic missense susceptibility allele for HGSC of the ovary and supports the use of targeted PARPi therapies in OC patients carrying missense *RAD51D* mutations.

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Extended RAS minor variant detection by Sanger sequencing from FFPE samples to a 5% Limit of Detection

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RAS mutational testing is frequently performed by clinical researchers because of a demonstrated strong correlation between RAS mutational profiles of colorectal cancers and their anti-EGFR response. Sanger sequencing is an ideal choice for detection of RAS mutations due to its high accuracy, fast turnaround time, simple workflow and cost-effectiveness. When combined with Minor Variant Finder software, Sanger sequencing enables high sensitivity with variant detection down to a 5% Limit of Detection.

We have developed an extended RAS Sanger sequencing panel targeting eight hot-spot regions of KRAS and NRAS genes (codons 12–13, 59–61, 117 and 146). Besides these hotspot codons, the panel is capable of detecting any variants along the entire amplicons. The panel was optimized for low amount of FFPE DNA input, down to 1 ng/reaction.

To further streamline the workflow for the user, we designed and developed 96-well plates pre-loaded with the eight hot-spot primer pairs so only the PCR mix and templates need to be added. The same plate migrates through the entire workflow, minimizing risk of sample mix-up or contamination.

The 96-well plates were tested using hot-spot positive FFPE control DNAs, and Minor Variant Finder successfully identified the expected 5% variant allele frequency. Additionally, variants from numerous FFPE DNAs derived from colon cancer biopsies with variant allele frequencies ranging from approximately 5% to 80% were also successfully identified.

These extended RAS plates performed well on all models of Applied Biosystems capillary electrophoresis instruments that were tested.

For Research Use only. Not for use in diagnostic procedures.

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P12.173A

Expression profiles of miRNA genes in clear cell renal cell carcinoma patients

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Introduction: Renal cell carcinoma (RCC) constitutes about 3% of all human cancers. Clear cell renal cell carcinoma (ccRCC) is the most common subtype at diagnosis and accounts for 75–88% of RCCs. MicroRNAs (miRNAs) are small noncoding RNAs that play a key role in cancer pathogenesis and are involved in several human cancers, including ccRCC.

Materials and Methods: A global miRNA profiling study to identify a specific miRNA signature characterizing ccRCC was performed at 18 samples of tumor and 6 samples of normal kidney tissue of ccRCC patients using OpenArray technology at QuantStudio 12K Flex Real-Time PCR System. In total, 758 miRNA genes were analyzed using TaqMan OpenArray MicroRNA Panel. Validation study was performed using TaqMan qRT-PCR assays at 48 ccRCC samples.

Results: Microarray analysis identified two over-expressed microRNA genes in tumor kidney tissue - miR-210 (FDR p-value=0.033) and miR-642 (FDR p-value=0.021). After validation only miR-210 showed statistically significant expression levels between normal and tumor kidney tissue (p-value=0.0134). There is a large amount of data that miR-210 is the most ubiquitously

upregulated miRNA in different cancers including renal cancer. Recently a few studies showed that changing of the microRNA-210 expression levels is directly related to hypoxia and HIF1α activity, key components of tumorigenesis.

Conclusions: Our results suggest that microRNA gene expression alteration may contribute to the genetic predisposition for kidney cancer and may serve a diagnostic marker of the disease. The work was supported by RFBR grant №14-04-97083.

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Germline nonsense mutations of the *SMARCB1* gene in Russian patients with rhabdoid renal tumors

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The malignant rhabdoid tumor (RT) is one of the most aggressive childhood neoplasms. RTs are characterized by the presence of inactivating mutations in the *SMARCB1* (*hSNF5/INI1/BAF47*) gene - a tumor suppressor localized in 22q11.2. The *SMARCB1* gene is involved in chromatin remodeling. Up to 30% of RTs are caused by germline mutations of this gene, to date those cases are considered as a manifestation of the rhabdoid tumor predisposition syndrome type 1 (RTPS1). We have analyzed the *SMARCB1* mutations for improving of genetic laboratory diagnostics of the RTPS1, as well as searching of genotype-phenotype correlations in this disease. Genomic DNA was isolated from blood samples of 18 patients with RT in different localizations. Then *SMARCB1* exons 2–9 were amplified by PCR and subsequent Sanger sequencing with the 3500xl ABI capillary genetic analyzer was performed. Three patients had *de novo* nonsense mutations c.157C→T (p. R53*), c.669_670del (p.C223*) and c.843G→A (p. W281*), confirming RTPS1, which were associated with RT in the kidney, early age at diagnosis (median 2.6 months) and poor prognosis. High frequency of the nonsense mutations corresponds to data obtained by other authors in the studies of renal RTs, but not of RTs in other localizations. This study is a first experience of *SMARCB1* mutation testing in Russian population to our knowledge. Identification of germline *SMARCB1* mutations in the patients with RTs is essential to assess the risk of metachronous tumors and for genetic counseling of other family members.

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RET-gene rearrangements in papillary thyroid carcinoma subtypes

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RET gene rearrangement is considered the second most important mutational event involved in the occurrence of papillary thyroid carcinoma (PTC). Aim of the study was to investigate the occurrence of RET gene rearrangements in tumor cells from different PTC subtypes: classic PTC (cPTC), follicular variant (fvPTC) and aggressive forms (AGR). Patients and methods: Paraffin-embedded tumor tissues from 57 PTC patients were examined for RET rearrangements using fluorescence *in situ* hybridization (FISH) technique: 12 cPTC cases, 31 fvPTC cases and 14 aggressive PTC cases. FISH was performed with the ZytoLight SPEC RET Dual Color break-apart probe (ZytoVision GmbH, Germany). At least 100 non-overlapping nuclei were taken into account and were analyzed with Leica CW4000 CytoFISH program to indicate the percent of positive RET-split cells. **Results:** The percent of positive RET-split cells ranged from 5% to 95%. We established 10% RET-split positive cells as the cutoff level to consider this event as a clonally one. In cPTC cases the positivity was registered in 8 cases (66.67%), in 13 fvPTC cases (41.94 %), and in 6 AGR cases (42.86%). Overall, RET gene rearrangements was encountered in 47.37% of cases, with 52.63% of tumors having positive RET-split cells below 10%. Split level over 20% is correlated with the invasiveness of tumor. Conclusion: The frequency of RET gene rearrangements in this study is similar with those