TOWARDS THE IDENTIFICATION OF THE MECHANISM OF ACTION OF ANTITUMOR 1-METHYL-D-TRYPTOPHAN

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Background: In recent years, tryptophan degradation has received increasing attention as a potent immunosuppressive mechanism involved in the maintenance of immunological tolerance in both tumor draining lymph nodes and tumors. Indoleamine 2,3-dioxygenase 1(IDO1) is a tryptophan metabolizing enzyme and its mechanisms of action as an immune regulator involves tryptophan deprivation, production of immunosuppressive metabolites (kynurenines), and activation of signaling events through binding of tyrosine phosphatases (SHPs). IDO1is chronically activated in many cancer patients and its expression and enzyme activity correlate with a poor prognosis in patients with various cancers. In the past, IDO1inhibition was mostly achieved using the racemic mixture of 1-D,L-methyltryptophan (1-MT). As it became apparent that IDO1inhibition may be a promising target for cancer therapy, the individual stereoisomers of 1-MT were investigated in more details. 1-L-MT was shown to more effectively inhibit IDO1in enzyme assays and in cancer cell lines. However, 1-D-MT showed superior anti-tumor activity in mouse models and was therefore chosen for clinical trials. 1-D-MT is currently being tested in phase II clinical trials (indoximod) as an adjunct to conventional chemotherapy, although its immunostimulatory mechanism is still unknown. We here investigated whether 1-D-MT could interfere with IDO1signaling rather than catalytic activity.

Methods: The enzymatic activity of IDO1was measured in vitro in terms of the ability of both purified protein or stably transfected cells to metabolize tryptophan to kynurenine. In these cells we also evaluated IDO1protein turnover and its ability to bind SHPs through both FRET analysis and immunoprecipitation.

Results: 1-D-MT did not inhibit IDO1catalytic activity, but affected IDO1-SHPs binding.

Conclusion: Our data indicated that 1-D-MT anticancer activity may rely on the inhibition of IDO1signaling but not catalytic activity.