ABSTRACT

Melanoma is a skin tumour arising from melanocytes - skin cells bearing pigment melanin. Melanoma belongs among immunogenic tumours, which is probably associated with a relatively high incidence of partial spontaneous regression (SR). Melanoma-bearing Libechov Minipigs (MeLiM) represent a specially bred animal model that is mostly affected by nodular melanomas, which fully regressed in about 2/3 of the affected animals. Our interest was to examine immune response (associated with melanoma cell destruction) and the role of proteins related to the extracellular matrix (reflecting tissue remodeling) during SR of MeLiM melanoma.

We performed an extensive time-lapse study of skin melanomas taken from individuals of the MeLiM strain at 3, 4, 6, 8, 10, 12, 20 and 32 weeks (5-10 samples in each age category) in which we immunohistochemically detected the expression of collagen IV, laminin, fibronectin, tenascin C, as well as MMP-2 and we monitored the proportion of basic immune subpopulations in blood and tumour by flow cytometry. The higher expression of collagen IV, laminin and MMP-2 positively correlated with the appearance of melanoma cells. The expression of collagen IV and laminin indicates a possible survival of tumour cells due to the interaction with these proteins, the presence of MMP-2 in these areas most likely indicates the continuous remodelling of tumour tissue. In contrast, fibronectin and tenascin C showed opposite results than in human melanoma. Their expression did not confirm the role of potential melanoma markers, because in the animals with SR a high expression of these proteins was found in areas devoid of melanoma cells. Due to the drift in the expression of collagen IV and tenascin C, we concluded that the age of 10 weeks represents the turning point in which the initially growing melanoma transits into the SR stage.

The results of flow cytometry analysis showed the first signs of anti-tumour immune response at the 6th week of pig age, when we found a higher proportion of

CD3-CD8+ (NK) and CD4+CD8+ (effector/memory T helper) cells in the tumour compared to the peripheral blood. The proportion of CD3-CD8+ cells reached three time higher representation in tumour than in the blood of 8 weeks old pigs contrary to CD4+CD8+ cells, wich showed temporal decrease at the age of 8 weeks. Both subpopulations gradually increased in older animals. At the 10^{th} week of pig age, a higher, roughly double, proportion of CD4-CD8+ cells (mainly cytotoxic T lymphocytes) appeared for the first time in melanomas compared to peripheral blood. These cells significantly dominated in the tumours of older animals until the end of the follow-up (32 weeks of age).

We conclude that the SR of melanoma in pigs of the MeLiM strain is a highly dynamic and long-term process probably initiated by immune response against melanoma. Firstly, non-specific (NK cells) and then specific immunity (cytotoxic T lymphocytes) recognize melanoma tissue and their activity gradually destroys tumour cells. This subsequently leads to the transformation of the tumour into connective tissue accompanied by a change in ECM protein expression.