

DIFFERENTIAL EFFECTS OF PROTON THERAPY AND PHOTON THERAPY ON HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) POST-TREATMENT AGGRESSIVENESS

Marilena Lupu-Plesu, Audrey Claren, Sonia Martial, Papa-Diogop N'Diaye, Kevin Lebrigand, Damien Ambrosetti, Isabelle Peyrottes, Julien Feuillade, Joël Hérault, Maeva Dufies, Jérôme Doyen, and Gilles Pagès

Head and neck cancers, the 7th cause of death worldwide, are currently treated with a combination of surgical resection of the primary tumor, chemotherapy and radiotherapy, depending on the disease stage. Conventional photon therapy nevertheless remains difficult to apply to tumors such as head and neck squamous cell carcinomas (HNSCC), due to the proximity of numerous organs at risk (i.e. salivary glands, esophagus, larynx). Proton therapy has been proposed to treat such sensitive tumors, due to its high precision in tumor targeting.

Despite the current therapeutic strategies, the five-year overall survival rate of HNSCC patients is only 53%, with a high percentage of poor response to therapy and a high recurrence rate. Lymph node metastasis, the first sign of tumor progression, has been directly correlated to Vascular Endothelial Growth Factor-C (VEGF-C) expression levels in HNSCC and to VEGF-C-dependent tumoral lymphatic vessel development.

In the present study, we investigated the hypothesis that, beside the advantage in dose deposition, proton therapy may show distinct biological properties than photon therapy (at similar doses). We thus examined several *in vitro* biological behaviors of HNSCC-derived cells when exposed to photons or protons, focusing on molecules with key roles in the progression and prognosis of HNSCC, such as genes/proteins involved in (lymph)angiogenesis/metastasis, inflammation, tumor cell proliferation and anti-tumor immunity, tumorigenic potential.

We showed that cell proliferation decreased with the irradiation dose, both in proton and photon irradiated cells. Proton and photon irradiations increased VEGF-C and PD-L1 expression in HNSCC cells. In cells surviving multiple irradiation, key (lymph)angiogenesis and inflammation genes were down-regulated (except for VEGF-C) after proton therapy and up-regulated after photon therapy. Both irradiation types stimulated VEGF-C promoter activity via NF- κ B-dependent transcriptional regulation. We conclude that cell resistance, tumor progression and lymphangiogenesis induction is less pronounced after proton irradiation than after photon irradiation.

We validated these results by *in vivo* experiments: Photon- or proton-irradiated HNSCC-derived cells were xenografted subcutaneously into immunodeficient mice. Cells surviving to multiple irradiations by protons or photons generated tumors with higher volume, anarchic architecture and increased density of blood vessels than non-irradiated cells. Increased lymphangiogenesis and a transcriptomic analysis in favor of a more aggressive phenotype were observed in tumors generated with X irradiated cells. Detection of a denser lymphatic vessel network in relapsed tumors from patients receiving conventional X radiotherapy is consistent with these results.