Blockade of the CD39 immunoregulatory pathway by monoclonal antibodies

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Abstract

The CD39-CD73-adenosine pathway is an important regulator of effector immune cell response. We previously demonstrated that, in human cancer specimen, CD39 is expressed by infiltrating regulatory T cells, tumor cells and the tumor associated stroma. CD39 enzymatic activity decreases peritumoral ATP, a potent tumor cell toxicity and immunogenic inducer, and generates immunosuppressive adenosine that binds adenosine receptors and inhibits CD4, CD8 T cell and NK cell responses. We and other demonstrated that CD39-mediated decrease of extracellular ATP and increase of adenosine promote tumor progression and immune escape as well as resistance to chemotherapy-induced immune response. We therefore generated several CD39-blocking monoclonal antibodies and present here the latest developments of these antibodies. We provide evidence that CD39-blocking mAbs restore the proliferation of CD4 and CD8 T cells inhibited by melanoma cells expressing CD39 and increase the generation of CD8 cytotoxic T cells. Treatment with CD39-blocking mAbs or CD39 inhibitor alleviated CD39+ tumor cell-mediated inhibition of CTL and NK cell-mediated cytotoxic activity. In conclusion, CD39-blocking antibodies may represent a novel immunotherapy strategy for inhibiting regulatory T cells and tumor cell-mediated immunosuppression. The results presented here support the ongoing development of CD39-blocking monoclonal antibodies as potential anticancer drugs to restore anti-tumor immune response.

Methods summary

CD39 IHC staining was performed on formalin fixed, paraffin embedded tissues using an anti-CD39 antibody suitable for IHC (clone 22A9, Abcam).

CD39 expression in cell lines was assessed by flow cytometry using a PE Cyanine 7 coupled anti-CD39 antibody (clone A1, Ebioscience).

Cell lines and treatments: Cells are from ATCC and cultured in RPMI or DMEM. SK-MEL-5 cells were irradiated at 80-100 Gy to block proliferation. POM1 or ARL (CD39 inhibitors) were used at 100 or 250 µM; antibodies at 5 µg/mL.

CFSE labeling (proliferation): cells were incubated with 0.5µM 5,6 CFSE (Molecular Probes) for 11 min at 37°C. Proliferation was assessed by flow cytometry.

Polyclonal activation: 4x10^4 CD4 or CD8 T cells were cultured with immobilized anti-CD3 antibody (10 µg/mL, UCHT1).

Expression of Foxp3 : Human CD4 T cells were stained with PE coupled anti-human Foxp3 antibody (clone 236/E7, Ebioscience).

Expression of CD107a : 5-days cultured CD8 T cells were activated with PMA (10ng/ml) and ionomycine (1µg/ml) and incubated with PE Cyanine 7 coupled anti-CD107a antibody (clone H4A3, Ebioscience).

ATPase activity : 5x10^4 cells were treated or not with antibodies or inhibitors for 16h. Cells were cultured for 30 min with 10 µM ATP. The concentration of unhydrolysed ATP was determined using the ATPlite Assay System (Perkin Elmer).

AMP quantification : 10^5 CD39+ cells were treated or not with antibodies or inhibitors for 16h and cultured 30 min with 50 µM ATP. AMP was quantified using Maldi-Tof.

CTL cytotoxic activity assay: 5.10^6 PBMC were cultured with 10^6 irradiated (80 Gy) SK-MEL-5 cells for 6 days with 20 UI/ml of IL-2 alone or with POM-1 (100µM). Then, CD8+ T cells are purified and their cytotoxic activities tested by a retargeted cytotoxic assay using anti-CD3 mAb and mouse P815 target cells as described (Le Bouteiller P, et al., PNAS, 2002).

NK cytotoxic activity assay: 4.10^4 isolated CD56+ cells were cultured for 2h in the presence or not of 4x10^4 SK-MEL-5 melanoma cells treated or not with POM1 at 100µM. 0.8 10^4 (^{51}Cr)K562 cells (target cells) were added to the cultures.

CD39-immunosuppressive environment in human cancer

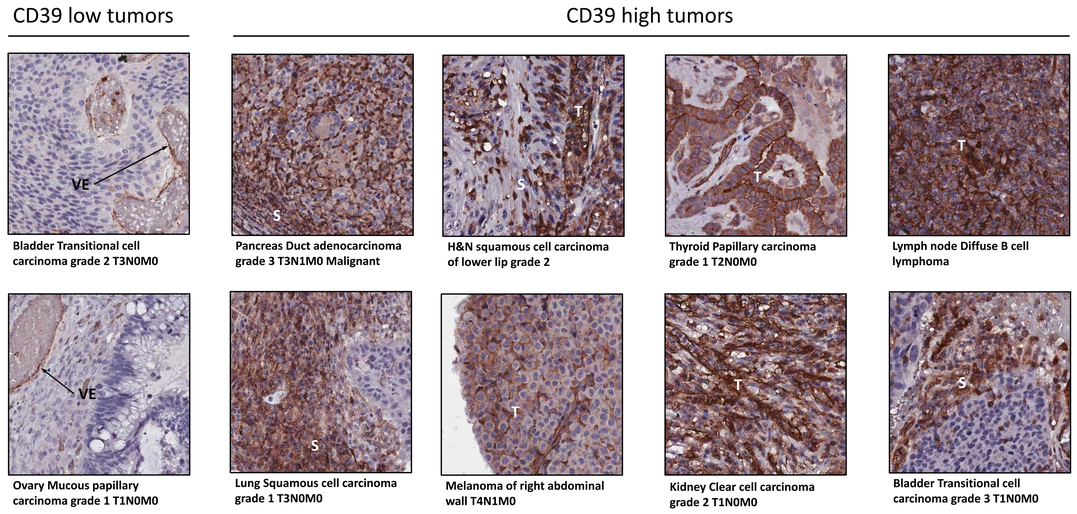
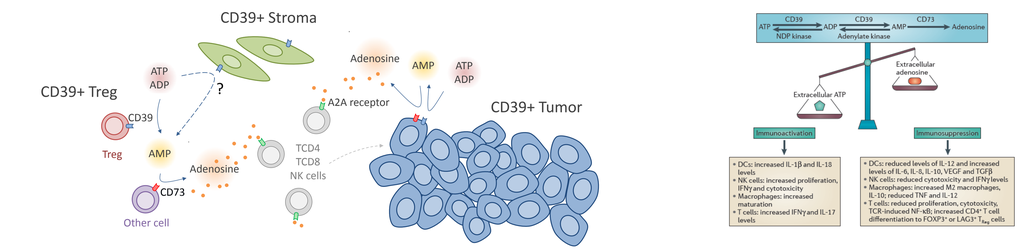


Figure 1: Representative IHC staining of CD39 expression in CD39 low/negative and CD39 high human cancer cases. In CD39 low/negative tumors, note the expression of CD39 by endothelial cells. In CD39 high tumors, CD39 is expressed by immune cells, tumor cells and/or stromal cells. T, tumor; S, stroma; VE, vascular endothelium; H&N, head and neck.

CD39-mediated immunosuppression in cancer : working hypotheses and take-home messages



CD39 expressed by regulatory T cells as well as tumor cells (and potentially stromal cells) exerts potent immunosuppressive functions by promoting the accumulation of immunosuppressive adenosine and decreasing peritumoral levels of ATP, a potent immunoactivator. CD39-blocking antibodies may therefore trigger potent immune response and are developed as potential immunotherapeutics for cancer. Cartoon (right) from Krysko VD et al., Nature Reviews Cancer (2012).

CD39-blocking monoclonal antibodies

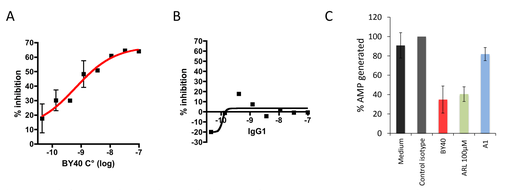


Figure 2: (A) Dose-dependent inhibition of CD39 enzymatic activity by BY40 assessed by mass spectrometry. (B) Isotype control IgG1 has no effect. (C) The CD39 inhibitor ARL (100µM) and BY40 (33nM) inhibit CD39 activity. Another CD39-binding antibody, A1 (33nM), does not inhibit CD39 activity.

Inhibition of Treg and CD8+ Treg-mediated immunosuppression by BY40/OREG-103 mAb

PoC studies have been published:

- Reversal of Treg-mediated suppression by BY40/OREGA-103: Mikolova M et al., PLoS Pathog. 2011 Jul;7(7):e1002110
- Reversal of CD8+ regulatory T cells-mediated suppression by BY40/OREGA-103 : Boer MC et al., Eur. J. Immunol. 2013. 43: 1925–1932
- Review of the role of CD39 in oncology: Bastid J et al., Oncogene, 2013 32, 1743–1751

Inhibition of CD39+ tumor-mediated immunosuppression by BY40/OREG-103 mAb

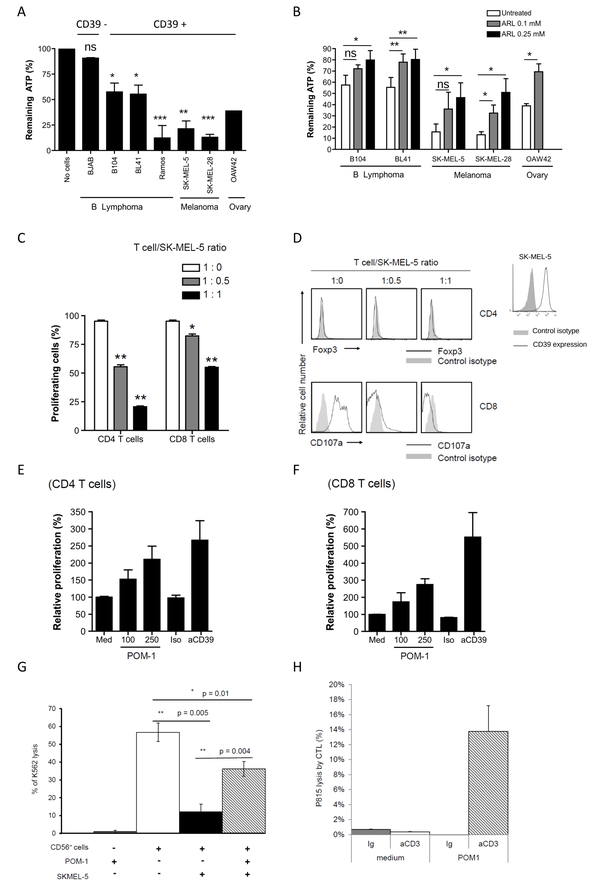


Figure 3: Similarly to Tregs, several human cancer cell lines express CD39 and exert ATPase activity in a CD39-dependent manner (A and B). CD39+ SKMEL5 melanoma cells suppress CD4 and CD8 T cell proliferation (C) as well as CTL and NK cell responses (D, G, H). CD39-blocking antibodies and/or inhibitors increase T cell proliferation (E, F) as well as cytotoxic activity of NK cells (G) and CTL (H).

Conclusion & perspectives

- CD39 is expressed in several human cancers by Tregs, tumor cells and stromal cells
- CD39 promotes accumulation of adenosine, a potent immunosuppressor
- CD39 decreases peritumoral ATP, which is vital for chemo-induced immune response
- We have generated antibodies that block CD39 enzymatic activities with nanomolar activity

These results support the ongoing development of CD39-blocking monoclonal antibodies as potential anticancer drugs.

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