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Zbadanie roli arginaz i mechanizmów przeciwnowotworowego działania inhibitorów arginazy

Rozprawa na stopień naukowy doktora nauk medycznych

w dyscyplinie nauki medyczne

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Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2020

ABSTRACT

Accumulating evidence indicates that the immunoregulatory mechanisms in the complex tumor microenvironment are among the main obstacles to successful cancer immunotherapy. One of the most prominent features of the tumor microenvironment that dysregulates the local adaptive immune response against cancer is amino-acid metabolism, also that involving L-arginine. Arginase (ARG) is an enzyme degrading L-arginine, which plays a role in the expansion and proper functioning of T-cells to exert a successful antitumor immune response. High ARG activity in the tumor microenvironment of various types of malignancies has been reported and an increasing number of observations indicate that it correlates with poor clinical outcomes of cancer patients. Therefore, this project focused on investigating the role of ARG in the tumor microenvironment and on studies of ARG inhibition mechanism to reduce the immunosuppressive properties of cancer.

ARG1 expression was studied in detail in the tumor microenvironment of a murine lung carcinoma at the different tumor progression stages. As a result, ARG expression was found in tumor-associated myeloid cells, mostly macrophages. Elevated ARG expression was linked with advanced tumor stage and correlated with impaired *in vivo* proliferation of the antigen-specific T-cells. Additionally, L-arginine plasma concentration decreased with tumor progression, suggesting elevated ARG activity. In *in vitro* settings, the influence of lack of L-arginine in medium or addition of recombinant ARG1 on the process of T-cells expansion was investigated. Depletion of L-arginine caused impaired T-cells proliferation, down-regulation of CD3ε and CD3ζ chains expression and reduced cytokines production. The observed negative changes were abrogated in the presence of ARG inhibitors. Transgenic mice with ARG1 deficiency were used to elucidate the immunomodulatory impact of ARG1 on the development of antigen-specific immune response. The obtained results indicate that mice with *knock-out* of ARG1 develop an improved immune response and have a higher percentage as well as the number of tumor-infiltrating lymphocytes. This study also demonstrates the negative effects of ARG1 overexpression on the *in vivo* tumor growth, causing accelerated progression of lung carcinoma and melanoma. Furthermore, the *in vivo* antitumor efficacy of the novel small-molecule ARG inhibitor OAT-1746 was investigated in the murine lung tumor model. Antitumor activity of OAT-1746 was studied in monotherapy as well as in combination with other immunotherapies, including checkpoint inhibitor

anti-PD-1 and stimulator of interferon genes (STING) agonist. OAT-1746 treatment as monotherapy significantly inhibited tumor growth as well as prolonged the survival of mice and these effects were enhanced in combination therapy. Finally, the mechanism of action of OAT-1746 was investigated. The obtained results suggest that OAT-1746 acts by changing the proportions of specific T-cell populations in the tumor microenvironment, especially by switching the balance towards a less immunosuppressive T-cell phenotype.

Altogether, this study provides the evidence that ARG1 activity impairs T-cell response and that modulation of tumor microenvironment properties by targeting ARG enzymatic activity is a promising immunotherapeutic approach to enhance the antitumor immune response.