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Analysis of therapy resistance after continuous treatment of pancreatic cancer cells with gemcitabine or bioactive agents

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Pancreatic ductal adenocarcinoma (PDA) is the most aggressive cancer entity with a marked resistance to gemcitabine (GEM) chemotherapy, which further enriches cancer stem cells (CSCs). Bioactive food-derived agents have attracted much attention, especially quercetin (Q) and sulforaphane (SF), which were effective to target pancreatic CSCs. However, whether continuous exposure to these bioactive agents also mediates resistance and enrichment of CSCs, as known from GEM, is unknown. I exposed the PDA cell line BxPC-3 to increasing concentrations of GEM, Q or SF for 6 months, selected the corresponding subclones BxPC-3/GEM, BxPC-3/Q and BxPC-3/SF and characterized them. Whereas BxPC-3/GEM cells acquired pronounced resistance to GEM, BxPC-3/SF or BxPC-3/Q cells acquired only a slight resistance toward SF or Q, respectively. The analysis of the self-renewal, differentiation and migration potential revealed that CSC features were enriched in GEM-resistant cells, but decreased in SF- and Q-long-time treated cells. I confirmed these results *in vivo* by orthotopic xenotransplantation of the 4 different cell lines to the mouse pancreas and evaluation of the tumor volume. To examine the underlying reason for GEM-resistance, I performed mRNA and miRNA expression arrays at the DKFZ. The expression of mRNAs and miRNAs between BxPC-3 and BxPC-3/GEM cells was further analyzed by bioinformatics tools *in silico*. The RRM1 was one of the top up-regulated mRNAs in BxPC-3/GEM cells and its overexpression was confirmed by western blot and immunofluorescence analysis. In addition, the miR-132-3p and miR-101-3p were the top miRNA candidates targeting RRM1.

Overexpression of miR-132-3p and miR-101-3p inhibited the expression of RRM1 and partly reversed GEM resistance. Therefore, I conclude that miRNA-regulation of RRM1 expression mediates GEM resistance in BxPC-3/GEM cells. Translated to the clinic, patients suffering from PDA may benefit from intake of fruits and vegetables enriched in the bioactive agents Q and SF, and from miRNA-mediated targeting RRM1 to sensitize GEM-resistant PDA cells.