Abstract

The treatment of metastatic melanoma was revolutionized by the approval of inhibitors of immune-checkpoints, including antibodies targeting the programmed cell death protein 1 (PD-1), such as nivolumab and pembrolizumab. Anti PD-1 agents improved the survival of patients with advanced melanoma; however, a great percentage of patients do not benefit from treatment with these drugs and understanding the mechanisms influencing the response to anti PD-1 agents is an urgent need.

Extracellular adenosine is a potent anti-inflammatory mediator able to impair anti-tumor immune response. The adenosine pathway has been indicated as one of the mechanisms causing immune suppression and resistance to immune-checkpoint inhibitors. The main enzyme responsible for extracellular adenosine production is the ectonucleotidase CD73, which hydrolyzes AMP into adenosine and inorganic phosphate.

CD73 is anchored to the membrane of many cell types, and its expression is upregulated in several human cancers. This ectonucleotidase is also found on the membrane of exosomes, extracellular vesicles (30-150 nm) that are involved in cell-to-cell communication and are produced by almost all cell types, including cancer cells and immune cells. CD73 can be cleaved from the cell membrane and the soluble form is free to circulate in biological fluids.

The main goal of this PhD project was to investigate the potential of all the forms of CD73 as predictive factors of response in patients with advanced melanoma receiving anti- PD-1 agents (nivolumab, pembrolizumab) alone or in combination with anti CTLA-4 (ipilimumab). The project was divided in four parts, each one focused on a different form of CD73: the cell-bound form expressed by circulating lymphocytes, the soluble and the exosomal form in serum and the cell-bound form within the melanoma lesion.

For the first part of the project, I analyzed the frequency of CD8+ lymphocytes phenotypes in a cohort of 100 patients with melanoma, using blood samples collected prior to the start of treatment (baseline) with nivolumab. High frequency of baseline circulating CD8+PD-1+CD73+ lymphocytes resulted associated with worse survival and no clinical benefits to nivolumab treatment.

In the second part, I characterized the expression and the activity of soluble CD73, in a retrospective study involving 546 melanoma patients, treated with nivolumab or pembrolizumab, or nivolumab plus ipilimumab. Both the activity and the expression of CD73 resulted higher in patients than in healthy donors, but the ROC curve analysis revealed that the enzymatic activity better stratifies patients from healthy donors. High CD73 activity resulted associated with no-response to therapy with anti-PD-1 monotherapy and its combination with anti-CTLA-4. After three months of treatment, soluble CD73 activity remains unchanged from baseline, and it is still higher in non-responders than in responders. High CD73 enzymatic activity resulted associated with reduced overall survival and progression-free survival in patients treated with anti-PD-1 monotherapy. Furthermore, CD73 activity emerged as an independent prognostic factor in multivariate cox regression analysis.

The third part of this project was dedicated to the study of the exosomal form of CD73. Firstly, I optimized a protocol, based on Size Exclusion Chromatography and ultrafiltration, to isolate biologically active exosomes from human serum. Analyses were performed in a single-center cohort of 41 patients with melanoma, treated with pembrolizumab or nivolumab. Exosomes express CD73, which maintains its enzymatic activity. In vitro assays revealed that CD73+ exosomes are able to suppress, in presence of AMP, the production of IFN-γ in activated peripheral blood mononuclear cells (PBMCs) isolated from healthy donors. This effect is mediated by the activation of A2A adenosine receptor expressed on PBMCs.

While no differences were observed in terms of exosomal CD73 expression at baseline between patients responding to therapy and those not responding, interestingly, the exosomal CD73 expression significantly increased after 4 weeks of treatment, compared to baseline levels, in non-responders group. These results indicate that exosome-derived adenosine can suppress T cell functions and the expression of CD73 on exosomes may impact the response to anti PD-1 therapy in patients with advanced melanoma.

The last part of the project was focused on studying the expression of CD73 within melanoma lesions, by performing co-detection by indexing multiplexed tissue imaging (CODEX®) using the PhenoCycler™ instrument (Akoya Biosciences). A panel of antibodies enabling the individuation of the different phenotypes populating the tumor lesion has been developed to obtain a complete overview of the CD73 expression in the tumor microenvironment of melanoma, and thus a better understanding of the prognostic value.

Taken together, the results discussed in this thesis indicate that the measurement of CD73 activity and/or expression could be informative to identify a suppressive mechanism that influence the anti-tumor immune response, impairing the therapeutic effectiveness of immune checkpoint inhibitors.

Both the pretreatment activity of serum CD73 and the pretreatment frequency of circulating CD8+CD73+ T cells emerged as prognostic factors, that may guide the therapeutic choices in patients with advanced melanoma. In addition, this thesis strongly supports the notion that targeting CD73 in combination with anti-PD-1 agents could further improve the clinical response in melanoma patients.