Deficiency of *Bach2* results in improved tumor immunity by enhancing effector function in CD8+ T ce

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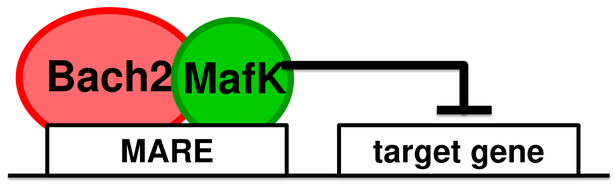
Abstract

Bach2 is a transcription repressor which belongs to the basic region-leucine zipper family and binds to Maf-recognition elements (MAREs). Bach2 plays essential roles in B cell development, immunoglobulin class-switch recombination and somatic hypermutation of immunoglobulin encoding genes. Bach2 is also required for development of effector T cells and regulatory T cells. These findings suggest that Bach2 plays important role in development, differentiation or function of various immune cells.

We speculated that the deficiency of Bach2 would result in an altered immune response in tumor rejection. A subcutaneous transplantation model revealed that the tumor transplanted into the Bach2 KO mice grew more slowly than the wild-type (WT) mice. These observations suggested that tumor immunity in the Bach2 KO mice was upregulated. We examined tumor-infiltrating cells with flowcytometry. The flowcytometric analysis revealed that significantly more CD8+ T cells infiltrated into the tumors in Bach2 KO mice than WT mice. Cell trace viollet (CTV) assay revelaed that the Bach2 KO CD8+ T cells exhibited stronger cytotoxicity against B16F10 compared to the WT CD8+ T cells. The expression level of Gzmb and Ifng was elevated in tumor-infiltrating Bach2 KO CD8+ T cells than WT cells. An electrophoretic mobility-shift assay revealed that Bach2 bound to the MARE-like sequence of FasL and Gzmb . The binding of Bach2 to MARE-like sequence of Gzmb required the hetero-dimer formation with MafK. The analysis of DNA micro array and ChIP-seq revealed that Bach2 may represses the klra family which is important for activation of NK cells. These results suggest that Bach2 represses the effector function of CD8+ T cells by repressing expression of effector genes directly and Klra family might be important for not only NK cells but also for CD8+ T cells.

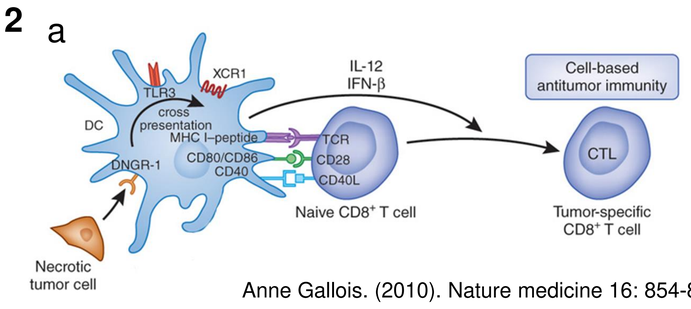
Background

Fig. 1



- Bach2 is a transcription repressor which belongs to the basic region-leucine zipper family and binds to Maf-recognition elements (MAREs). Oyake T. et al. (1996). Mol. Cell. Biol. 16:6083-6095
- Bach2 promotes lymphocytic differentiation by repressing granulocytic genes. Ari Itoh-Nakadai. et al. (2014). Nature Immunology 15,12:1171-1180
- Bach2 is essential for efficient formation of regulatory T cells (Tregs) by repressing differentiation of other lineages of effecter CD4 T cells. Roychoudhuri R et al. (2013). Nature 498:506-510
- Bach2 plays essential roles in B cell development, immunoglobulin class-switch recombination and somatic hypermutation. Muto A. et al. (2004). Nature 429:566-571; Muto A. et al. (2010). EMBO J 29:4048-4061

Fig. 2



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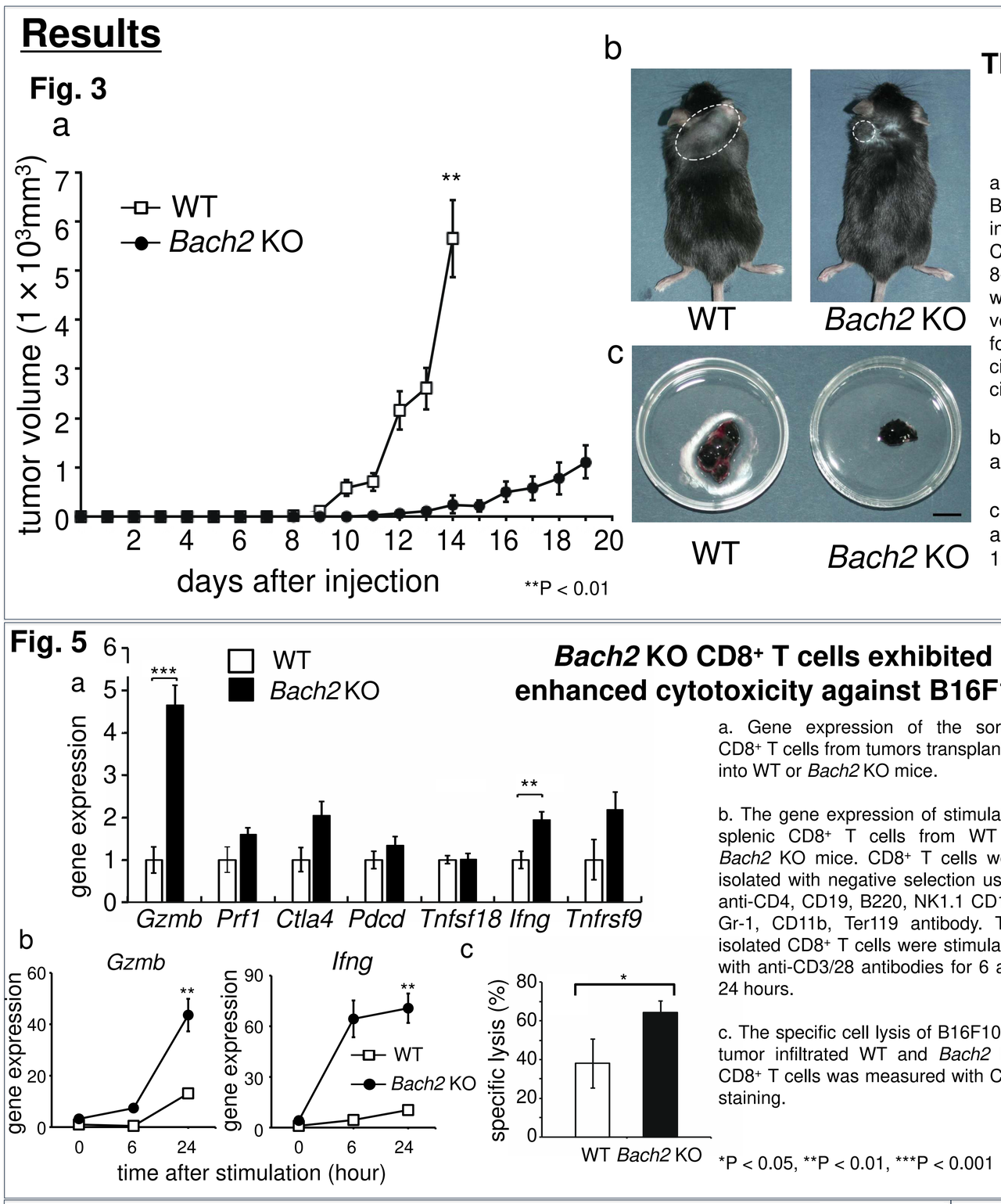
a. $CD8^+$ T cells are stimulated by the TCR signaling by DC.
b. Activated CD8+ T cells express granzyme-B, perforin1, FasL which induce apoptosis of target cells such as tumor cells.

Results

Fig. 3



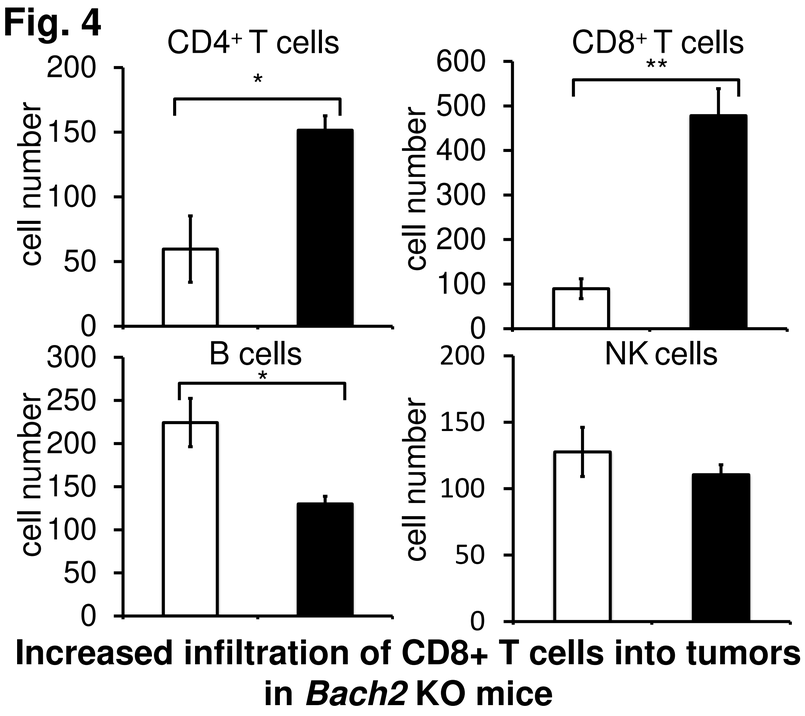
The tumor transplanted into *Bach2* KO mice grew slowly

a. The murine melanoma cell line B16F10 (0.5 × 10^6 cells/body) was injected subcutaneously into WT C57BL/6 mice and *Bach2* KO mice of 8-10 weeks old. The tumor growth was measured over time. The tumor volume was calculated using the following formula : (major circumference × minor circumference^2)/2.

b. The WT and *Bach2* KO mice after 12 days of tumor injection.

c. The tumors transplanted into WT and *Bach2* KO mice. Scale bar, 1cm.

Fig. 4



Increased infiltration of CD8+ T cells into tumors in *Bach2* KO mice

Flowcytometric analysis of homogenized tumor cells after 12 days of injection. *P < 0.05, **P < 0.01

Fig. 5

Bach2 KO CD8+ T cells exhibited enhanced cytotoxicity against B16F10

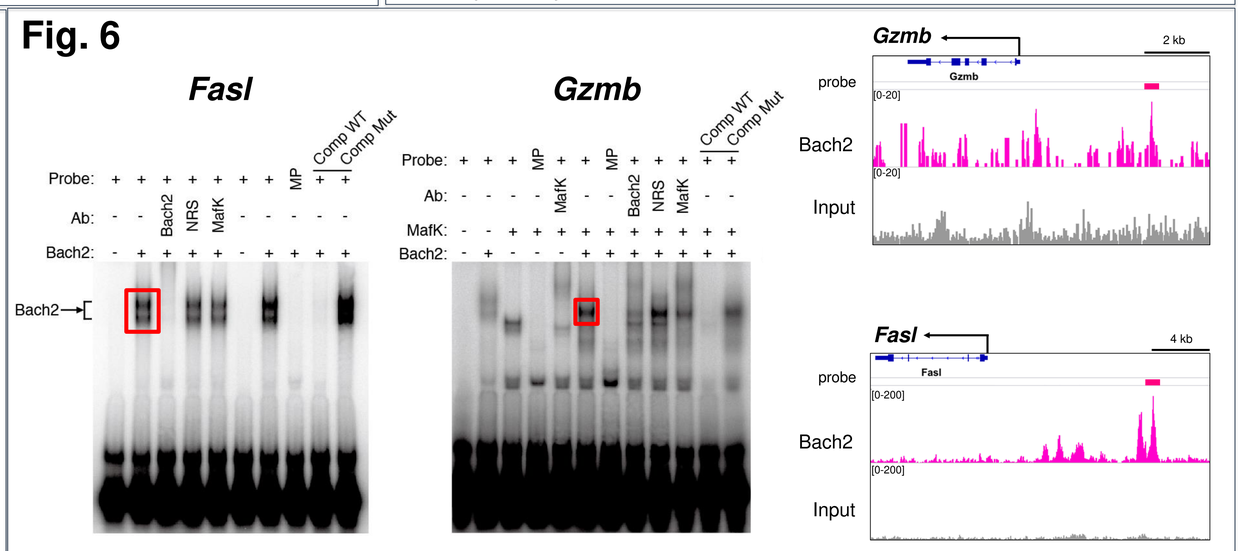
a. Gene expression of the sorted $CD8^+$ T cells from tumors transplanted into WT or *Bach2* KO mice.

b. The gene expression of stimulated splenic $CD8^+$ T cells from WT or *Bach2* KO mice. $CD8^+$ T cells were isolated with negative selection using anti-CD4, CD19, B220, NK1.1 CD11b Gr-1, CD11b, Ter119 antibody. The isolated $CD8^+$ T cells were stimulated with anti-CD3/28 antibodies for 6 and 24 hours.

c. The specific cell lysis of B16F10 by tumor infiltrated WT and *Bach2* KO $CD8^+$ T cells was measured with CTV staining.

*P < 0.05, **P < 0.01, ***P < 0.001

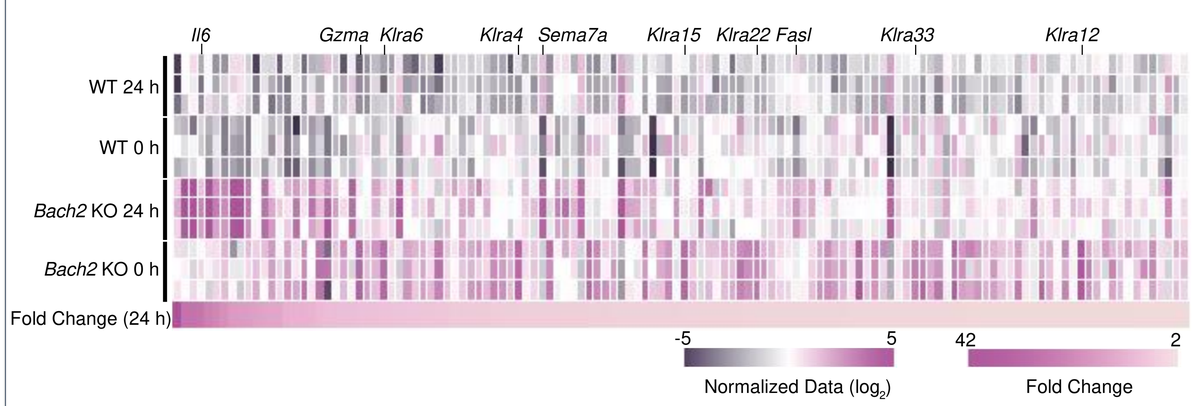
Fig. 6



Bach2 directly bound to genes associated with cytotoxicity in $CD8^+$ T cells

Electrophoretic mobility-shift assay in the presence (+) or absence (-) of recombinant Bach2 (above lanes), plus oligonucleotide probes containing the MARE-like sequences (+) or mutated sequences (MP) of *Fasl* and *Gzmb* or competitor oligonucleotides with wild-type (Comp WT) or mutated (Comp Mut) sequence, incubated without antibody (Ab -) or with antibody to Bach2 (Bach2), normal rabbit serum (NRS) or antiserum to MafK (MafK).

Fig. 7

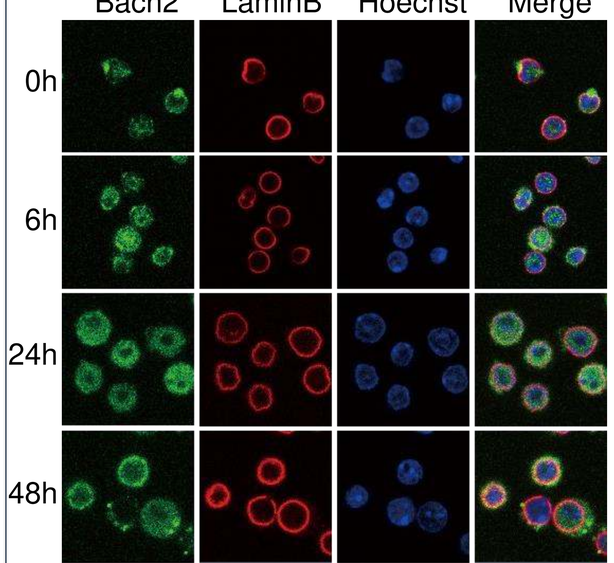


Bach2 possibly represses various effector gene in $CD8^+$ T cells

The list of genes of which is upregulated in Bach2 KO $CD8^+$ T cells compared to WT CD8+ T cells with DNA micro array and has some peaks in TSS with ChIP-seq.

Klra family is important for activation of NK cells and is expressed in also activated KO $CD8^+$ T cells. IL6 is one of the pro-inflammatory cytokine which activates B cell or Th17 differntiation and represses regulatory T cell function.

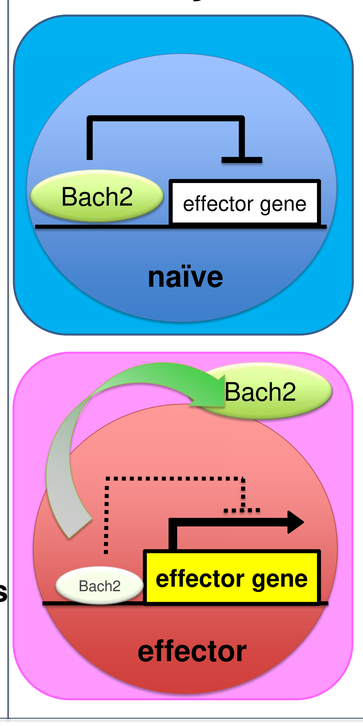
Fig. 8



Bach2 was re-localized from nuclear regions into cytoplasmic regions after stimulation

The immunofluorescent staining of stimulated splenic $CD8^+$ T cells by anti-CD3/28 antibody.

Summary



early phase

Bach2, localized in intra-nuclear region, represses the expression of effector genes such as *Gzmb*, *Ifng*, and *Fasl*.

late phase

Bach2 is localized in cytoplasmic regions. The expression of effector genes which are repressed by Bach2 is upregulated.

The change of localization of Bach2 may be important for the acquirement of effector function in $CD8^+$ T cells