A Promising Pathway in Immuno-Oncology: CD73-Adenosine Axis Highlighted by Quantitative Mass Spectrometry Imaging

Lauranne Poncelet1, Rima Ait-Belkacem1, Bruno Gomes2, and Jonathan Stauber1

1ImaBiotech, MS Imaging Dept., Loos, France; 2iTeos Therapeutics SA, Gosselies, Belgium

Introduction

The CD73-Adenosine (CD73-ADO) axis constitutes one of the most promising pathways in immuno-oncology. CD73 catalyzes the conversion of adenosine monophosphate (AMP) to ADO. CD73 is believed to play a role in mediating the inhibitory function of regulatory B and T cells. It is widely expressed and up-regulated in many cancerous tissues. The presence of extracellular ADO within tumor microenvironment has been described as an immunosuppressive halo surrounding the tumor, permeating the tumor microenvironment and preventing antitumor immunity. In this study, the endogenous level of AMP and ADO were analyzed by quantitative mass spectrometry imaging (QMSI) and LC-MS/MS in CT26 tumor mouse model. Clones with different CD73 expression levels were selected (low, medium & high), which mimed different CD73-inhibitors efficacies. Then, ADO metabolic pathway was targeted in order to highlight the tumor microenvironment response linked to a mimic immunotherapy effect.

Methods

Undifferentiated colon carcinoma CT26 cell line was implanted into C57BL/6 mice. Then, tumors were harvested and frozen at -80°C until use. 1,5-diaminonaphthalene (1,5-DAN) and 2,5-dihydroxybenzoic acid (2,5-DHB) matrices mixed to internal standards were sprayed onto tumor sections of 10 µm thickness with the automatic TM Sprayer (HTX Technologies, LLC) prior analysis. Data acquisition was performed using 7T MALDI-FTICR (SolarIX XR, Bruker Daltonics) and analyses were set at 120 µm spatial resolution in full scan positive and negative modes. Acquired data were treated with Multimaging™ software (ImaBiotech). All quantitative results were confirmed by LC-MS/MS analysis. Histological and immunohistochemistry staining anti-CD73 were applied on adjacent section.

Results

A validation of CT26 mouse model occurred since an increase of ADO/AMP ratio of 77% was relative to the high CD73 expression. In other words, CT26 mouse models with high CD73 expression showed higher ADO/AMP ratio (2.1) than CT26 medium (1.7) and low (1.6) clones. Then, as an immunosuppressive halo, the impact of ADO expression on tumor microenvironment was also characterized using MSI analysis. A large number of metabolites that are implicated in this pathway were highlighted (ADP, IMP, GMP, Guanosine, Inosine, Hypoxanthine, Xanthine, Uric acid…) and differences were also observed.

Conclusion

The combination of all these data extended our understanding on the relative contribution of ADO signaling in suppressing antitumor immunity. Because the development of immunotherapies such as CD73 inhibitor requires a deep understanding of the interplay between the immune system and cancer cells in the tumor microenvironment, these immune/tumor metabolites and nutrients can now be followed by QMSI as biomarkers of response to treatment towards enhancing immunotherapies efficacy.