Analysis of infiltrating gamma delta and T-reg cells in cutaneous melanoma: a hypothetic crosstalk for new immunotherapy.

BACKGROUND

Melanoma is one of the most frequently occurring skin cancer and remains an important cause of mortality mainly in Caucasian populations. It is characterized by an extremely high metastatic potential, leading to 5 year-survival rates of less than 5 % after the onset of metastatic spread [1]. In clinical practice, the standard treatment for localized melanoma is surgical resection but for metastatic melanoma, few treatments are available, with immunotherapies playing a central role, while chemo and radio-therapies are rarely indicated. Advanced melanoma in stages III and IV still display a poor prognosis, and further research is needed to provide new more effective therapeutic protocols [2], [3]. Immunotherapy has revolutionized the management of metastatic melanoma that, as in others tumors, uses your body's own immune system to help fight cancer. In fact, after immunotherapy treatment, the median survival for metastatic melanoma diminished in 6 years from 18 to 9 months. Immune checkpoint inhibitors such as anti-cytotoxic T-lymphocyte protein 4 (CTLA-4) and anti-programmed cell death protein 1 (PD-1) were widely used in several types of cancer, included melanoma. However, more than half of the patients do not respond to these treatments [4]. The importance of the immune response in the clinical outcome was correlated with increased number of tumor-infiltrating lymphocytes (TILs) in melanoma and in several other tumors [5], [6], [7]. The predominant types of lymphocytes within TILs are NK, T cells, such as CD4+ and CD8+, T-regs but recently γδ T lymphocytes have been detected too [8], [9]. Many studies underline the role of γδ T lymphocytes in the anti-tumor surveillance [10] with a marked cytotoxic effect towards tumor cells. A recent study of Cordova et al. demonstrated that γδ T lymphocytes are well represented amongst TILs in cutaneous melanomas; they produce pro-inflammatory cytokines such as TNF-α and IFN-γ and exert a strong cytotoxic activity against melanoma cells in vitro [11]. T regulatory cells (Treg) regulate immune responses and maintain self-tolerance. Moreover Tregs inhibit T cell proliferation and IFN-γ production, but, on the other hand, Vg9Vd2 T cells antagonize expansion of Tregs and subsequent suppression of T-cell responses [12].

Therapies targeting the immune checkpoint molecules have achieved objective responses in melanoma. Several receptors and/or ligands, such as PDL1, PD-1 and CTLA-4, have been identified as important regulatory molecules but these therapies are not effective for a large number of patients.

Current research focuses on identifying new immune checkpoints to potentially use their inhibitors alone or in combination with current therapies.

Research Plan

In this project proposal, I plan to investigate the expression of some novel immune checkpoint molecules, such as TIM3 (T-cell immunoglobulin and mucin-domain containing-3) and TIGIT (T-cell Ig and ITIM domain), potentially expressed on T lymphocytes. TIGIT is highly expressed on Treg cells, but its presence in Vγ9Vδ2 T cells is still unclear. In particular I wanted isolated T-regs and a specific subset of γδ cells, Vγ9/Vδ2, from tumor infiltrating immune cells derived from tumor samples. The Vγ9Vδ2 T cells are significantly correlated with the melanomas in the initial and advanced phase [11] and open new horizons in immunotherapy field for that patients that actually do not benefit from any therapy. In this regard, a recent study of Cordova's group showed that the percentages of tumor-infiltrating Vγ9Vδ2 T cells were correlated negatively with stage of disease and positively with Relapse Free Survival (RFS) and Overall Survival (OS) of melanoma patients. In particular, then the same group highlighted the presence of Vγ9Vδ2 T cells in TILs of melanoma samples and the amount was correlated with early stage of melanoma (stage 0-I-II) and without metastasis [13]. Moreover, patients who progressed to stage III or IV showed a decreased frequency of circulating Vγ9Vδ2 T cells compared to values at diagnosis. All these evidences lead to hypothesize the possibility of using combination of adoptive γδ T cell therapy with immune checkpoint inhibitors as a useful strategy to enhance the anti-tumor activity of these cells. Because currently very little is known about these signalling pathways in γδ T and T-reg cells it would to investigate downstream effectors and its presence in Vγ9Vδ2 subsets. .

Materials and methods

The study involves the enrolment of patients diagnosed with a primary melanoma or skin melanoma metastasis (first 18 months of the study period). After the informed consent, the examination will be performed on samples of peritumoral skin (in close proximity to the excised melanoma) of primary and metastatic melanomas. In all patients, a venous blood sample will be performed at the time of diagnosis of melanoma and at 1 year follow-up(from month 12 to month30 of the study period) in order to correlate the obtained data with clinical stage and evolution of disease.

Perilesional skin will first be minced into small pieces and then digested with collagenase type IV and DNAase. Then, tumor-infiltrating cells will be obtained by Ficoll-Hypaque density gradient centrifugation and will be stained for live/dead discrimination. Then, expression of surface markers

will be determined by flow cytometry. The composition of γδ T cells will be evaluated with anti-CD45, anti-CD27, anti-CD19, anti-CD3 antibodies, anti-pan γδ TCR, anti-Vδ1, anti-Vδ2, anti-CD45RA and anti-CD14, while the T-reg with used anti-CD3, anti-CD4, anti-CD45, anti-CD25 and FoxP3 and other markers as Helios. Then, a subsequent analysis will be carried out on γδ T cells populations in order to evaluate the presence of an immune-checkpoints molecules with specific antibodies. To confirm the presence of these molecules and their localization in γδ T cells within TILs, it will perform an immunofluorescence analysis in melanoma sections. These investigations will be achieved on primary and metastatic melanoma samples and compared them with healthy controls.

In the last 6 months, the data will be processed through statistical analysis.

Expected project impact

The search for tumor-infiltrating lymphocyte cells belonging to the innate immunohistocompatibility system could be fundamental in the development of alternative therapies based on immunosurveillance as a mechanism of action against advanced malignant melanoma, when surgery does not improve the Overall survival (OS). The proposed study could provide further evidence to support the use of new checkpoints in melanoma immunotherapy.

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