

Biomarker Monitoring by Quantitative MALDI Imaging: Application to the Tryptophan-Kynurenine Pathway in Immuno-Oncology

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Introduction

Tryptophan (Trp) is an essential amino acid for cell proliferation and survival that can be metabolized through different pathways, a major route being the kynurenine (Kyn) pathway. The first and rate-limiting enzyme of this pathway is the indoleamine-2,3-dioxygenase 1 (IDO1), that is a natural endogenous molecular mechanism of immune suppression acting through modulation of the Trp degradation pathway. Inhibition of T-cell functions, activation of the regulatory T-cells, and inhibition of Natural Killer cells are among the important immunosuppressive effects of IDO1. Thereby, IDO1 enzyme is proposed to have a therapeutic potential in immunodeficiency-associated abnormalities, including cancer. The standard quantification methods are based on the total level of Trp and its metabolites determined by LC-MS/MS analysis in plasma and tumors. We describe here the setup, development and application of a new method based on MSI to detect, localize, and quantify Tryptophan and Kynurenin in the microenvironment of the tumors.

Methods

Results

This method allows both the study of the sub-tissular localization and the detection/quantitation of metabolites of interest in tumor tissues. In the present study, an experimental tumor model overexpressing IDO1 and its wild-type counterpart were implanted in mice. Then, tissue sections of different tumors were realized and used for mass spectrometry imaging analysis. MALDI FTICR high resolution imaging followed by data analysis enabled an absolute on tissue quantitation. Internal standards of tryptophan (Trp-d5) and kynurenine (Kyn-d4) metabolites were used for normalization. As expected, our results showed an increase of Kyn in parallel to a decrease of Trp amount in IDO1-positive tumors. Following, immunostainings of IDO1 and Trp-depletion sensor pathways were carried. Overlaying images between the immunostainings and the molecular MS images allowed co-localization studies and underlined both the biology and the tumor heterogeneity. This study allowed us to highlight key metabolites of the Trp pathway that are responsible for the immunosuppressive tumor microenvironment. Furthermore, this study illustrated the heterogeneity of tumor immune areas. Because the development of immunotherapies such as IDO1 inhibitors requires a deep understanding of the interplay

between the immune system and cancer cells, these immune endogenous metabolites can now be followed by quantitative MALDI imaging and as biomarkers towards enhancing immunotherapies efficiency.