Abstract

The adenosinergic pathway plays a critical role in cancer development and progression, as well as in drug resistance to chemotherapy and/or targeted-therapy.

The goal of this PhD thesis was to investigate and fully characterize the role of CD73/adenosine A_{2A}-A_{2B} receptors axis in cancer, highlighting the therapeutic potential of inhibitors of the adenosinergic pathway.

We firstly characterized the mechanism/s by which A_{2B}R promotes immunosuppression and angiogenesis in tumor-bearing hosts, focusing on the role of myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts (CAFs). The results revealed that treatment of melanoma-bearing mice with Bay60-6583, a selective A_{2B}R agonist, is associated with 1. increased tumor VEGF-A expression and vessel density, and 2. increased accumulation of tumor-infiltrating CD11b+Gr1+cells (MDSCs). MDSCs strongly contribute to the immunosuppressive and angiogenic effects of Bay60-6583. Melanoma-bearing mice treated with a selective A_{2B}R antagonist PSB1115 showed reduced tumor growth compared to controls and this effect was associated with reduced tumor angiogenesis, low levels of MDSCs and increased number of tumor-infiltrating CD8+ T cells. Furthermore, blockade of A_{2B}R increased the anti-tumor effects of VEGF-A inhibitors. Next, we verified that A_{2B}R activation also drives fibroblasts activation within melanoma tissues, by increasing the number of FAP positive cells within tumor lesions. FAP is a common marker of activated fibroblasts also named cancer-associated fibroblasts. These cells produce and secrete various tumor-promoting factors, including fibroblast growth factor (FGF)-2 and CXCL12 or stromal-derived factor 1 α (SDF1α), that were increased both in melanoma tissue and fibroblasts isolated from melanoma tissue or from skin upon Bay60-6583 treatment. Bay60-6583-induced FGF-2 from fibroblasts contributed to melanoma cells proliferation. The CXCL12/CXCR4 pathway, instead, was involved in the pro-angiogenic effects of A_{2B}R agonist, but not in its immunosuppressive effects. These effects were significantly blocked by the A_{2B}R antagonists PSB1115. Taken together, these data elucidate the pivotal role of A_{2B}R in establishing a positive cross-talk between tumor-infiltrating immune cells, fibroblasts and endothelial cells that sustain tumor growth, reinforcing the therapeutic potential of A_{2B}R blockers for cancer therapy.

We next investigated the immunosuppressive mechanism mediated by A_{2A}R that occurs through the Notch signalling pathway in CD8+ T cells. A_{2A}R stimulation with CGS-21680 downregulated Notch1 expression in CD3/CD28-stimulated CD8+ T cells at the protein level but not at transcriptional level. The inhibitory effects of CGS-21680 on effector functions of CD8+ T cells were enhanced in presence of the Notch1 inhibitor, PF-03084014. Similar results were obtained

also in CD8+ T cells treated with forskolin, an activator of adenylate cyclase, which mimics the effects of CGS-21680. The $A_{2A}R$ agonist did not influence the expression of Notch1 in CD8+ T cells after TCR activation, suggesting that stimulation of $A_{2A}R$ affects the expression of TCR-induced Notch expression. These results were confirmed by using transgenic CD8+ T cells from N1IC mice (N1IC CD8+ T cells) that were less sensitive to the inhibitory effects of CGS-21680. CD8+ T cells deficient of $A_{2A}Rs$ were instead sensitive to the inhibitory effects of the Notch inhibitor PF-03084014. Overall, our results indicate that the inhibitory effects of adenosine via A_{2A} receptor occurs, at least in part, by reducing the Notch1 expression and activity, by blocking the TCR-mediated signalling transduction in CD8+ T cells.

Finally, we evaluated the associations of soluble CD73 (sCD73) enzyme activity with clinical outcomes of patients with metastatic melanoma receiving the anti-PD1 agent nivolumab. In a retrospective study we found that melanoma patients stage IV with high basal serum level of sCD73 enzyme activity, before starting nivolumab treatment, had a lower response rate to nivolumab, shorter survival and higher rates of progression of disease. Patients obtaining partial response to nivolumab or stable disease had low levels of sCD73 activity in the serum, thus suggesting a predictive role of response to nivolumab therapy for sCD73. This evidence could be very helpful to select patients to an appropriate therapy and then, neutralizing CD73 with clinically validated CD73 monoclonal antibodies could represent a highly potent and innovative therapy in combination regimen.

In conclusion, we provide new insights into the mechanisms by which adenosinergic molecules modulate immune- and stromal-cells responses in the tumor environment, that is important for developing combination strategies for cancer therapy.