The transcription factor BACH2 promotes tumor immunosuppression

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The immune system has a powerful ability to recognize and kill cancer cells, but its function is often suppressed within tumors, preventing clearance of disease. Functionally diverse innate and adaptive cellular lineages either drive or constrain immune reactions within tumors. The transcription factor (TF) BACH2 regulates the differentiation of multiple innate and adaptive cellular lineages, but its role in controlling tumor immunity has not been elucidated. Here, we demonstrate that BACH2 is required to establish immunosuppression within tumors. Tumor growth was markedly impaired in Bach2-deficient mice and coincided with intratumoral activation of both innate and adaptive immunity. However, augmented tumor clearance in the absence of Bach2 was dependent upon the adaptive immune system. Analysis of tumor-infiltrating lymphocytes from Bach2-deficient mice revealed high frequencies of rapidly proliferating effector CD4+ and CD8+ T cells that expressed the inflammatory cytokine IFN-γ. Effector T cell activation coincided with a reduction in the frequency of intratumoral Foxp3+ Tregs. Mechanistically, BACH2 promoted tumor immunosuppression through Treg-mediated inhibition of intratumoral CD8+ T cells and IFN-γ. These findings demonstrate that BACH2 is a key component of the molecular program of tumor immunosuppression and identify therapeutic targets for the reversal of immunosuppression in cancer.

Introduction

While the immune system has a powerful ability to recognize and kill cancer cells, its function is often suppressed, preventing clearance of disease. A variety of innate and adaptive immune lineages causes immunosuppression within tumors, including immature DCs, plasmacytoid DCs, myeloid-derived suppressor cells, CD4+ Foxp3+ Tregs, IL-10-secreting type-I Tregs, and CD1d-restricted natural killer T cells (1–5). Thus, a relatively well-characterized network of innate and adaptive immunosuppressive cell types drives immunosuppression, but molecular mechanisms required for the development of immunosuppressive responses within tumors are poorly elucidated. TFs play key roles in cellular differentiation and bind to regulatory DNA to control gene expression. BACH2 is a 92-kDa transcription factor (TF) of the basic leucine zipper family and functions within multiple innate and adaptive lineages to control immune function. In B cells, BACH2 is critical for somatic hypermutation and class-switch recombination, and its absence leads to impaired formation of class-switched antibody responses (6, 7). In CD4+ T cells, BACH2 promotes the development of Foxp3+ Tregs by suppressing effector cell transcriptional programs (8, 9). Bach2-2 is also required for normal development and function of alveolar macrophages (10). Consistent with its role in regulating the development and function of diverse immune cell types, the BACH2 gene in humans is a prominent susceptibility locus for multiple autoimmune and allergic diseases (11–14). Thus, BACH2 functions in a variety of cellular lineages that can either promote or suppress immune responses against tumors. However, its function in controlling tumor immunity has not been elucidated.

In this study, we have found that BACH2 is required to establish immunosuppression within tumors. We found that growth of B16 melanoma and EL-4 lymphoma tumors was markedly impaired in Bach2-deficient mice and coincided with intratumoral activation of both innate and adaptive immunity. However, augmented tumor clearance in the absence of Bach2 was dependent upon adaptive immunity. Analysis of tumor-infiltrating lymphocytes in Bach2-deficient mice revealed high frequencies of rapidly proliferating CD4+ and CD8+ effector cells expressing the inflammatory cytokine IFN-γ. Lymphocyte activation coincided with reduction in the frequency of intratumoral CD4+ Foxp3+ Tregs. Treg-dependent inhibition of intratumoral CD8+ T cells and IFN-γ was required for Bach2-mediated tumor immunosuppression. These findings identify BACH2 as a key component of the molecular program of tumor immunosuppression and identify a target for therapies aimed at reversing immunosuppression in cancer.

Results and Discussion

BACH2 promotes tumor immunosuppression. To determine the function of BACH2 in regulating tumor immunity, we implanted syngeneic B16 melanoma cells subcutaneously into littermate
cient animals were uniformly distinct from those of WT animals (Figure 1C). Analysis of transcriptional profiles for global differences in gene expression identified 3,623 differentially expressed transcripts (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI82884DS1), which enabled tumors to be distinguished based upon hierarchical cluster analysis (Figure 1D). Gene set enrichment analysis (15) indicated induction of transcriptional signatures of both innate and adaptive immune systems (Figure 1E and F). These findings suggest that BACH2 plays a critical role in modulating immune responses within tumors.
adaptive immune activation in tumors from Bach2-deficient mice (Figure 1, E and F, and Supplemental Tables 2–4). Collectively, these data indicate that BACH2 promotes tumor growth in a tumor cell–extrinsic fashion and suppresses induction of transcriptional profiles indicative of innate and adaptive immune.

**BACH2 suppresses adaptive immunity to promote immunosuppression within tumors.** CD4+ and CD8+ T cells form a key component of adaptive immune responses against tumors. Whole-tumor transcriptional profiles indicated striking induction of genes associated with CD4+ and CD8+ effector T cells in tumors from Bach2-deficient mice. In particular, we noted elevated expression of genes that are expressed by T cells (including Cd3g, Il2rg, Thy1, and Cd8a) (16) or associated with effector differentiation (including Tbx21 and Prdm1) (17, 18) and function (including Ifigg, Gzm, and Fasl) (ref. 16 and Figure 2A). We therefore measured infiltration of CD4+ and CD8+ T cells within tumors using flow cytometry. Normalized for differences in tumor mass (Figure 2B), the density of both CD4+ and CD8+ T cells was higher in tumors from Bach2-deficient animals (Figure 2C). Increased density of CD4+ and CD8+ T cells was also apparent from immunohistochemical analysis of tumor sections (Figure 2D and Supplemental Figure 1A). Consistent with their increased density, we observed elevated frequencies of proliferating Ki67+ cells among both CD8+ and CD4+ Foxp3– effector T cells within tumors from Bach2-deficient mice (Figure 2E). Moreover, intracellular cytokine staining following brief restimulation of tumor-infiltrating cells ex vivo showed strikingly elevated frequencies of CD4+ and CD8+ T cells expressing the effector cytokine IFN-γ within tumors from Bach2-deficient mice (Figure 2E). Moreover, intracellular cytokine staining following brief restimulation of tumor-infiltrating cells ex vivo showed strikingly elevated frequencies of CD4+ and CD8+ T cells expressing the effector cytokine IFN-γ within tumors from Bach2-deficient mice (Figure 2E). Thus, BACH2 limits the proliferation of CD8+ and CD4+ effector T cells and their expression of effector cytokines within tumors.

The protein encoded by recombination-activating gene 1 (Rag1) is required for rearrangement of T and B cell receptor loci, and its loss results in a complete defect in generation of mature T and B lymphocytes (19). To test whether decreased tumor growth in the absence of BACH2 was dependent upon lymphocytes, we measured growth kinetics of subcutaneously implanted B16 melanoma cells in Bach2 Rag1 double-deficient animals (Figure 2G).
Consistent with our previous observations, tumor growth was markedly impaired in Bach2-deficient animals. However, this was dependent upon the activity of lymphocytes, since augmented tumor clearance was not seen in Bach2 Rag1 double-deficient animals. Thus, decreased tumor growth in the absence of Bach2 requires the adaptive immune system.

**BACH2 causes tumor growth through Treg-mediated suppression of CD8+ T cells**. While CD8+ T cells primarily function to promote tumor clearance, CD4+ T cells can either drive or constrain immune responses against tumors through the reciprocal function of effector and regulatory cell lineages, respectively (2, 3, 20). Within tumors, Tregs can powerfully inhibit the accumulation and function of CD4+ and CD8+ effector T cells (3). We have previously found that Bach2 promotes Foxp3+ Treg development through repression of helper cell transcriptional programs (8, 21). We wished to determine whether Bach2-mediated tumor immunosuppression was dependent upon its role in Treg development. In mice, Tregs can be identified by their expression of the TF Foxp3 (22–24). We observed substantially diminished Foxp3+ Treg populations in tumors from Bach2-deficient animals when expressed as either their frequency relative to total CD4+ T cells or CD8+ T cells within tumors or as their absolute number (Figure 3, A and B). The frequency of Foxp3+ Tregs was similarly reduced in both tumors and spleens of Bach2-deficient mice (Supplemental Figure 2), suggesting that defective Treg-mediated tumor immunosuppression in Bach2-deficient mice may correspond to the generalized Treg deficiency in these animals.

We wished to determine the relative contribution of Foxp3+ Tregs to Bach2-mediated tumor immunosuppression. Since Tregs suppress immune function in an immunodominant fashion, phenotypes resulting from their deficiency are amenable to rescue by provision of WT Tregs. Tregs from mice expressing...
the human diphtheria toxin (DTx) receptor (DTR) under the control of the endogenous Foxp3 locus (Foxp3<sup>DTxt</sup> mice) are sensitive to depletion following administration of DTxs (25). To test whether Bach2-mediated immunosuppression is dependent upon the function of Bach2 in Treg development, we reconstituted irradiated Rag1<sup>−/−</sup> mice with 1:1 mixtures of bone marrow (BM) cells from Foxp3<sup>DTx</sup> mice and either WT or Bach2-deficient mice, resulting in WT:Foxp3<sup>DTx</sup> and KO:Foxp3<sup>DTx</sup> mixed chimeric animals. This system allows experimental interrogation of Treg-dependent phenotypes attributable to Bach2 deficiency, since Bach2-deficient Tregs are provided by the Foxp3<sup>DTx</sup> compartment, except in cases in which DTxs is administered. Using this system, we observed equivalent chimerism of WT and Bach2-deficient CD4<sup>+</sup> and CD8<sup>+</sup> T cells within reconstituted WT:Foxp3<sup>DTx</sup> and KO:Foxp3<sup>DTx</sup> mixed chimeric animals, respectively (Supplemental Figure 3, A and B), and near-complete DTx-induced depletion of Foxp3<sup>+</sup> Tregs within Foxp3<sup>DTx</sup> compartments (Supplemental Figure 3C). Strikingly, Bach2-mediated tumor immunosuppression was dependent upon Foxp3<sup>+</sup> Tregs, since impaired tumor growth and loss of immunosuppression was only observed when DTx was administered to KO:Foxp3<sup>DTx</sup> mice (Figure 3C). Growth of subcutaneous tumors in reconstituted Rag1<sup>−/−</sup> radiation chimeras occurred with distinct kinetics compared with that in WT nonirradiated animals, as is consistent with our previous experience (our unpublished observations), and the duration of measurement in these experiments was limited by systemic effects of Treg depletion in mice reconstituted with mixtures of KO and Foxp3<sup>DTx</sup> BM and administered DTx. Collectively, these findings suggest that Bach2-mediated tumor immunosuppression is dependent upon Foxp3<sup>+</sup> Tregs.

We observed increased frequencies of CD8<sup>+</sup> T cells and elevated production of IFN-γ within tumors of Bach2-deficient animals. This led us to ask whether Treg-dependent tumor immunosuppression mediated by Bach2 is caused by suppression of CD8<sup>+</sup> T cells or IFN-γ. Strikingly, augmented tumor immunity observed upon administration of DTx to KO:Foxp3<sup>DTx</sup> mice was partially reversed upon depletion of CD8<sup>+</sup> T cells and fully reversed upon simultaneous depletion of CD8<sup>+</sup> T cells and blockade of IFN-γ (Figure 3D). Thus, we conclude that Bach2 causes tumor growth through Treg-mediated suppression of CD8<sup>+</sup> T cells and concomitant IFN-γ-dependent effector mechanisms.

In this study, we have found that the TF Bach2 is required for establishment of immunosuppression within tumors. Despite its activity in a diversity of innate and adaptive immune cell types, it predominantly exerts this function through its role in CD4<sup>+</sup> Tregs, thus potently suppressing the antitumor activity of CD8<sup>+</sup> T cells. These findings identify a molecular axis of tumor immunosuppression and provide targets for design of immune-based therapies aimed at reversing deleterious immunosuppression in cancer.

**Methods**

Additional details can be found in the Supplemental Methods. Animals. C57BL/6 and Rag1<sup>−/−</sup> (B6.129S7–Rag<sup>flac×10</sup>) mice were purchased from The Jackson Laboratory. Bach2-deficient mice, which have been previously described (6), were backcrossed >16 times with C57BL/6 mice.

Data deposition. Data from whole-transcriptome analyses are deposited under GEO accession number GSE74653.

Statistics. Two-tailed Student’s t tests were used to calculate statistical significance of the difference in sample means. P values of less than 0.05 were considered significant and are provided.

Study approval. Experiments were approved by the Institutional Animal Care and Use Committee of the National Cancer Institute and performed in accordance with NIH guidelines.

**Author contributions**

RR, RLE, DC, CAK, LG, and NPR conceived the study and wrote the manuscript. RR, RLE, DC, GM, ZY, HL, JHP, and PJ performed experiments. PJ and RR performed bioinformatic analyses. MS, F MG, ZY, YJ, DCP, AC, JGC, SJP, DS, EW, FMM, KO, and NPR edited the manuscript.

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