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Anti-tumor and immune regulatory function of interferon – alpha in adjuvant chemoradioimmunotherapy for pancreatic carcinoma in an orthotopic mouse model

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Data from a phase II trial combining chemoradiotherapy with interferon-alpha (CapRI scheme) for adjuvant treatment of pancreatic carcinoma are very encouraging. Compared with other published adjuvant trials such as ESPAC-1, it is supposed that interferon-alpha (IFN-α) is the agent turning a slightly effective treatment (chemoradiotherapy in ESPAC-1) into a potent therapy (Virginia Mason chemoradioimmunotherapy).

IFN-α is a well studied therapeutic agent for unspecific active immunotherapy. It achieves its function through various strategies. The role and mechanism of interferon alpha in CapRI protocol is not clear. This project is conducted in murine models to: 1) determine the effect of addition of IFN-α to monochemotherapy on tumor growth, survival rate, and metastasis; 2) investigate the immune responses in IFN-α treated animals; 3) testify the efficacy of adoptive immune cell transfer from treated donors as a potent anti-tumor agent; and 4) elucidate the anti-angiogenic effects of IFN-α and its influence on leukocyte-endothelium interactions. Five days after inoculation of murine, syngeneic Panc 02 pancreatic carcinoma cells in the

pancreas, C57/BL6 mice were treated with 5-Fluorouracil (5-FU) , Cisplatin, radiation, IFN-α and their different combinations according to experimental plan, and kept alive till they died naturally or were sacrificed when in very bad condition. Survival rate, tumor volume and metastasis were three basic evaluative parameters; immune reaction analysis includes mouse splenic cell flow cytometry, tumor tissue immunohistochemistry (IHC), adoptive cell transfer (ACT) and Th1 cytokines test (ELISpot). Intravital microscopy (IVM) was employed for tumor blood vessel study and for interaction between leucocytes and tumor blood vessel investigation. Finally IFN-α dependent anti-angiogenesis in tumor was carefully investigated. Because of severe side effects, Cisplatin and radiation treatment were cancelled, most data were from experiments with control, 5-FU and 5-FU+ IFN-α groups.

5-FU as well as Cisplatin treatment had a significant life-prolonging effect. After addition of IFN-α to chemo- or radiotherapy, a significant decrease in tumor volume was observed coupled with significant prolonged survival rate and reduced metastasis.

Immune analysis showed a significant increase of CD4, CD8 and dendritic cells in 5-FU+IFN-α treated mice. As a result of that, a significant shifting from naïve T cells to central memory T cells has been seen in these mice. Furthermore, a significant increase of interferon gamma (IFN-γ) secretion after stimulation with tumor antigens was observed after IFN-α treatment. These data could be interpreted that. more effective tumor antigen presentation was performed by dendritic cells and more effective cytotoxic T lymphocytes (CTLs) were recruited.

Results from adoptive cell transfer experiments displayed that anti-tumor response could be transferred by injection of leukocytes from 5-FU+IFN-α treated mice into untreated tumor-bearing animals. The transferred leukocytes homed into the tumors and proliferated there, i.e. IFN-α pre-treated immune system can fight tumor more effectively and is more specific to tumor antigens.

Intravital microscopy (IVM) provided a direct insight into tumor. With IVM a significant lower blood vessel density in tumor after addition of IFN-α to monochemotherapy was revealed. This powerful anti-angiogenic effect in 5-FU+IFN-α treated mice was confirmed by IFN-α dependent lower RGS-5 expression, and can be pursued to IFN-α dependent significant lower VEGF level in sera and lower VEGF receptor in tumor. Additionally, IFN-α promoted the interaction between tumor blood vessel and active leucocytes that ensures the migration of effective cells into tumor. This observation is consistent with what discovered in tumor immunohistochemistry investigation: more effective T cells and dendritic cells infiltrated into tumor. The powerful direct "touch" on tumor cells ensures cytotoxic T lymphocytes to execute their anti-tumor duty.

Unlike IFN-α, 5-FU reduced the interaction between tumor blood vessel and active leukocytes, this might be another reason for resistance of pancreatic carcinoma to chemotherapy.

In conclusion, as the main component in CapRI scheme, IFN-α improves tumor antigen presentation thus inducing more antigen-specific T cell enrolling in immune reaction. In the meantime, IFN-α also limited tumor-angiogenesis that results in a smaller tumor volume, which contributed to prolonged survival rate and reduced metastasis.

The CapRI-scheme is tested in a phase III clinical trial in the Surgery Department of University Heidelberg, Germany. We are optimistic that this treatment scheme might bring benefit to the patients.