Inhibition of glucose turnover by 3-bromopyruvate counteracts pancreatic cancer stem cell features and sensitizes cells to gemcitabine

Considering the still devastating prognosis of patients with PDA, the development of novel therapeutic strategies is a prerequisite to achieve better outcomes. It is confirmed by several studies that gemcitabine as single therapy is not successful, because of high chemoresistance in pancreatic cancer. According to the cancer stem cell (CSC) hypothesis, the aggressive growth and early metastasis of pancreatic ductal adenocarcinoma (PDA) is due to the activity of CSCs, which are not targeted by current therapies. Otto Warburg suggested that the growth of cancer cells is driven by a high glucose metabolism. Here, I investigated whether glycolysis inhibition targets CSCs and thus may enhance therapeutic efficacy. Four established and 3 primary PDA cell lines, non-malignant cells, and 3 patient-tumor-derived CSC-enriched spheroidal cultures were analyzed by glucose turnover measurements, MTT and ATP assays, flow cytometry of ALDH1 activity, annexin and DNA fragmentation positivity, colony and spheroid formation, western blotting, antibody protein array, electrophoretic mobility shift assay, xenotransplantation, and immunohistochemistry. The effect of siRNA-mediated inhibition of LDH-A and LDH-B was also investigated.

I found a direct dependence of gemcitabine-resistant CSCs on glucose metabolism. I observed the strongly inhibited growth of highly malignant PDA cells upon glucose deprivation, whereas less aggressive PDA cells or normal cells were barely affected. Additionally, colony formation as a feature of the self-renewal potential was strongly inhibited by glucose deprivation or LDH-A inhibition by siRNA transfection. In an attempt to replace the siRNA approach with a more clinically suited strategy, I implemented the anti-glycolytic agent 3BrP because several animal studies and a translational case study with 3BrP showed exciting results with this putative future anti-cancer agent. 3BrP strongly reduced the viability of the highly malignant PDA cells in a pyruvate- and lactate-dependent manner, which confirmed the specificity of the pyruvate/lactate analog 3BrP. Although 3BrP strongly inhibited viability and self-renewal potential in the highly malignant and gemcitabine-resistant PDA cells, it had a reduced effect in less aggressive and gemcitabine-sensitive PDA cells and only marginally affected normal cells.

Moreover, I examined the sensitizing effect of 3BrP to gemcitabine treatment by in vitro and in vivo xenotransplantation studies. A concentration of 50 µM 3BrP was very effective in depleting NAD(P)H and ATP levels in highly malignant PDA cells, as concluded from the reduced viability measured using MTT and Cell Titer Glo assays. Furthermore, the ability to grow as colonies and spheres and the tumor-forming potential in vivo was strongly reduced and almost completely abolished with the combination of 3BrP and gemcitabine. This finding is supported by my results obtained using Western blot analysis and antibody protein arrays, which showed that 3BrP, but not gemcitabine, strongly inhibited several markers involved in stemness and pluripotency, specifically ALDH1 expression and activity and such factors as Notch-1, Oct-4, Sox2, c-Met, CD44, and CxCR4. My in vivo findings that 3BrP mediated total inhibition of tumor growth, invasion, and metastasis of MIA-PaCa2 and PANC-1 cells after xenografting to the CAM of fertilized chicken eggs or mice. I did not observe side effects because the weight of the chicken embryos or mice was not diminished after treatment and liver necrosis was not detected. Most importantly, my in vitro experiments revealed that...
NF-κB binding activity was strongly inhibited after 3BrP-treatment, similar to the observed inhibition of the expression of the NF-κB subunit c-Rel in the xenografted tumor tissue of MIA-PaCa2 cells. Finally, I confirmed my findings in primary CSC-enriched spheroidal cultures, which I selected from patient tumors after serial transplantation into immunodeficient mice. 3BrP, but not gemcitabine, strongly inhibited spheroidal growth and sensitized the CSC-enriched spheroidal cultures toward gemcitabine, as verified by morphological studies and expression studies. The combination of 3BrP with gemcitabine eliminated secondary spheroid formation along with the strong downregulation of c-Met, CD44, and Sox2 expression; in contrast, the levels of the cleaved fragment of active caspase 3 were enhanced, indicating the induction of apoptosis.

In conclusion, my findings extend the knowledge about the remarkable anti-cancer properties of 3BrP against CSCs. The combination of 3BrP with gemcitabine may lead to a complete eradication even of advanced PDA due to the elimination of the more differentiated tumor cells by gemcitabine and of CSCs by 3BrP. Because 3BrP affects more differentiated (less malignant) tumor cells and normal cells only marginally and thus has low side effects, there is an urgent need to bring 3BrP to clinical trials.