

Does neoadjuvant radiotherapy and the timing of surgery modify metastatic dissemination?

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Neoadjuvant radiotherapy (RT) is used in many treatments and aims at improving tumor local control and patient overall survival. RT schedule and the timing of surgery are mostly empirical based on clinical experiences. Tumor microenvironment plays a crucial role in tumor growth and metastatic dissemination. Modifying this environment with radiotherapy may influence the tumor phenotype and the formation of metastases. With an original pre-clinical model of neoadjuvant RT, we study the impact of different RT schedules on tumor inflammatory microenvironment and on tumor dissemination according to the timing of surgery.

Human mammary tumors, implanted into the flank of SCID mice, were irradiated with different neoadjuvant RT schedules (i.e. 5x2Gy and 2x5Gy). We surgically removed carefully tumors 4 or 11 days after the end of RT and kept the mice alive during 5 weeks for metastatic growth. Then we sacrificed the mice and searched for lung metastases thanks to human Ki-67 immunohistochemical staining.

After 2x5Gy, the size and the number of lung metastases were smaller when surgery was performed at 11 days after the end of RT, compared to 4 days. Inversely, in the 5x2Gy schedule, applying surgery at 4 days protected the mice against lung metastases compared to surgery at 11 days. These results suggest that the timing of surgery and RT schedules are both important factors that influence the formation of metastases.

We first investigated several pathways well known to contribute to cancer progression and metastatic dissemination such as hypoxia, vessel density, proliferation and necrosis. No obvious difference was seen between the 4 experimental groups. We next focused our interest on the inflammation, a process known to play a crucial role in cancer progression and, which could be influenced by RT.

Thanks to computer assisted quantification performed on F4/80 stained tumor sections, we observed a significant difference in tumor macrophage infiltration according to the timing of surgery. Macrophage infiltration appears to increase at day 11 compare to day 4 after 2x5Gy RT and to decrease at day 11 compare to day 4 after 5x2Gy RT. In sharp contrast, no difference was seen according to the RT schedule. RNA level of iNOS (a marker M1 macrophage subtype) and Arginase 1 (a marker of M2) by real time PCR appeared correlated with the occurrence of metastases. Thus, we studied the different subsets of tumor-associated macrophages and inflammatory cells by FACS analyses. Although many differences were observed between the experimental groups, none of them explained the difference in lung metastases occurrence.

We developed a powerful pre-clinical model to study the impact of neoadjuvant RT schedules and the timing of surgery on tumor microenvironment and metastatic dissemination. This model reveals the importance of the tumor inflammatory microenvironment at the time of surgery for tumor dissemination. Further investigations are needed to highlight the precise role of inflammatory microenvironment in the metastatic phenotype .