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Studying the influence of microenvironmental NGFR in melanoma progression and metastasis

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Introduction:

The Nerve Growth Factor Receptor (NGFR), also known as CD271 and $p75^{NTR,}$ is a neurotrophin receptor from the TNFa receptor superfamily. NGFR expression has been reported in many tissues, and it has been showed to play a role in several different biological processes: induction of migration of neural crest cells during embryo development, apoptosis promotion during the generation of the nervous system, or pluripotency maintenance in stem cells among others (1). NGFR has also been involved in melanoma progression, associated to a stem cell-like phenotype, increased tumorigenicity and promotion of proliferation and migration (2). However, the role of NGFR in the microenvironment has been poorly studied. The crosstalk process of the microenvironment with the tumor is highly complex due to the contribution of different factors in the tumor-host interaction including several cell types (e.g. immune cells, stromal cells, etc…) which may promote multiple effects on tumor cells.

Objectives:

We aim to characterize the role of NGFR in melanoma progression analyzing primary tumor growth and metastasis in NGFR knock out mouse models. We hypothesize that NGFR depletion in the host microenvironment would have an impact in tumor formation and metastatic spread.

Methodology:

We have developed a NGFR monoclonal antibody in collaboration with the Monoclonal antibodies and Histopathology unit at CNIO. We have assessed the expression of NGFR in male and female C57BL/6 wild type mice from 10 to 15 weeks, and in the NGFR KO mouse model B6.129S4-*Ngfr*^{tm1Jae}/J. We have analyzed the expression of NGFR in mouse melanoma models by immunohistochemistry, immunofluorescence, Western blot and flow cytometry. In addition, we have studied the influence of NGFR in melanoma metastasis to lymph nodes, lung and liver.

Results:

We have tested the expression of NGFR during normal development in the brain of adult mice. Our data show that NGFR is expressed in basal forebrain neurons as previously reported (3), hence validating the utility of this antibody to analyze NGFR expression in mice. We have also

validated the use of our antibody in mouse models founding NGFR expression in wild type mouse E11.5 embryos, and in the liver, spleen, suprarenal gland and lymph nodes of adult mice.

To analyze the role of microenvironmental NGFR in melanoma metastasis we used B16-F1, and F10 cell lines as models. We have observed accelerated subcutaneous tumor growth in NGFR KO animals as well as significantly higher incidence of metastasis in lymph nodes and lungs in this mice compared to wild type controls. We then looked for immune cells populations that could explain the observed phenotype. Our study of the lymph nodes of NGFR KO animals revealed a significant decrease in different dendritic cells type 1 subsets, specifically in migratory cells while resident dendritic cells frequencies seems to remain unaffected. We have also analyzed the stromal cell populations in lymph nodes and we found alterations in the frequencies and structures in fibroreticular cells and high endothelial venules.

Conclusions:

Our work reveals an unknown role of endogenous NGFR in the immune regulation of tumor development and metastasis formation as a result of the deficient function of dendritic cells in this mouse models. We have also observed that NGFR is normally expressed in fibroreticular cells and high endothelial venues in the lymph nodes, and that it is crucial to maintain the normal structure of these organs. Finally, we have developed an antibody against NGFR, useful for immunohistochemistry in mouse paraffin sections, as well as immunofluorescence and Western blot.

References:

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