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CD39: A complementary target to immune checkpoints to counteract tumor-mediated immunosuppression

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We report that CD39–expressing-melanoma cells inhibited both T-cell proliferation and the generation of cytotoxic effectors in an adenosine-dependent manner, and that treatment with a CD39-blocking antibody alleviated tumor-mediated immunosuppression. Thus, blocking CD39 ectonucleotidase may represent a novel immunotherapeutic strategy to restore antitumor immunity.

Introduction

One hallmark of cancer cells is their capacity to evade immune-mediated destruction via the acquired expression of multiple negative regulators of the immune response, termed immune checkpoints, such as CTLA4 and PD1/PDL1. Targeting such key regulators of the immune response recently emerged as a new treatment option to prevent tumor-mediated immunosuppression and to establish a long-lasting cancer-specific immune response, possibly by reactivating mutant tumor-antigen-specific T cells.[1] Antibody-mediated blockade of these immunoregulatory pathways has provided outstanding clinical results with durable responses and survival benefits in cancer patients. However, because an objective response is observed in less than 50% of the patients and is tumor dependent, the identification of alternative non-redundant inhibitory/immunosuppressive pathways that could be targeted in synergistic therapeutic association schemes is of major interest. Among molecules involved in the regulation of the immune response, the CD39 ectonucleotidase triphosphate diphosphohydrolase 1 (Entpd1) represents a new promising target for cancer immunotherapy.

CD39 hydrolyzes adenosine triphosphate (ATP) and adenosine diphosphate (ADP) into adenosine monophosphate (AMP), which is then processed into adenosine by the CD73 ecto-5′-nucleotidase (Nt5e). Adenosine, bound to the A2A receptor (A2AR), is a critical regulator of both innate and adaptive immune responses and protects normal tissues from inflammatory damage and autoimmunity. Interestingly, the CD39–CD73–adenosine–A2A receptor pathway is aberrantly activated in tumor tissues, notably in response to hypoxia. Several seminal works evidence the key role of this pathway in cancer promotion. Indeed, immunogenic tumors are rejected in A2AR-deficient mice[2] and tumor-resistant phenotypes are observed in CD39 or CD73 knockout (KO) mice.[3,4] Through the modulation of pericellular levels of adenosine, $CD39^+$ serves as an integral component of the suppressive machinery of regulatory T cells (Tregs) within the tumor microenvironment. CD39 disruption (CD39KO mice) or blockade with pharmacological inhibitors inhibits Treg activity and facilitates natural killer (NK) cell-mediated antitumor response, which in turn inhibits tumor growth and metastasis in mice.[4] Beyond its contribution to the suppressive activity of Tregs, it has been shown that Th17 cells isolated from various experimental tumor models express not only CD39, but also CD73, and that the expression of these ectonucleotidases determines the immunoregulatory function of Th17 cells.[5] Furthermore, an examination of ovarian cancer specimens revealed that tumor cells express CD39 and that $CD39^+$ ovarian cancer cells generate adenosine, which suppresses T- and NK-cell antitumor responses.[6]

Using a large cohort of 500 human tumor and normal histologic samples from 18 of the most common types of cancer, we reported that CD39 is absent or weakly expressed in normal samples, except in endothelial cells. Strikingly, CD39 is expressed in several human cancers, including kidney, lung, ovarian, pancreatic, thyroid, testicular, endometrial, and prostate tumors, as well as in lymphoma and melanoma. The infiltration of $CD39^+$ immune cells was clearly

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observed; however, in kidney, lung, testicular, and thyroid cancer as well as lymphoma and melanoma, CD39 was also strongly expressed by the tumor cells themselves. The expression of CD39 by tumor cells was further validated by flow cytometry on several human cancer cell lines, especially in B lymphoma, B-cell chronic lymphocytic leukemia cell lines, and melanoma. By measuring either extracellular ATP degradation or the release of free phosphate in cell culture supernatants, we demonstrated that all CD39-expressing cells display strong ATPase activity that is counteracted by the chemical CD39 inhibitors ARL 67156 and POM-1.[7]

As previously described for ovarian tumor cells,[6] we found that some cancer cell lines also express CD73, suggesting that tumor cells co-expressing CD39 and CD73 may exert potent immunosuppressive actions via adenosine. Supporting this hypothesis, we demonstrated that the degradation of ATP into AMP and adenosine by the SK-MEL-5 melanoma cell line is associated with the suppression of $CD4^+$ and $CD8^+$ T-cell proliferation as well as the generation of cytotoxic effector $CD8^+$ T cells. Similarly, we observed that the SK-MEL-5 melanoma cell line inhibits the lytic activity of peripheral blood $CD56^+$ NK cells toward target cells. Underlining the essential roles of CD39 and adenosine, we showed that treatment with the CD39-blocking antibody OREG-103/BY40, currently in preclinical development, or with the A2AR antagonist SCH58261, alleviated the tumor-induced inhibition of $CD4^+$ and $CD8^+$ T-cell proliferation and increased cytotoxic T lymphocyte- and NK cell-mediated cytotoxicity.[7] Taken together, these results reinforce the concept that the CD39-adenosine pathway is an efficient immunotherapeutic strategy for inhibiting tumor cell-mediated immunosuppression and demonstrate that the OREG-103/BY40 antibody represents a good candidate for CD39-based immunotherapy in cancer patients.

Beyond its ability to neutralize tumor-mediated immune resistance by limiting generation of adenosine, another important biological effect of the CD39-blocking antibody OREG-103/BY40 on antitumor immunity may result in its capacity to limit the degradation of extracellular ATP. Indeed, ATP is a key component of the "immunogenic cell death" process. Extracellular ATP, released by dying tumor cells following treatment with chemotherapeutic agents or ionizing radiations, is required for the efficient recruitment and differentiation of immature dendritic cells (DCs) into mature DCs able to present tumor-associated antigens and prime an adaptive antitumor immune response.[8,9] Pointing to the role of CD39 in this process, it has been shown that its expression by tumor cells prevents the establishment of an efficient antitumor immune response, unless CD39 pharmacologic inhibitors are used to prevent ATP degradation.[10]

Overall, the expected efficacy of CD39-based immunotherapy could be based on both the inhibition of the production of immunosuppressive adenosine together with the degradation of extracellular ATP in the tumor microenvironment. Such an approach may represent a promising immunotherapeutic strategy with potential to improve the activity of immune-checkpoint inhibitors in combinational treatments.

Disclosure of Potential Conflicts of Interest

NB, AB and J-FE are co-founders of OREGA Biotech.

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