In vitro analysis of the combination of APR-246 and carboplatin in triple negative breast cancer (TNBC) and high grade serous ovarian cancer (HGSOC) cell lines and its impact on Aurora kinase A (AURKA)/p38 pathway

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Background: AURKA is a protein that regulates mitotic spindle formation and is p53 involved in cell cycle regulation. p53 mutations and AURKA overexpression is a frequent alteration in both TNBC and HGSOC cell lines which lead to carboplatin sensitivity. APR-246 is a new targeted agent that modulates abnormal p53 in mutated cells. The aim of our study was to assess the effect of the combination of carboplatin (carbo) plus APR-246 in TNBC and HGSOC cell lines.

Methods: We selected two TNBC (MDA-MB-231 and MDA-MB-436) and one HGSOC cell line (Kuramochi). Next-generation sequencing was performed in order to assess more relevant mutations. MITT experiments were performed to calculate the IC50 for APR-246 and carboplatin in both cell lines. The combination of both drugs was assessed in the presence of a constant concentration of each other. Western-blot (WB) and PCR analysis was performed in each line after exposure to APR-246, carbo or the combination to determine expression of AURKA and p53.

Results: The mutation profile of each line and the IC50 of APR-246, carbo and combo is shown in the table.

Table 1: P53 - IC50

<table>
<thead>
<tr>
<th>Carbo</th>
<th>APR-246</th>
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<tr>
<td>P53</td>
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| MDA-MB-231 | Mut wt | 242.6 uM | 41.77 uM | 8.85 uM | <0.0001 | 21.78 uM | <0.0001 |
| MDA-MB-436 | Mut wt | 38.73 uM | 5.01 uM | 8.85 uM | <0.0001 | 21.78 uM | <0.0001 |
| Kuramochi  | Mut wt | 35.86 uM | 18.86 uM | 13.97 uM | <0.0001 |

Our results showed that IC50 of carbo and APR-246 decreased when administered in combination with constant doses of each other regardless of the subtype (TNBC vs HGSOC) and p53 mutations but more evident in the MDA-MB-231 BRCAwt cell line. PCR in MDA-MB-231 and MDA-MB-436 showed that AURKA was overexpressed by exposure to APR-246 and carbo. For the combination (combo) increasing IC50 of either APR-246 or carbo was assessed in the presence of a constant concentration of each other. Western-blot (WB) and PCR analysis was performed in each line after exposure to APR-246, carbo or the combination to determine expression of AURKA and p53.

Conclusions: Addition of APR246 to carboplatin increased apoptosis in both TNBC and HGSOC cell lines regardless of p53 mutations status in this in vitro study. AURKA and p53 expression was modified after exposure to carbo or combo.
Legal entity responsible for the study: The authors.
Funding: Has not received any funding.
Disclosure: All authors have declared no conflicts of interest.
https://doi.org/10.1016/j.annonc.2021.08.297

20P Molecular mechanism of MK2-CRBAP2 signaling pathway mediating the progression of lung adenocarcinoma
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Background: The morbidity and mortality of lung cancer rank the first among malignant tumors in China. EGFR-TKIs has achieved good efficacy in advanced lung adenocarcinoma with EGF-R-positive, however, drug resistance and rapid progress of some patients are still inevitable. Therefore, it is of great significance to find new therapeutic targets. Abnormally activated transcriptional regulators are involved in many biological processes of the development of malignant tumors, further exploration of their functional mechanisms is expected to provide a new direction for targeted tumor therapy.

Methods: TRRUST and CHIPBase databases of transcriptional regulatory factor and TCGA databases were integrated to screen the transcriptional regulatory factors with significantly different expression in lung adenocarcinoma. The prediction of interacting molecules was performed on the Linkedomics, and preliminary verification was performed using WB. The clinicopathological data of lung adenocarcinoma patients with survival data from GEO and TCGA databases were downloaded and analyzed to test the effect of CRABP2 and MK2 expression on survival prognosis.

Results: Transcription and protein expression of CRABP2 were significantly up-regulated in lung adenocarcinoma. Bioinformatics analysis showed that MK2 interacted with CRABP2, and CRABP2protein decreased significantly after knockdown of MK2 protein expression. The overall survival of lung adenocarcinoma patients with high CRABP2 and MK2 expressions were worse than that of the low expression group (P = 3.4e-04 and 6.5e-05, respectively).

Conclusions: MK2 may phosphorylate CRABP2 to mediate the progression of lung adenocarcinoma, and the MK2-CRBAP2 signaling pathway is expected to become a biomarker or a new target for therapy of lung adenocarcinoma. However, further molecular experiments are needed for further exploration and verification.

Legal entity responsible for the study: The authors.
Funding: Has not received any funding.
Disclosure: All authors have declared no conflicts of interest.
https://doi.org/10.1016/j.annonc.2021.08.298

21P Analysis of germline variants in the immune response-related genes in BRCA1 mutation carriers: Possible modifying effect on age-dependent BRCA1 penetrance
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Background: Most female carriers of germline BRCA1 mutation develop breast/or ovarian cancer during the lifetime. However, the penetrance of BRCA1 pathogenic variants does not reach 100%, and the age of BRCA1-associated breast cancer (BC) onset varies widely. BRCA1-driven tumors are chromosomally unstable and may have excessive antigenicity. We hypothesized that hereditary variations in immune response genetic pathways may contribute to the variability of BRCA1 penetrance. We evaluated whether genetic variations in the immune response-related genes contribute to this heterogeneity.

Methods: The entire coding sequence of 353 immune response genes was analyzed by NGS in 54 young (<39 y.o.) and 59 senior (>57 y.o.) BRCA1-mutated BC patients. Newly identified candidate variants were genotyped in the extended study, which included 185 young and 167 senior BC Slavic patients affected by BRCA1-driven BC.

Results: NGS analysis identified 54 allelic variants with gnomAD frequency ≤5% and a CADD-score of at least≥:−25, which were found exclusively either in young or in senior patients. The prevalence of 26 top candidates was analyzed in the extension study. The known pathogenic variant in the perforin (PRF1 p.Ala91Val) was significantly overrepresented in young BC patients compared with senior women (20 /carriers from 239 (8.4%) vs. 8/226 (3.5%), p = 0.032). PRF1 p.Ala91Val heterozygosity was associated with significant elevation of the risk of acquiring BC before the age of 39 years as compared to the BRCA1 mutation carriers with the wild-type PRF1 genotype (OR = 2.49, 95% CI: 1.073 - 5.771, p = 0.034).

Conclusions: PRF1 is one of the principal cytotoxic proteins responsible for cell lysis mediated by T-lymphocytes and NK cells. PRF1 p.Ala91Val heterozygosity is known to be associated with subclinical symptoms of immunodeficiency. This study suggests that the PRF1 p.Ala91Val substitution may compromise antimouse immune response and support the development of tumors in BRCA1 carriers. This study revealed that the inherited haploinsufficiency of immunodeficiency-related gene PRF1 caused by pathogenic missense p.Ala91Val may increase the risk of early breast cancer manifestation in BRCA1 mutation carriers.

Legal entity responsible for the study: The authors.
Funding: RSF grant #19-15-00207
Disclosure: All authors have declared no conflicts of interest.
https://doi.org/10.1016/j.annonc.2021.08.299

22P Functional characterization of DIRC3 long non-coding RNA in differentiated thyroid cancer
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Background: Differentiated thyroid cancers (DTC) are characterized by poorly characterized hereditary susceptibility. Some germline variants augmenting DTC risk (in particular rs11693806) locate in a long non-coding RNA (IncRNA) gene termed disrupted in renal cancer 3 (DIRC3). To date no studies have evaluated the biological function of DIRC3 IncRNA in DTC.

Methods: DIRC3 expression and rs11693806 genotype was tested in 75 patient-matched DTC/non-malignant thyroid tissue pairs. Public RNA-seq data (TCGA) was used to test correlations between expression of DIRC3 and protein-coding genes in DTC. We evaluated DIRC3 expression, its subcellular localization and genotype in a panel of DTC cell lines. Antisense oligonucleotides were used to silence DIRC3; the effect on expression of putative target gene(s), cell viability, migratory potential and invasiveness was tested. The significance of rs11693806 locus was evaluated with CRISPR.

Results: Study performed in DTC/non-malignant thyroid tissue pairs indicates that DIRC3 is downregulated in cancer (p < 0.01). Lower DIRC3 levels were noted in DTC manifesting with capsular invasion or metastasis. TCGA data revealed that DIRC3 is co-expressed with IGFBP5 (insulin-like growth factor binding protein 5), a nearby cancer suppressor. The gene co-expression was confirmed in our material (Spearman 0.73). In cancer cell lines, DIRC3 IncRNA preferentially localizes in nuclear cellular fraction. DIRC3 silencing induced IGFBP5 downregulation, and augmented cancer cell viability, migration and invasiveness. A strong chemotactic response to insulin-like growth factor 1 (IGF-1) was also noted. Using CRISPR we produced isogenic MDA-T32 cell line clones harboring small monoallelic deletions in the rs11693806 locus. We observed >2-fold reduction in IGFBP5 mRNA in the edited cells as compared to the parental cell line. Transcriptome changes (RNA-seq) induced by DIRC3 silencing will be tested shortly.

Conclusions: DIRC3 emerges as a putative thyroid cancer suppressor producing a cis-acting IncRNA that governs expression of IGFBP5, a modulator of IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway. We propose an interplay between the germline variants, DIRC3 and IGF-1 signaling pathway.