

Burden of Liver Metastases by Gene Expression and Immune Response in Experimental Model of Breast Carcinoma

*Džeina Mežale*¹, *Ilze Štrumfa*¹, *Andrejs Vanags*²,
*Jekaterina Pankova*³, *Maria Issagouliantis*⁴

¹ Rīga Stradiņš University, Department of Pathology, Latvia

² Rīga Stradiņš University, Department of Surgery, Latvia

³ N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russia

⁴ Karolinska Institute, Department of Microbiology, Sweden

Introduction. 4T1 breast carcinoma is a highly tumourigenic and invasive transplantable tumour cell line resembling human triple negative breast cancer. It can spontaneously metastasise from the primary tumour to multiple distant sites including lymph nodes, liver, lung, brain, and bone. Thus, 4T1 is a relevant tumour model including the general field of immunisation studies in oncology as well as liver metastases, in particular.

Aim, Materials and Methods. The aim of the present study was to characterise capacity of breast carcinoma cells 4T1 to metastasise to the liver by tumour burden evaluation after experimental introduction of different HIV genes in cell line and / or immunisation. Liver samples (n = 62) were analysed from mice transplanted with 4T1luc2 adenocarcinoma cells expressing variants of HIV-1 FSU_A enzyme with and without drug resistance mutations. 15 mice were transplanted with 4T1luc2 expressing reverse transcriptases (RT) (RT-DNA immunised 9, naïve 6); 23, proteases (PR) (PR-DNA immunised 15, naïve 8); 16 integrases (IN) (PR-DNA immunised 10, naïve 6). Controls (n = 8) received parental 4T1luc2 adenocarcinoma cells. Metastases were diagnosed and evaluated in formalin-fixed, paraplant-embedded liver tissues. For each mouse, the area of tumour metastases was quantified in five high power (400x) microscope fields 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. Liver micrometastases were found in livers of 11/15 4T1luc2_RT- (73.3%; CI = 48.0–89.1); 9/16 of 4T1luc2_IN- (69.2%; CI = 42.3–87.3); and 18/23 of 4T1luc2_PR implanted mice (78.2%; CI = 58.1–90.3). RT immunised mice developed metastasis in 5/9 (55.6%; CI = 26.7–81.2); IN-immunised, in 3/10 (30.0%; CI = 10.7–60.3); PR-immunised, in 10/15 (66.7%; CI = 41.7–84.8), and naïve, in 8/8 examined cases. Inflammatory cells, including neutrophils and lymphocytes, were observed morphologically, surrounding liver metastases, as well as spreading between tumour cells. No stromal desmoplasia was evident, indirectly suggesting rapidly evolving process. The mean size of 4T1luc2_RT metastases was 0.03 mm² (SD ± 0.01), 0.04 mm² (SD ± 0.08) of RT-immunised and 0.03 mm² (SD ± 0.04) of naïve; 4T1 luc2_IN, 0.08 mm² (SD ± 0.14), 0.04 mm² (SD ± 0.05) of IN-immunised and 0.1 mm² (SD ± 0.17) of naïve mice metastases; 4T1luc2_PR, 0.11 mm² (SD ± 0.13), 0.12 mm² (SD ± 0.15) PR-immunised and 0.09 mm² (SD ± 0.1) of naïve; and 0.04 mm² (SD ± 0.05) in naïve 4T1luc2 implanted mice.

Conclusions. Number of subjects with metastases among HIV DNA-immunised mice implanted with HIV-expressing tumours was significantly lower than among naïve animals (18/34 vs. 20/20, p = 0.0003). DNA-immunisation with IN protected (p = 0.006), and with RT, tended to protect against liver metastases (p = 0.058). No protection was offered by PR-DNA immunisation. Furthermore, 4T1luc2_PR tumours tend to form larger metastases and spread to the liver more aggressively, compared to other groups, which may relate to a strong cellular response against anti-PR induced by DNA immunisation with migration of inflammatory cells to liver.



Vactrain Twinning on DNA-based cancer vaccines.