Immunosuppression Mediated by CD39+ Cancer Cells is Reversed by CD39-Blocking Antibodies

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Abstract

The CD39 and CD73 ectonucleotidases hydrolyze extracellular ATP and ADP into immunosuppressive adenosine that binds adenosine receptor and inhibits T cell and NK cell responses. It has been demonstrated that CD39+ Tregs are increased in some human cancers and participate to immunosuppression. The importance of CD39+ Tregs in promoting tumor growth and metastasis has been evidenced in several models *in vivo* (for a review, see Bastid J et al., Oncogene, 2012). Here, we addressed whether CD39 is expressed by tumor cells and whether CD39+ tumor cells mediate suppression through the CD39-adenosine pathway. Immunohistochemical staining of normal and tumor tissues revealed that CD39 is upregulated in several types of human cancer. In cancer specimens, CD39 is expressed by infiltrating lymphocytes, tumor stroma and tumor cells, whereas its expression in normal samples is absent or weak and mostly limited to vascular endothelia. The expression of CD39 at the cell surface of tumor cells was further directly demonstrated by flow cytometry in human cancer cell lines. We evidenced that CD39+ tumor cells express functional CD39 and inhibit CD4 and CD8 T cell function in a CD39-dependant manner. Treatment with CD39-blocking antibody OREG-103/BY40 was able to alleviate CD39+ tumor cell-mediated inhibition of CD4 and CD8 T cells in co-culture experiments. In conclusion, interfering with the CD39-adenosine pathway could represent a novel immunotherapy strategy for inhibiting Treg and tumor cell-mediated immunosuppression. Furthermore, these results support the ongoing development of CD39-blocking monoclonal antibodies as potential anticancer drugs.

Methods summary

CD39 expression was assessed by IHC in ~500 normal and cancer clinical samples (18 cancer types) using multiple tissue Microarray (MC5002, US Biomax). 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 100 Gy to block proliferation. ARL and POM1 (CD39 inhibitors) were used at 100 or 250 µM; antibodies at 5 µg/mL. OREG-103/BY40 is a CD39-blocking monoclonal antibody in preclinical development.

CFSE labeling (proliferation): cells were incubated with 0.75µM 5,6 CFSE (Molecular Probes) for 12 min at 37°C. Proliferation was assessed by flow cytometry.

Polyclonal activation: $4x10^4$ CD4 or CD8 T cells were cultured in flat-bottom plates in presence of immobilized Anti-CD3 antibody (1 µg/mL, clone UCHT1) and soluble anti-CD28 antibody (1 µg/mL, clone CD28.2).

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).

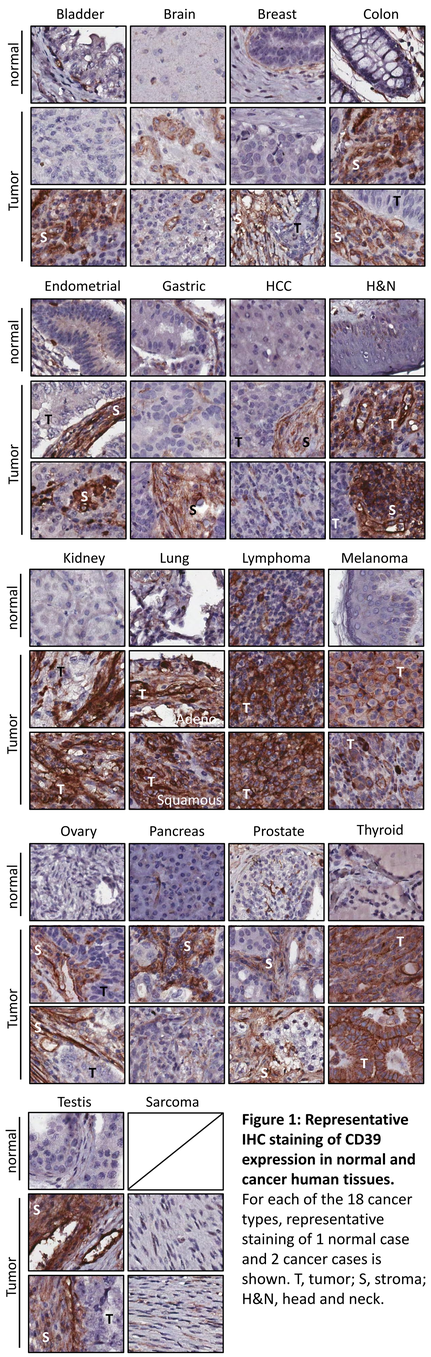

Figure 1: Representative IHC staining of CD39 expression in normal and cancer human tissues. For each of the 18 cancer types, representative staining of 1 normal case and 2 cancer cases is shown. T, tumor; S, stroma; H&N, head and neck.

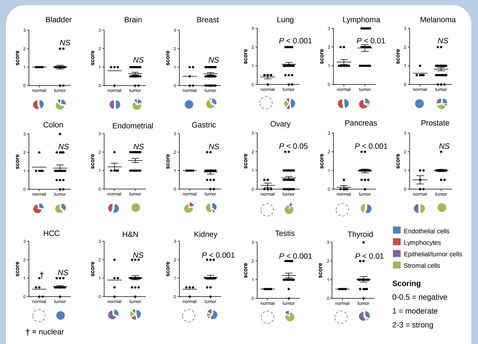

Figure 2: Scoring of CD39 expression in normal and cancer human tissues. Scoring of intensity and localization of staining was completed by a pathologist and is presented as follow: 0-0.5: negative, 1: moderate, 2-3: strong. The distribution is indicated in the graphs: blue: vascular endothelial cells, red: lymphocytes, purple: epithelial/tumor cells, green: stromal cells.

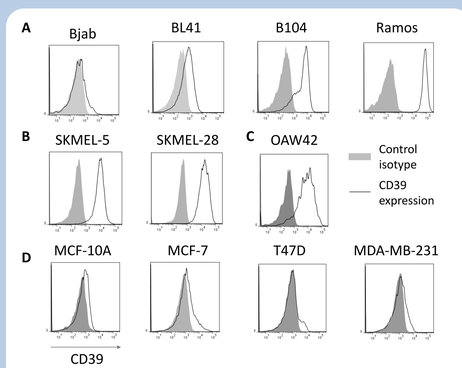

Figure 3: Expression of CD39 by human cancer cell lines. Lymphoma (A), melanoma (B), ovarian cancer (C) and breast cancer cell lines (D) were stained with anti-CD39 antibody and CD39 expression was analyzed by flow cytometry.

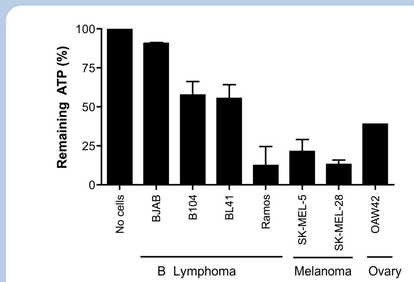

Figure 4: CD39+ tumor cells exert ATPase activity. $5x10^4$ cells were cultured for 30 min in presence of 10 µM ATP. The concentration of unhydrolysed ATP was determined using the ATPlite luminescence ATP Detection Assay System.

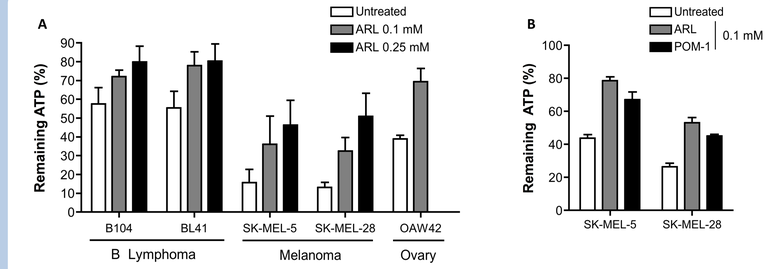

Figure 5: ATPase activity of CD39+ cell lines is dependent of CD39. (A) $5x10^4$ cells were treated or not with the CD39 inhibitor ARL. (B) Cells were treated or not with CD39 inhibitor ARL or POM-1. ATPase activity was then assessed using the ATPlite luminescence ATP Detection Assay System.

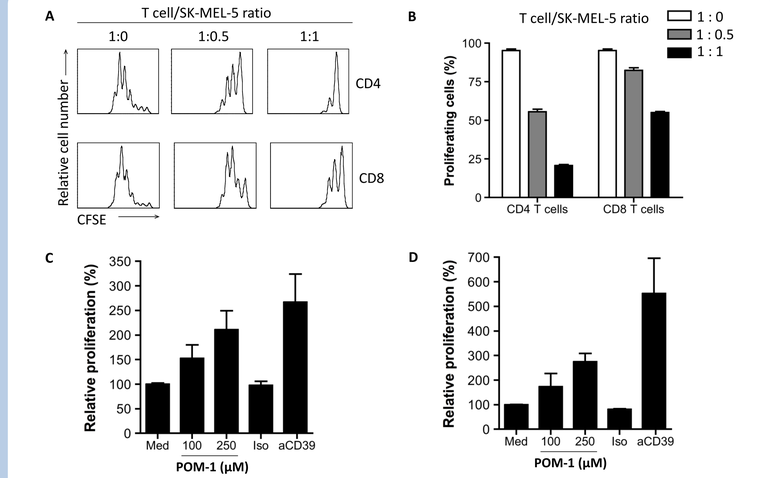

Figure 6: Suppression of T cell proliferation by CD39+ melanoma cells is reversed by CD39-blocking antibody OREG-103/BY40. CD39+ melanoma cells inhibit T cell proliferation: $4x10^4$ activated and CFSE labeled CD4 or CD8 T cells were cultured with irradiated SK-MEL-5 cells at indicated ratio. CFSE profiles (A) and percentage of proliferating cells (B) were analyzed after 4 days of culture. OREG-103/BY40 alleviates melanoma cell-mediated suppression: $5x10^4$ CFSE-labeled PBMC were cultured with $5x10^3$ irradiated SK-MEL-5. POM-1, anti-CD39 antibody OREG-103/BY40 (aCD39) or control isotype (iso) were used. After 5 days, proliferation of CFSE-labeled CD4 (C) and CD8 (D) T cells was analyzed by flow cytometry.

Conclusions and perspectives

- CD39 is overexpressed in some human cancers
- CD39 is expressed by some tumor cells
- CD39+ tumor cells suppress T cell proliferation
  - in a CD39-dependent manner
  - suppression is reversed by CD39-blocking antibody OREG-103/BY40

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