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Enhancement of Chemotherapeutic and Radio-Immunotherapeutic Sensitivity on Human Pancreatic Cancer Cells due to Interferon-alpha (CapRI scheme In Vitro)

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Human pancreatic carcinoma has an especially bad prognosis whose five-year survival rate is less than 1% with a median survival of 4-6 months. Even after surgical operation with a curative intention in specialized centers, the two-year survival rate is 25% at best.
Various chemo- and/or radiation regimens have been tested in small studies for treatment of adjuvant resected pancreatic carcinoma such as 5-FU or gemcitabine as a chemotherapeutic agent, sometimes in combination with other agents such as CDDP. However, none of the adjuvant treatment of pancreatic adenocarcinoma has worked well so far to produce long-lasting benefits.
The authors from the ESPAC-1 trial with large numbers of patients concluded that radiochemotherapy shows only limited success. Investigators from the Virginia Mason Clinic have reported toxicity and intermediate term event-free outcome from a phase II trial of postoperative 5-FU, cisplatin, Interferon alpha-2b (IFN-α), and external-beam radiation administered following pancreaticoduodenectomy. The results from this phase II trial are very encouraging. We termed this regimen as **CapRI** for adjuvant **C**hemo**R**adio**I**mmunotherapy to **p**ancreatic **a**denocarcinoma.
Comparing the data from ESPAC-1 and CapRI, we hypothesize that IFN-α is the key agent which plays a pivotal role in this encouraging therapy. We decided to test effects of Interferon-alpha in vitro on human pancreatic cancer cell lines according to this CapRI scheme and at the meantime to look for possible predictive markers and as well to investigate possible working mechanisms of Interferon-alpha based upon this protocol. Eight human pancreatic cancer cell lines were treated with single agent and combinations of each of these. Possible roles of IFN-α such as a) direct inhibitory effects; b) radio- and chemosensitizing effects; c) anti-angiogenic properties; d) enhancement of immunogenicity and e) enhanced cytotoxicity of the immune system were investigated.
Our results show that Interferon-alpha has direct inhibitory properties and some synergistic effects as determined by AnnexinV/PI stain and cell count. Interferon-α is also able to prevent the increase in proliferation rate and VEGF secretion of CDDP resistant cells by MTT assay and ELISA. We saw an increased proliferation rate of hPBLs after IFN-α stimulation and a significant increase in cytotoxic activity against pancreatic carcinoma cell lines after one single overnight IFN-α stimulation investigated by standard ^{51}Cr release cytotoxicity assays and flow cytometry-based cytotoxicity assays. We found that tumor cells which are normally resistant to PBLs could be lysed by IFN-α stimulated hPBLs. Besides, high expression of MHC molecules, CD118, EGF-R and Fas tested by FACS was predictive for a good response. Furthermore, our RT-PCR and the Western Blotting show that both on RNA level and on protein level Interferon-alpha upregulates the expressions of immunoproteasome subunits hLMP-2, hLMP-7 and hMECL-1. Interestingly but not surprisingly, 5-FU downregulates their expressions. Here, interferon-alpha induced the switch of proteasome of the investigated

human pancreatic cancer cell lines into immunoproteasomes which implicates for their immunogenicity.

In conclusion, IFN-α demonstrates direct cytotoxic, radio- and chemosensitizing, antiangiogenic and immunogenic effects in vitro on human pancreatic cancer cell lines and circumvents tumor cell-regrowth after CDDP treatment. hPBLs could be activated by IFN- against pancreatic carcinoma cells and pancreatic carcinoma cells are more susceptible against immunological attacks after 5-FU treatment. IFN- induces the immunoproteasome in pancreatic carcinoma cells which are then more immunogenic for hPBLs. This mechanism may be responsible for the good clinical outcome of CapRI scheme. All in all, we could presume that there is a complex network of enhancing interactions between the different agents used in this CapRI scheme.