IRRADIATION AND GENE ELECTROTRANSFER OF PLASMID DNA ENCODING CHEMOKINES CCL5 AND CCL17 INDUCE IMMUNOMODULATORY EFFECTS IN MURINE TUMORS

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Chemokines regulate immune cell migration. The degree and the type of immune cells in the tumor affects disease progression and correlates with the efficacy and outcome of immunotherapies. Similarly, beneficial immunomodulatory effects were also observed after irradiation. Therefore, we sought to investigate gene electrotransfer (GET) of proinflammatory chemokines CCL5 or CCL17 in combination with irradiation, as a potential therapeutic strategy for cancer therapy.

Tumor models were chosen to correspond to an inflamed (CT26 murine colon cancer) or immunosuppressive (4T1 murine breast cancer) immunophenotype. First, chemotactic properties of investigated chemokines were examined *in vitro*. Next, the potential of chemokines to induce the extravasation of fluorescently labelled splenocytes was determined using intravital microscopy of tumors in dorsal window chamber model (DWC). The antitumor effectiveness of combined therapy utilizing GET of chemokines and two irradiation regimes (single dose of 10 Gy and fractionated dose of 3× 5 Gy) was then determined *in vivo*. Lastly, qRT-PCR was used to evaluate gene expression of several cytokines in tumors after the therapies, while changes in the abundance of CD4+, CD8+ cells and vasculature (CD31+ cells) were determined with immunofluorescent staining.

Both chemokines CCL5 and CCL17 induced the migration of murine macrophages RAW264.7 *in vitro*. Similarly, in both CT26 and 4T1 tumors growing in DWC, GET of chemokines showed increased retention of splenocytes compared to control. CT26 tumor growth delay after combined therapy of GET of chemokines and both irradiation regimes was significantly longer compared to control and led to tumor cures. In the case of 4T1 tumors, only GET of chemokines combined with irradiation led to a pronounced tumor growth delay but without tumor cures. Gene expression analysis showed increased expression of both chemokines after corresponding therapies. Moreover, increased expression of CXCL9 and CXCL10, two potent chemoattractants of cytotoxic CD8+ T lymphocytes, was determined in tumors after

most of the combined therapies. Immunofluorescence showed increased numbers of CD4+ and CD8+ T lymphocytes in tumors after GET of chemokines, however their numbers decreased whenever irradiation was used. Our results show that combined therapy elicits an antitumor immune response in inflamed tumors CT26 and to some extent in immunosuppressive tumors 4T1, indicating the potential of chemokines in cancer immunotherapy.