Tailoring the timing of surgery and the neoadjuvant radiotherapy schedule for decreasing tumor dissemination.

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Purpose : Neoadjuvant radiotherapy is the standard of care for locally advanced rectal cancer and allows to reduce local recurrence below 10%. Even though, radiotherapy improves local control more than 50% compared to surgery alone, patient overall survival is not improved. The incidence of distant recurrences is the same with or without neoadjuvant treatment and patients still die from metastases. We hypothesize that neoadjuvant radiotherapy could modify tumoral microenvironment and that may promote tumor dissemination at the time of surgery. Such modifications may depend on the radiotherapy schedule and the timing of surgery after radiotherapy.

Methods: To test the hypotheses we used a specific clone of MDA-MB231 cell line, a human mammary carcinoma cell line, that generates spontaneous metastases *in vivo.* We injected subcutaneously tumor cells mixed with Matrigel into the flank of SCID mice. Tumors were irradiated with different radiotherapy schedules, a hypofractionated schedule, 2X5Gy and a more conventional radiotherapy schedule used in clinics, 5X2Gy. The number of fraction delivered is different than in clinics because it was tailored to murin model. A non irradiated group served as control. We removed tumor 4 or 11 days after the end of the radiotherapy, and collected them for immunohistological staining, RNA and protein extraction. We sacrified mice 45 days after the beginning of radiotherapy, lungs and liver were collected for metastases analysis by immunohistological staining of human KI-67.

Results: We observed differences in lung metastases incidence according to the radiotherapy schedule and the timing of the surgery. Our first experiment showed that operating mice 11 days after hypofractionated radiotherapy (2x5Gy) decreases the incidence of lung metastases than operating after 4 days. On the other hand, a second experiment showed that with more conventional radiotherapy schedule (5x2Gy), operating mice 11 days after radiotherapy slightly increase metastases incidence then operating 4 days after radiotherapy. Tumor volumes were the same in all groups and therefore can't be incriminated in the difference of lung metastases. In a third experiment which aims to compare hypofractionated to conventional radiotherapy schedule, we observed a reduced metastases incidence with the hypofractionated schedule when operating mice 11 days after radiation treatment. We are studying, on RNA and protein extracts from tumors, several molecules implicated in microenvironment remodeling in the aim to pinpoint a cascade responsible of tumor dissemination.

Conclusions: We report here that the fractionation of the neoadjuvant radiotherapy and the timing of the surgery influence the tumor dissemination but the mechanisms are still unknown. Understanding the mechanisms by which radiotherapy induce modifications of the tumor microenvironment responsible of tumor dissemination is essential for the development of new strategy and the application of new protocols of radiotherapy to patients affected by colorectal cancer.