

## Inflammatory Activity in Liver Metastases of Modified Breast Carcinoma

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**Introduction.** Inflammation is recognised as one of the key players in cancer development, which can promote growth of the primary tumour as well as metastatic spread. Therefore, it is not surprising that neutrophils, representing key inflammatory cell type, are recruited to a wide variety of tumours. Furthermore, infiltration of neutrophils is often associated with poor clinical outcomes in cancer patients. Mature neutrophils are now recognised as possible mediators of metastatic spread and could be considered as a therapeutic target to prevent the pro-metastatic activity in breast cancer.

**Aim, Materials and Methods.** The aim of the present study was to characterise inflammatory activity in liver metastases of breast carcinoma 4T1 after experimental introduction of different HIV genes in cell lines and / or immunisation. A retrospective study was done of 62 liver samples of mice implanted with 4T1luc2 murine breast adenocarcinoma cells expressing HIV-A reverse transcriptase (RT), protease (PR), and integrase (IN). Mice were transplanted with 4T1luc2\_RT (RT-DNA immunised 9, naïve 6); 4T1luc2\_PR (PR-DNA immunised 15, naïve 8); 4T1luc2\_PR (PR-DNA immunised 10, naïve 6); and parental 4T1luc2 cells (naïve 8). Liver metastases were diagnosed and evaluated microscopically in formalin-fixed, paraffin-embedded tissues of immunised and naïve animals. Presence of inflammatory cells in liver metastases was assessed within five high power microscope fields (400x) of haematoxylin-eosin-stained slides by computer-assisted morphometry using specialised NIS-Elements software (Nikon, Tokyo, Japan). IBM SPSSv23 was applied for statistical analysis, including descriptive assessment as detection of mean values, standard deviation (SD) and 95% confidence interval (CI).

**Results.** The average number of liver metastases detected per five high power microscopy fields was 2.3 (SD ± 1) for RT-, 3.8 (SD ± 1.4) for IN-, 7.8 (SD ± 1.9) for PR-expressing, and 2.2 (SD ± 1) for parental 4T1luc2 cells. Inflammatory infiltrate (IINF) consisting of neutrophils and lymphocytes were found in 14/25 metastases of 4T1luc2\_RT cells (56.0%; CI = 37.0–73.3) including 6/8 in immunised (75.0%; CI = 40.9–92.9) and 8/17 in naïve mice (47.0%; CI = 26.2–69.0). For 4T1luc2\_IN, IINF were present in 21/34 (61.8%; CI = 45.0–76.1) metastases, 3/9 in immunised (33.3%; CI = 12.0–64.6) and 18/25 (72.0%; CI = 52.4–85.7) in naïve mice. For 4T1luc2\_PR, IINF were present in 70/86 metastases (81.4%; CI = 71.9–88.2), 43/50 (86.0%; CI = 73.8–93.0) of immunised and 27/36 (75.0%; CI = 58.9–86.3) of naïve mice. Finally, in control mice, IINF were present in 8/18 metastases (44.4%; CI = 24.6–66.3). Diffuse inflammation of the liver (10.7%; SD ± 2.79) mostly consisting of lymphocytes was observed in all groups.

**Conclusions.** Both the number of liver metastases per five high power microscopy fields and occurrence of neutrophil-based inflammatory infiltrate were higher in metastases of 4T1luc2\_PR carcinomas. Thus, liver metastases from HIV PR-expressing carcinomas showed higher inflammation activity, compared to control group. The effects of HIV gene immunisation were variable demonstrating the role of inflammatory response in shaping tumour-host interactions. In an early or rapidly growing liver metastasis lacking stromal desmoplasia, inflammatory infiltrate can compromise the morphological recognition. Immunohistochemical visualisation is strongly recommended in such cases, both for experimental and clinical diagnostic purposes.



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