RESEARCH REPORT OTKA 108655

Deciphering the role of the RALA signal transduction pathway in colon cancer progression

2014-2016

The proposed research plan was completed successfully and a paper directly describing the research results was published. In addition, two highly related studies were carried out, both focusing on prognostic biomarkers and genes in colon cancer (CRC). Additional 28 papers were published during this time where the OTKA support was listed. One of the PhD fellows in the group finished her PhD work by working directly on our CRC studies. Below a short summary is presented for the three most important results.

In the first study our focus was on an experimental investigation of the RALA gene in colon cancer. To date, our understanding of oncogenic signaling pathways has strongly fostered current concepts for targeted therapies in metastatic colorectal cancer - the RALA pathway was a novel candidate due to its independent role in controlling expression of genes downstream of RAS.

In our study, we compared RALA GTPase activities in three colorectal cancer cell lines by GTPase pull-down assay and analyzed the **transcriptional and phenotypic effects of transient RALA silencing**. Knocking-down RALA expression strongly diminished the active GTP-bound form of the protein. Proliferation of KRAS mutated cell lines was significantly reduced, while BRAF mutated cells were mostly unaffected. By microarray analysis we identified common genes showing altered expression upon RALA silencing in all cell lines. None of these genes were affected when the RAF/MAPK or PI3K pathways were blocked.

To investigate the **potential clinical relevance of the RALA pathway** and its associated transcriptome, we performed a meta-analysis interrogating progression-free survival of colorectal cancer patients of five independent data sets using Cox regression. In each dataset, the RALA-responsive signature correlated with worse outcome in CRC.

In summary, we uncovered the impact of the RAL signal transduction on genetic program and **growth control in KRAS- and BRAF-mutated colorectal cells** and demonstrated prognostic potential of the pathway-responsive gene signature in cancer patients.

This study was published in: Győrffy B. et al.: Effects of RAL signal transduction in KRAS- and BRAF-mutated cells and prognostic potential of the RAL signature in colorectal cancer, Oncotarget, 2015 (IF=5.1)

At the end of our first study, a signature of multiple genes was set up. The signature included the RALA responsive genes and had a strong prognostic potential. **In the last decade, multiple gene-expression-based prognostic classifiers have been already proposed for the molecular subdivision of colon cancer.** One can raise the issue of which of these has the highest prognostic potential.

We aimed to **cross-validate a large set of such classifiers** to explore their concordance and their power to predict survival. For this, a gene-chip-based database comprising 2,166 samples from 12 independent datasets was set up. A total of 22 different molecular subtypes were re-trained including the CCHS, CIN25, CMS, ColoGuideEx, ColoGuidePro, CRCassigner, MDA114, Meta163, ODXcolon, Oncodefender, TCA19, and V7RHS classifiers as well as subtypes established by Budinska, Chang, DeSousa, Marisa, Merlos, Popovici, Schetter, Yuen, and Watanabe (first authors). Correlation with survival was assessed by Cox proportional hazards regression for each classifier using relapse-free survival data.

Of the above classifiers, the highest efficacy at predicting survival in stage 2–3 patients was achieved by Yuen (p = 3.9e-05, HR = 2.9), Marisa (p = 2.6e-05, HR = 2.6) and Chang (p = 9e-09, HR = 2.35). At the end of this analysis **we assigned 61 colon cancer cell lines from four independent studies to the closest molecular subtype**. This will enable to select the most representative preclinical model for each of the classifiers in future CRC studies.

The above work was published in: Sztupinszki and Győrffy: Colon cancer subtypes: concordance, effect on survival and selection of the most representative preclinical models, Scientific Reports, 2016 (IF=5.23)

Another key gene included in colon cancer is the Yes-associated protein 1 (YAP1) which is a transcriptional coactivator in the Hippo signaling pathway. **Increased YAP1 activity**

promotes the growth of tumors, including that of colorectal cancer. Verteporfin, a drug that enhances phototherapy to treat neovascular macular degeneration, is an inhibitor of YAP1.

We investigated the role of YAP1 in a new collaboration with the University of Michigan (USA) which we set up during the OTKA grant. In our study we found that verteporfin inhibited tumor growth independently of its effects on YAP1 or the related protein TAZ in genetically or chemically induced mouse models of CRC, in patient-derived xenografts, and in enteroid models of CRC. Verteporfin exhibited in vivo selectivity for killing tumor cells in part by impairing the global clearance of high – molecular weight oligomerized proteins, particularly p62 (a sequestrome involved in autophagy) and STAT3 (signal transducer and activator of transcription 3; a transcription factor). Verteporfin inhibited cytokine-induced STAT3 activity and cell proliferation and reduced the viability of cultured CRC cells.

Although verteporfin accumulated to a greater extent in normal cells than in tumor cells in vivo, experiments with cultured cells indicated that the normal cells efficiently cleared verteporfin-induced protein oligomers through autophagic and proteasomal pathways. Culturing CRC cells under hypoxic or nutrient-deprived conditions (modeling a typical CRC microenvironment) impaired the clearance of protein oligomers and resulted in cell death, whereas culturing cells under normoxic or glucose-replete conditions protected cell viability and proliferation in the presence of verteporfin. Finally, verteporfin suppressed the proliferation of other cancer cell lines even in the absence of YAP1, suggesting that verteporfin may be effective against multiple types of solid cancers.

This work was published in: Zhang, Győrffy et al: Tumor-selective proteotoxicity of verteporfin inhibits colon cancer progression independently of YAP1, Sci Signal, 2015 (IF=6.28)