The interplay between Spliceosomal Components and Cellular Stress mediated by chemo- and radiotherapy

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Chemo- and radiotherapy are the main treatments of cancer, but their effectiveness is limited by the resistance of cancer cells to the treatment [1, 2]. With proper selection of treatment, the main population of cancer cells are drug sensitive tumor cells. It is assumed that the signaling molecules secreted by these cells into the extracellular environment, accelerate proliferation of neighboring tumor cells. Hereupon neighboring tumor cells obtain more aggressive phenotype [3, 4]. Analyzing proteomic data obtained in our laboratory, ovarian cancer patient ascites, proteome and secretome of cell lines after radio- and chemotherapy, we have found an increased representation of spliceosome proteins after treatment with radio- and chemotherapy. As chemotherapy we used different DNA-damaging agents: cisplatin, radiation. We found that splicing proteins in the secretomes after chemotherapy, are coded by the same splicing genes, the loss of which causes the highest phosphorylation of histone H2AX [9]. So it can be argued that the cell perceives the lack of splicing proteins as a DNA damage signal. The cell secreting proteins outside thereby it can quickly respond to DNA damage.

We were interested in how transcription changes in cancer cells after the treatment with drugs that cause DNA damage, resulting in secretion of splicing proteins. We did differential gene expression meta-analysis of 104 cell lines of various cancers after different types of stresses: radiation, platinum agents, hypoxia, tyrosine kinase inhibitors and topoisomerase. According to the results of our expression meta-analysis and the time clusterization results of cancer cell genes expression after various kind of stresses relative to untreated cells, we observed a simultaneous reduction in expression of genes involved in the splicing and mitotic cell cycle. The only exception was the

effect of taxanes - expression of these genes increased co-directionally. Pursuant to our results, the key transcription factors that regulate such co-directional expression change of splicing and cell cycle genes are MYC and E2F5.

After we had found a significant change in the expression of splicing genes in response to different types of chemotherapy, we suggested that the chemotherapy might also cause changes in alternative splicing. To do this, we took RNA-seq data of two lung adenocarcinoma patient-derived xenograft tumors before and after different chemotherapy treatments: carboplatin, docetaxel, afatinib, BEZ235, BKM120, DAPT, erlotinib, tivantinib, or selumetinib. Overall we took 12 sample pairs: before and after chemotherapy. When we analyzed alternative splicing changes after exposure to these drugs, we noticed the overlap of differential splicing events. Different types of chemotherapy agents significantly affected alternative splicing of genes belonging to the path of spliceosome, and genes involved in cell cycle. The most frequent splice events were intron retention of splicing and cell cycle genes. It is known that the intron-containing mRNAs are retained in the nucleus and / or subjected to degradation. Thus, our analysis showed that genes involved in the spliceosome and the cell cycle paths were the most suppressed by chemotherapy.

In response to stress cell have to quickly change the concentration of important proteins. It can do this by several levels of regulation: chromatin remodeling, transcription and alternative splicing, mRNA localization and degradation, translation and post-translational modification of proteins and protein degradation, as well as protein export. Probably three processes: expression, and secretion splicing – are complementary, i.e. run parallel, in order to dramatically reduce the amount of splicing proteins as a result of severe stress. Our analysis indicates that a lack of spliceosome proteins causes high histone H2AX phosphorylation, thereby cell signal of DNA damage. All this lead to cell cycle arrest and DNA repair.

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