4 Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the most lethal common cancer, usually diagnosed at an advanced stage when curative therapy is almost impossible. It is the fourth most common cause of cancer-related death in Europe and in the US. The pancreatic adenocarcinoma typically has a poor prognosis with a five year relative survival rate of 4-5%. In fact, incidence and mortality of PDAC are almost equal. Surgical resection is the only potentially curative therapy for pancreatic cancer. Because of the poor outcome associated with surgery only, the role of adjuvant therapies has been extensively evaluated. A series of studies revealed, that chemotherapy with gemcitabine or fluorouracil improves the overall survival of patients with pancreatic adenocarcinoma. In the majority of cases a complete resection of the tumor is impossible. Therefore a palliative chemotherapy may be conducted to prolong survival and improve the quality of life. In these cases new combination therapies like FOLFIRINOX are investigated in clinical trials or are in use. However, current chemotherapeutic agents are still disappointing due to their poor response and high toxicity. New and innovative agents have to be found to expand the therapeutic opportunities.

Therefore the aim of this study was to characterise the anti-neoplastic effect of the recently developed substance 2250, which is a derivative of the main metabolite of TRD, in different pancreatic cancer celllines in vitro and in vivo. In the first part of this study we determined the cytotoxic effects, the inhibition of proliferation and analyzed the relative contribution of apoptosis and necrosis during substance 2250 induced cell death by MTT assay, BrdU assay and FACS analysis. It could be shown, that the substanz 2250 has comparable cytotoxic and proliferation inhibiting effects like its mothersubstanz TRD. The flowcytometric analysis resulted in a higher amount of necrotic cells after treatment with substance 2250. A possible explanation for this effect is the induction of programmed necrosis (necroptosis). Programmed necrosis is, besides apoptosis and autophagy, another type of programmed cell death, which was previously shown for TRD. The next part of this study deals with the contribution of reactive oxygen species (ROS) to substance 2250 induced cell death and the analysis of another major cell death associated pathway, the caspase pathway. Hence, co-incubation experiments with either the radical scavenger NAC for inhibition of oxidative stress or the pan caspase inhibitor z-VAD for involvement of the caspase pathway were performed. It could be shown, that the generation of ROS and the consequent activation of following pathways is an obvious explanation of the induction of cell death by substance 2250 in pancreatic cancer cell lines. Thus, ROS induced cell death may not be the universal mechanism of substance 2250, but our experiments highlight its central role in PCD. The major importance of ROS generation via cell death induction of substance 2250 was underlined by the previous results of FACS analysis, which showed a high amount of necrotic cells, because ROS triggers the necroptosis. In contrast the observed response in pancreatic
cancer cell lines regarding the inhibition of 2250 induced cell death, via the pan-caspase inhibitor zVAD, leads to the assumption, that especially for pancreatic cancer cell lines the caspase dependent PCD plays only a minor role.

Furthermore the anti-neoplastic effect of substance 2250 and TRD to putative cancer stem cells was analysed for the first time. The cells were cultivated in a 3D-spheroide model, which was able to enrich the putative cancer stem cells. For analysing the stem cell marker CD133 was used. Thereby it was shown that both substances in contrast to the chemotherapeutic agent gemcitabine were able to reduce the amount of CD133⁺ cells.

On the basis of this foregone promising in vitro results, we analysed the anti-neoplastic effects of substance 2250 and TRD in vivo. For the experiments xenograft models of established pancreatic cancer cell lines and PDX models were used in athymic nude mice. A significant reduction of subcutaneous tumor growth by treatment with substance 2250 or TRD could be observed, however according to RECIST 1.1 criteria a tumor progress was still noticed. In addition, the chemotherapeutic agent gemcitabine and combinations of gemcitabine with substance 2250 and TRD were analysed. After a first progress until the tumorvolume was doubled no further tumorgrowth could be observed after treatment with gemcitabine. The combinations of gemcitabine and substance 2250 resp. TRD were the most hopeful treatments with an according to RECIST 1.1 criteria partial response.

In conclusion, this is the first study providing an evaluation of substance 2250 induced cell death among several pancreatic cancer cell lines in vitro as well as inhibition of pancreatic tumor growth in vivo. Substance 2250 is characterized by a clear anti-neoplastic potential. Therefore substance 2250 especially in combination with gemcitabine seems to effectively inhibit pancreatic cancer tumor growth in mice. These encouraging results are the basis for a further evaluation of substance 2250 as a promising new therapeutical option in the treatment of pancreatic cancer.