Identification of Actionable Cancer Genes and Treatment Options for Metastatic Ovarian Carcinomas using Patient Derived Xenografts (PDXs) and PDX Derived Tumor Cells (PDTCs)

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ABSTRACT

Patients with advanced ovarian cancers have experienced little improvement in overall survival and standard treatment has not much progressed beyond cytoreductive surgery and platinum-based combination chemotherapy. Besides targeted anti-angiogenic and anti-PARP1 therapies, matching individual most critical genomic alterations with the best available drugs has not advanced as in other cancers, likely because a handful of cancer-related genes are mutated at high frequency, while many more are found mutated at much lower frequencies. This so-called “mutation tail” is not only long but also mostly unexplored. We used Patient Derived Xenografts (PDXXs) and PDXX Derived Tumor Cells (PDTCs) to identify actionable cancer genes and to accelerate the discovery of treatment options. We envisioned that the alleged weakness of PDX models, i.e., lack of human stromal and immune cells, might be instrumental to identify mutations in cancer genes and to test approved or experimental targeted drugs as monotherapy or in different combinations to link genetic biomarkers to treatments. Forty-three PDXX lines from metastatic epithelial ovarian carcinomas have been propagated and fully characterized as far as histology, immunohistochemistry of epithelial and high-grade serous-specific markers and NGS of TP53 and BRCA1/2. Whole Exome Sequencing (WES) and copy number analyses of NGS samples were performed on 12 PDXX lines derived from naive metastatic high-grade-serous epithelial ovarian carcinomas. We studied non-synonymous mutations with suitable allele frequencies in cancer genes reported in databases. TP53 and PIK3CA mutations were detected in PDXX lines 1.4 actionable genes were identified. In one line a possibly driver mutation was found in the PIK3R1 gene, encoding the p85alpha regulatory subunit of PI3K. This likely loss of function missense change had an allele frequency=0.9 in early and late passed PDXXs. Moreover, the mutation was also detected in DNA extracted from two microdissected FFPE samples of the source tumor, with an allele frequency nearly identical to that of metastatic TP53. Hence, this is a likely truncation mutation in the PDXX line and possible in the source tumor. Notably, ONVs of early and late passages of these PDXX line were almost identical. Treatment options were assayed ex vivo, on short-term cultivated PDTCs of this PDXX line. Buparlisib showed the ability to block proliferation of PDTCs and the growth in vivo of PDXX in regression preclinical trial. These data provided the concept that a PDXX-based pipeline is able to unveil actionable pathways for the treatment of advanced/metastatic ovarian cancer.

METHODS

RESULTS

(PDXX lines derived from EOC were analyzed using immunohistochemistry with antibodies against CT-7, EpCAM, WT1 to confirm the preservation of the histotypes of the source tumors. The targeted NGS of TP53 showed the presence of pathogenic mutations with AF=1 in all the 12 PDXX lines selected. WT1 staining and TP53 mutations confirmed the diagnosis of high-grade serous histology. Targeted NGS of BRCA1/2 showed the presence of BRCA1/2 mutations with the expected frequency (approx. 15%). Table 2 shows mutations in cancer genes reported in COSMIC found in 8/12 PDXX lines and the prediction of their possible pathogenic role based on SIFT, PROVEAN and FATHMM softwares. In the PDXX line #475 the WT2435R mutation of the PIK3R1 tumor suppressor gene was found with an AF=0.9 and predicted to be deleterious and damaging by softwares.

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EXO in vivo and in vivo assays of suscepibility to predictably active drugs

The analysis of biological effects of possible drugable mutations was carried out ex vivo in EOC, WT2. PDXX Derived Tumor Cells (PDTCs) were propagated as short-term cultures for ex vivo assays. These cell lines were treated with genetic control cells lines (A2780, OVCA8 and LNCaP) were exposed to different targeted drugs for 72 hours and analysed with cellTiter-Glo® Assay. Drug response was studied using GR metrics calculator. In vivo studies were carried out treating PDXX lines with different doses of selected targeted drugs.

CONCLUSIONS

The PDXX based pipeline shown here has been able to unveil mutated actionable cancer genes in epithelial ovarian cancer. Ex-vivo assays have been instrumental to accelerate the discovery of new treatment options, validated in the following in vivo assays. The W6245R mutation of the tumor suppressor PIK3R1 gene was found in one PDXX line as putative truncation mutation also in its source tumor. This mutation was previously reported in one colorectal cancer and mutations of the same residue in one stomach and one NSCLC. We show here that this mutation makes ovarian cancer cells responsive to PIK3R1 inhibitors, suggesting new potential therapeutic strategies for the treatment of advanced ovarian cancer carrying the PIK3R1 gene.

REFERENCES

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