CANCEROGENESIS

RAD50, A POTENTIAL PREDICTIVE MARKER OF CHEMOTHERAPY RESISTANCE

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Cancer is a morphologically and molecularly heterogeneous disease. Among different cancer types, the breast cancer is the most common one, and it is the leading cause of women death worldwide. Breast tumors belonging to the same intrinsic subtype could have different response to therapy, but reasons of this are still not clear. Moreover poor disease outcome after chemotherapy is often caused by resistance formation to most of commonly used drugs. Effectiveness of anti-neoplastic agents is not fully understood and could be influenced by DNA repair activity. RAD50 protein plays a key role in DNA double strand breaks repair (DSBs), it is crucial to safeguard genome integrity. The aim of this study was to determine whether RAD50 was capable of being a prognostic marker of tumor cells response to chemotherapy.

To directly investigate the association of chemotherapeutic drugs and gene expression or copy number alterations (deletion – log2<-0.3; gain – log2>0.3) of RAD50 in breast cancer, we analyzed the cell line expression and CNA data in 59 breast cancer cell lines; data was taken from Cancer Cell Lines Encyclopedia (https://portals.broadinstitute.org/ccle/home). The response information (IC_{50}) to 12 anti-cancer drugs, namely 5-fluorouracil, carboplatin, doxetaxel, doxorubici, gemicitabine, lapatinib, methotrexate, mitomycin, oxaliplatin, paclitaxel, tamoxifen, vinblastine, were downloaded from Genomics of Drug Sensitivity in Cancer (http://www.cancerrxgene.org) and Cancer Cell Lines Encyclopedia.

We determined the association between mRNA expression of RAD50 and response to drugs as well as between CAN and response to drugs using Pearson correlation and Wilcoxon-Mann-Whitney test. The analysis revealed a significant association between the mRNA expression of RAD50 and sensitivity to vinblastin in breast cancer cell lines (correlation = 0.3625; p-value 0.0215). Correlation directly in cell lines with basal like subtype was stronger and more significant than in not differentiated cohort (correlation = 0.6340; p-value 0.0199). Resistant (mean = 7.787; 25% of available cell lines with highest IC_{50}) to vinblastine cell lines have significantly higher mRNA expression (p-value = 0.0029) than sensitive (mean = 6.989; 25% of available cell lines with lowest IC_{50}). Analysis of cell lines sensitivity to chemotherapeutic compounds taking into account CNA showed a significantly better response to vinblastine in cell lines with deletions (p-value = 0.0143) than in cell lines with diploid RAD50 copy number.

Our data suggests that RAD50 might be a predictive marker in determining the benefit of vinblastin chemotherapy. However, further studies are needed to clarify the outputs using a larger sample group and more in-depth *in vitro*, *in vivo* and *ex vivo* studies.

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ADAPTOR PROTEIN RUK/CIN85 INDUCES GENOMIC REPROGRAMMING IN BREAST CANCER CELLS AND THEREBY INCREASES THEIR MALIGNANCY

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Adaptor proteins serve as molecular platforms for multimolecular complexes assembly and thereby regulate cell signaling. The information from public databases and our previous results demonstrated that tumors (including breast cancer) are characterized by increased expression of adaptor protein Ruk/CIN85. This ubiquitously expressed adaptor is involved in dynamic control of cell signaling in space-dependent manner and plays critical role in several cellular processes, such as ligand-induced endocytosis of RTKs, intracellular vesicular trafficking, adhesion, motility, and survival. In the present study we investigated the potential mechanisms of of adaptor protein Ruk/CIN85 involvement in breast cancer invasion and metastasis.

As a model we used mouse 4T1 breast adenocarcinoma cells with stable overexpression (RukUp cells) and downregulation (RukDown cells) of Ruk/CIN85. *In vitro* motility was investigated by scratch test and invasion - by Boyden chamber assay. The efficiency of RukUp and RukDown cells extravasation and metastasis *in vivo* was studied by using syngeneic mouse model. Gene expression was analysed by Real-time PCR.

We demonstrated that overexpression of Ruk/CIN85 increases both motility and invasiveness of 4T1 cells. Using animal model it was shown that RukUp cells are characterized by elevated ability to produce lung metastasis, while the effectiveness of RukDown cells metastasis was significantly suppressed. These changes in tumor cells behavior were accompanied by the differential expression of EMT-related genes, including vimentin, E-cadherin, SNAI1, Zeb-1, Zeb-2, Lcn2.

The obtained data suggest that adaptor protein Ruk/CIN85 is a critical regulatory component involved in EMT of breast cancer cells that arise through the reprogramming mechanisms.

METHYLATION OF *GPX3* AND *TIMP3* GENES OF DNA OF TUMORS IN PATIENTS WITH RENAL CELL CARCINOMA

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Approximately 250,000 cases of renal cell carcinoma are diagnosed every year in the world, and 100,000 patients die of the disease. Cancer is associated with a variety of molecular genetic changes. Hypermethylation of tumor suppressor genes is often associated with developing cancer and it can be used as biomarkers for early detection of the presence of cancer as well as for monitoring patients during and after therapy. It was indicated that tumor genomic DNA can be used for detection of hypermethylation of cancer marker genes, which have been applied in cancer risk assessment, early detection, prognosis, and prediction of response to cancer therapy.

The purpose of our study is to determine methylation of GPX3 and TIMP3 suppressor genes of tumors in patients with renal cell carcinoma.

The research was performed on biopsies of the tumor and surrounding tissues of fifty patients with clear cell renal cell carcinoma. Bisulfite modified genomic DNA was amplified using real-time quantitative methylation-specific polymerase chain reaction with specific primers for GPX3 and TIMP3 tumor suppressor genes.

Analysis of the results showed methylation of CpG islands of *GPX3* gene in 43 (86%) renal cancer tumor tissue samples and in 14 (28%) tissue samples around the tumor. At the same time methylation of the TIMP3 promoter was detected in 6 (12%) tumor DNA tissue samples and in 2 (4%) DNA tissue samples around the renal cancer tumor.

To sum up we observed hypermethylation of the GPX3 promoter and low methylation level of CpG islands of *TIMP3* gene. The obtained results indicate that hypermethylation of genomic DNA of *GPX3* gene are cancer-specific changes.

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