

## T-cell-mediated tumor immune surveillance and expression of B7 co-inhibitory molecules in cancers of the upper gastrointestinal tract

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**Abstract** Tumorigenesis can induce adaptive T-cell-mediated immune responses against malignant cells. Such cellular immune responses are actively suppressed by cancer cells via mechanisms of immune tolerance. We studied T-cell responses against tumor growth by examining tumor-infiltrating lymphocytes (TILs) in upper gastrointestinal (GI) cancers. The number of T-bet<sup>+</sup> TILs correlates with better survival of esophageal cancer patients. Using well-defined mouse models, we have further shown that T-bet and Eomes are both required for the adaptive anti-tumor immunity by regulating T-cell trafficking into the tumor tissue and their effector functions inside the tumor microenvironment. In order to gain further insight into the tumor immune microenvironment in the upper GI cancer, we have also studied expression levels of co-inhibitory molecules such as B7-H1/PD-L1 and B7-H4 in tissue specimens of esophageal and gastric cancers. These inhibitory B7 molecules were expressed at high but variable levels by cancer cells. The overexpression of these molecules correlates with poor clinicopathological parameters and shorter patient survival time. The number of CD3<sup>+</sup> and CD8<sup>+</sup> TILs correlates inversely with expression levels of B7-H4 in samples from esophageal cancer, supporting a role of active immune suppression by inhibitory B7 molecules in the tumor microenvironment. In addition, TILs show functional exhaustion and express high levels of PD-1 and Tim-3. We propose that metabolic competition mediated by phosphatidylinositol 3-kinases (PI3Ks) characterizes the immune suppression within cancer tissues. Future tumor vaccine design should combine blockade of B7 inhibitory molecules and enhancement of T-bet and Eomes levels within the tumor microenvironment.

**Keywords** Tumor immunosurveillance · Tumor-infiltrating lymphocyte · CD8<sup>+</sup> T cell · Co-inhibitory molecule · T-bet · B7 family

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### Introduction

Esophageal and gastric cancers remain a significant problem worldwide. Esophageal cancer is the fifth and eighth leading cause of cancer-related death for men and women, respectively [1]. There are two major types of esophageal cancer, namely squamous cell carcinoma (SCC) and adenocarcinoma. Squamous cell carcinoma is responsible for 95% of all esophageal cancers worldwide and incidence of adenocarcinoma is on the rise in Western countries [2, 3]. Gastric cancer is the third and fifth leading cause of cancer-specific mortality for men and women, respectively worldwide [1]. Despite availability of multiple treatment modalities, esophageal and gastric cancers together still cause over 1,000,000 deaths each year [1]. Cancer immune

therapy holds promises as an effective adjuvant therapy for these cancers. And understanding the molecular and cellular basis of tumor immune microenvironments is the key to advancement of tumor immunotherapy.

Cell-mediated immune responses against tumor are either present spontaneously in human cancer patients as a critical component of tumor immune surveillance or can be elicited by cancer vaccination and adoptive T-cell transfer [4]. Type 1 adaptive immune responses, mediated by Th1 cells and CTLs, are thought to be a critical component of cell-mediated immunity against cancer [5]. In humans, the presence of Th1 cells and CTLs within the tumor can be a favorable prognostic indicator [5]. It is well known, however, that many tumor-infiltrating T cells are in a state of non-responsiveness due to local mechanisms of immune suppression in the tumor microenvironment [6, 7]. Members of the B7 gene family provide crucial inhibitory signals that regulate T-cell responses within tumor sites [8]. B7-1 and 2 stimulate early T-cell responses via CD28. But they can also inhibit T-cell activation through CTLA-4 in lymphoid tissues [9]. The extended B7 family also include ICOS ligand (ICOSL, also known as B7h, ICOS-L, B7RP-1, and B7-H2), B7-H1 (also known as PD-L1, B7-H, CD274), B7-H2 (also known as PD-L2 and B7-DC), B7-H3 (also known as CD276 and B7RP-2), and B7-H4 (also known as VTCN1, B7X, and B7S1). They play variable roles in maintaining the intricate cellular network of the tumor immune suppressive microenvironment [8–13].

We have examined markers of active anti-tumor T-cell responses as well as expression of immune suppressive molecules such as PD-L1/B7-H1 and B7-H4 in human esophageal cancer and gastric cancer tissues. Our studies reveal that frequencies of T-bet<sup>+</sup> and CD8<sup>+</sup> lymphocytes within tumor tissues are positively associated with cancer patient survival. Conversely, the expression levels of PD-L1 and B7-H4 are inversely associated with patient survival [14, 15]. Using genetically defined mouse models, we demonstrated that T-bet and Eomes are essential for trafficking of tumor-reactive CD8<sup>+</sup> T cell into tumor tissues [16, 17]. Understanding the molecular network underlying the tumor microenvironment should help improving immunotherapy.

### Reactive T-cell changes in esophageal cancer and clinical outcome

Lymphoid infiltration in tumor tissues is believed to be the host immune response against cancer cells [18]. However, there are a plenty of evidence supporting that tumor reactive inflammation can promote cancer progression [19]. We decided to examine this question by quantifying the frequency of different subsets of tumor-infiltrating lymphocytes

(TILs) in esophageal cancer tissues. Since IFN $\gamma$ -producing cells such as Th1, CD8<sup>+</sup> T cells, and NK cells are the major cell types that have anti-tumor properties, we then examined, in cancer tissues, the number of lymphocytes expressing T-bet, a master transcription factor for Th1 responses, and the number of lymphocytes expressing Foxp3, a critical transcription factor of regulatory T cells (Treg), which suppress Th1 responses. We performed immunohistochemical analysis on tumor tissues from 112 esophageal squamous cell carcinoma (ESCC) patients. We found CD3<sup>+</sup> and CD8<sup>+</sup> T cells were present in both tumor nest and tumor stroma [15]. More importantly, the number of CD3<sup>+</sup> or 8<sup>+</sup> TILs correlates positively with patient survival. The patients with higher numbers of T-bet<sup>+</sup> lymphocytes in both tumor nest and tumor stroma survived much longer than patients with lower numbers of such cells. Foxp3<sup>+</sup> regulatory T cells can also be detected in these cancer tissues. Although the difference did not reach statistical significance, patients with higher numbers of Foxp3<sup>+</sup> cells showed a trend of shorter survival time than those with lower numbers of Foxp3<sup>+</sup> cells [15]. We have also recently performed similar histopathological studies on cancer specimens from gastric cancer patients. We have found that higher T-bet<sup>+</sup> cell frequencies are also associated with better clinical outcome in these patients (J. Jiang and B. Lu manuscript under review). Our results are consistent with the idea that cancer immune surveillance, mediated by the Th1 type immune responses, is induced in these cancers of the upper gastrointestinal (GI) tract. Cancer progression is associated with active suppression of anti-tumor cellular immune responses.

### T-bet and Eomes drive T-cell-mediated adaptive anti-tumor immune responses

Despite the ongoing debate about the existence of effective tumor immune surveillance in human, vaccination with a tumor antigen can evidently elicit effective anti-tumor immunity in animal models and human. Since higher levels of T-bet expression in human tumor tissues are associated with better patient survival, we decided to determine the role of T-bet in tumor vaccine-triggered cellular immune responses against tumor cells. T-bet is a hallmark transcription factor that is essential for differentiation and function of Th1 cells, CD8<sup>+</sup> T cells, and NK cells [20]. The role of T-bet in anti-cancer immune responses has been attributed to its function in innate cells such as NK cells and DCs [21–24]. Many prior studies have elucidated the function of T-bet and its closely related family member Eomes in infectious diseases and autoimmune diseases [25]. However, their roles in adaptive tumor-specific immunity were not clear. The tumor microenvironment is evidently different from tissues involved in infectious

diseases and autoimmune diseases [6]. In addition, the tumor microenvironment is quite dynamic and evolves as cancer progresses and also adapts to anti-tumor immune responses. Thus, it is quite important to study the roles of these important Th1 transcription factors in the setting of the tumor microenvironment.

We first used prophylactic vaccination approach and examined the individual and combined roles of T-bet and Eomes in eliciting anti-tumor immune responses. No tumor nodules were found in WT mice immunized with a tumor vaccine. Interestingly, no tumor nodules formed in vaccinated T-bet-deficient mice, suggesting T-bet was not essential for anti-tumor immunity in this experimental model. In contrast, ~30% of Eomes KO and ~60% T-bet/Eomes DKO mice showed tumor growth despite the vaccination [17]. These data showed that T-bet and Eomes play functionally redundant roles in mediating vaccine-induced anti-tumor immune responses. Our recent unpublished data showed that T-bet and Eomes are also required for the memory recall response against tumors. Thus, both T-bet and Eomes are required for the adaptive anti-tumor immunity at the effector and memory phases. Consistent with our data, another group has recently shown that over-expression of T-bet in T cells promotes anti-tumor immune responses [26].

The greatly reduced anti-tumor function in T-bet/Eomes DKO mice has allowed us to decipher the underlying molecular mechanisms. We first examined whether tumor vaccine failed to elicit an effective anti-tumor immune response in T-bet/Eomes DKO mice in the peripheral lymphoid system. To our surprise, both the number of tumor antigen-specific T cells in the spleen and *in vivo* CTL activities were similar between WT and T-bet/Eomes DKO mice that had been given tumor vaccines [17]. These data suggest that the tumor vaccination elicited anti-tumor immune responses in the peripheral lymphoid system independent of T-bet and Eomes [17]. In contrast, the number of tumor-infiltrating CD8<sup>+</sup> T cells drastically decreased in tumor bearing T-bet/Eomes DKO mice compared with WT mice. In addition, we found that CXCR3 decreased greatly in T-bet/Eomes DKO CD8<sup>+</sup> T cells when compared with WT CD8<sup>+</sup> T cells. We further demonstrated that CXCR3-deficient mice mounted reduced levels of anti-tumor immune responses and had reduced levels of CD8<sup>+</sup> TILs. These data suggest T-bet and Eomes likely control trafficking of CD8<sup>+</sup> T cell to the tumor site via CXCR3. The CXCR3 ligands CXCL-9, CXCL-10, and CXCL-11 are known to be induced by IFN $\gamma$  [27]. However, the tumor cells do not express IFN $\gamma$  in general. It is possible that CD8<sup>+</sup> T cells or NK cells engage tumor cells and then release IFN $\gamma$ , which subsequently induces expression of CXCL-9, CXCL-10, and CXCL-11. If this is indeed the case, CXCR3 is likely involved in amplifying Th1

responses in the tumor site rather than serving as the initial chemotactic signals for CD8<sup>+</sup> T cells. It remains to be elucidated which signal triggers the initial CD8<sup>+</sup> T-cell trafficking to tumor site and whether such initial signal is regulated by T-bet or Eomes. Careful examination of early trafficking of WT and T-bet/Eomes-deficient T cells into the tumor site should provide answers to these questions.

Although, combined T-bet and Eomes deficiency did not significantly reduce IFN $\gamma$  production in CD8<sup>+</sup> T cells cultured in Th1 polarizing conditions, it resulted in a large reduction in IFN $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells and an increase in IL17<sup>+</sup> CD8<sup>+</sup> T cells in the tumor site [17]. Thus, expression of T-bet and Eomes is critical for the effector function of CD8<sup>+</sup> T cells in the tumor microenvironment. More importantly, expression of T-bet and Eomes is important in suppressing dominance of other T subsets such as Th17, Tc17, and regulatory T cells in the tumor [17]. There is evidence suggests that Th17 cells are an indicator of favorable anti-tumor immune responses [28]. In addition, adoptive transfer of Th17 cells improves tumor vaccination [29]. Nevertheless, dependence of IFN $\gamma$  and functional CD8<sup>+</sup> T cells for the anti-tumor effect seems inevitable [28]. Thus, a reduction of T-bet and Eomes in TILs is an ominous sign of a failed anti-tumor immune response.

### Expression of PD-L1/B7-H1 and B7-H4 and cancer progression

B7-H1 (also known as PD-L1) and its receptor PD-1 negatively regulate the function of T cells [9]. PD-1 interacts with at least two ligands: PD-L1 and 2 [30–32]. The expression patterns of PD-L1 and 2 are distinct. PD-L1 is expressed on leukocytes, non-hematopoietic cells, and in non-lymphoid tissues, while PD-L2 is expressed exclusively on DCs and monocytes [9]. Due to its expression pattern, PD-L1 is thought to limit T-cell function within tissue sites. PD-L1 inhibits T-cell function including interferon- $\gamma$  (IFN- $\gamma$ ) production, proliferation, and increased T-cell apoptosis [33–36]. Interactions between PD-L1 and PD-1 are thought to maintain peripheral tolerance. PD-L1 expression in immune privileged sites including the eye and the placental vascular endothelial cells is consistent with this role [37, 38].

PD-L1/PD-1 has been shown to mediate T-cell exhaustion, an important mechanism underlying T-cell dysfunction during chronic inflammation and chronic infection [39, 40]. CD8<sup>+</sup> T-cell exhaustion was first described with chronic LCMV infection of mice. During the chronic phase of infection, virus-specific CD8<sup>+</sup> T cells persist but lose their effector function [41]. In human, exhaustion of CD4<sup>+</sup> and 8<sup>+</sup> T cells during HIV and other chronic viral infections is associated with expression of the

inhibitory molecule PD-1 and blockade of PD-1 augmented HIV-specific CD4<sup>+</sup> and 8<sup>+</sup> T-cell function [42–44], suggesting the PD-L1/PD-1 axis plays an important role in mediating T-cell exhaustion during chronic infection.

Most human cancer tissues express variable levels of PD-L1 protein (reviewed in [8]). PD-L1 mRNA is expressed at very low levels in many mouse and human tumor cell lines but both its mRNA and protein can be upregulated in response to IFN $\gamma$  [36]. This suggests that tumor expression of PD-L1 is influenced by inflammatory signals. Besides cytokines, a study shows that PD-L1 protein but not its mRNA increases in human glioma upon loss of phosphatase and tensin homolog (PTEN) and activation of the phosphatidylinositol-3-OH kinase (PI3K) pathway (Fig. 1). Tumor specimens from patients with glioblastoma multiforme (GBM) had high levels of PD-L1 protein that correlated with PTEN loss [45]. Signaling through the PI3K pathway can be initiated in tumor cells by growth factors that bind receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) [46]. PTEN negatively regulates this pathway [46]. Activation of the PI3K cascade is thought to promote cell survival and proliferation by altering cellular metabolism (Fig. 1). This pathway has been shown to be commonly activated in cancer, including esophageal cancer and gastric cancer [47]. Thus, the expression of PD-L1 mRNA and protein in tumor cells can be induced by cancer reactive inflammatory cells. Cancer progression can further increase PD-L1 protein through activation of PI3K (Fig. 1).

We have also investigated the expression of PD-L1 in specimens from individuals with upper GI cancers. We performed immunohistochemical studies on tissue samples collected from individuals who suffered from gastric cancer and had undergone surgical treatment. There was no PD-L1 immunostaining in normal gastric tissues and only very weak labeling in the gastric adenoma tissues; however, about 42.2% of gastric carcinoma tissues showed positive PD-L1 immunolabeling [48, 49]. In addition, PD-L1 was significantly correlated with lymph node metastasis, tumor size, and depth of tumor invasion. These findings suggest that PD-L1 expression levels are not high in all patients and can be induced as cancer progresses. Consistent with this idea, decreased probability of survival was observed in patients with PD-L1-positive tumors compared to patients with PD-L1-negative tumors [48, 49]. The multivariate analysis using the Cox regression model showed that PD-L1 immunolabeling could be considered as a significant prognostic factor [48]. Other investigators have investigated the expression of PD-L1 in esophageal cancer [50]. About (43.9%) of the 41 tumors evaluated in the study were positive for PD-L1 using real-time quantitative PCR

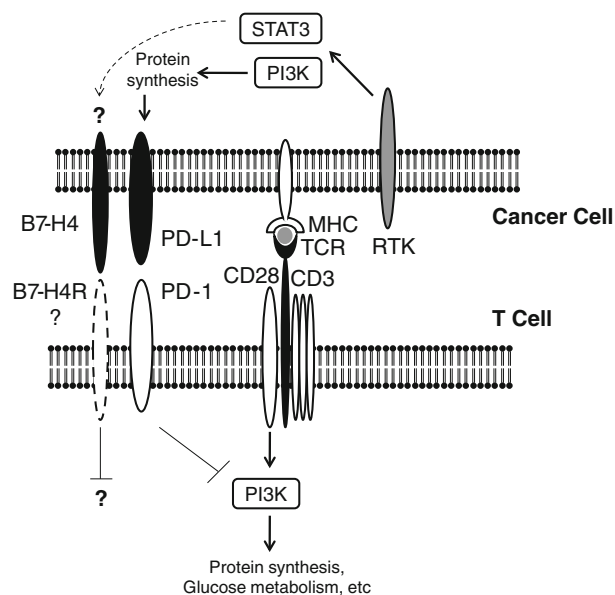


**Fig. 1** PD-L1 mediates suppression of T-cell metabolism by tumor cells. Activation of RTK in tumor cells leads to upregulation of PD-L1. PD-L1 can inhibit activation of PI3K in tumor-infiltrating T cells, and thereby inhibiting T-cell metabolism. How B7-H4 is induced in tumor cells is unknown. One candidate upstream molecule is STAT3. RTK receptor tyrosine kinase. TCR T cell receptor. PI3K phosphoinositide 3-kinase

analysis. The protein levels of PD-L1 were positively correlated with PD-L1 mRNA expression. PD-L1-positive patients had a significantly poorer prognosis than the negative patients. Together these findings establish PD-L1 as a key pathological factor in the tumor microenvironment of upper GI cancers.

B7-H4 was discovered based on its DNA sequence homology to other B7 genes [51–53]. The putative B7-H4 receptor was shown up-regulated in activated T cells [51, 52]. B7-H4 inhibits T-cell proliferation as well as cytokine production and is thought to be involved in peripheral tolerance [51–53]. B7-H4 KO mice develop stronger Th1 responses against *Leishmania major* infection with an increase in IFN- $\gamma$  production by CD4<sup>+</sup> T cells [54]. B7-H4KO mice are more resistant to infection by *L. monocytogenes* than WT controls due to an increase in neutrophils, suggesting B7-H4 also regulates innate immune responses [55].

B7-H4 is detectable in the human cancer tissues including upper GI cancers at variable levels [8, 14, 15]. B7-H4 mRNA and protein are highly expressed in human serous ovarian cancers and breast cancers with little or no expression in normal tissues [56]. Our quantitative RT-PCR analysis also revealed very little B7-H4 mRNA in normal gastric tissues but high levels of B7-H4 mRNA in gastric cancer tissues (J. Jiang and B. Lu manuscript

in preparation). These findings suggest that B7-H4 is regulated at the transcriptional level. Tumor-associated macrophages from ovarian cancer stroma express surface B7-H4 and suppress tumor-associated antigen-specific T-cell immunity in a B7-H4-dependent manner [57]. IL-6 and 10 stimulate macrophage B7-H4 expression. IL-6 and 10, however, cannot up-regulate B7-H4 in tumor cells [57]. STAT-3 is known to be activated by IL-6 and 10 in many cell types [58]. It can also be stimulated by receptor tyrosine kinases in tumor tissues [58]. It will be interesting to explore whether STAT-3 is required for B7-H4 expression in tumor tissues (Fig. 1).

B7-H4 expression on cancer cells was studied by us in both gastric cancer and esophageal cancer tissues. The positive staining of B7-H4 was found in 70 out of 156 gastric cancer specimens (the positive rate was 44.9%), whereas there was no or very weak B7-H4 staining in the gastric polyp tissues or adjacent normal tissues [14]. Interestingly, although B7-H4 was highly expressed in the infiltrating mononuclear cells within gastritis tissues, it was not expressed in the epithelial cells in these tissues. These data suggest that general inflammation does not induce B7-H4 expression on epithelial cells. B7-H4 overexpression was significantly associated with poor pathological parameters such as myometrial invasion and lymph node metastasis. Higher expression levels of B7-H4 were also associated with worse patient survival [14]. For esophageal cancer, positive B7-H4 immunostaining was observed in 107 of 112 (95.5%) of ESCC tissue sections. We found that high expression levels of B7-H4 on tumor cells were significantly associated with distal metastasis and advanced TNM stages. The overall survival rate of the patients with higher B7-H4 expression was significantly worse than that of the patients with lower B7-H4 expression [15]. Together these findings establish B7-H4 as a clinically significant factor in upper GI cancers and an important molecule in shaping the tumor immune suppressive microenvironment.

#### **T cells in the tumor microenvironment with high levels of PD-L1 and B7-H4**

Levels of PD-L1 and B7-H4 increase in cancer cells as cancer progresses. We have shown that the numbers of TILs are affected by PD-L1 and B7-H4 in human cancers [14, 15]. In esophageal cancer specimens, levels of B7-H4 expression on tumor cells are inversely correlated with the density of CD3<sup>+</sup> and 8<sup>+</sup> TILs [15]. Besides these quantitative differences, we have found large numbers of TILs-bearing surface markers for exhausted T cells in tumor-bearing animal models, consistent with recent findings by other groups [59, 60]. In addition, we found PD-1 and Tim-3 expression on large proportions of both CD4<sup>+</sup>

and CD8<sup>+</sup> TILs in cancer tissues from individuals suffering from gastric cancer, colorectal cancer, or lung cancer (X. Gao and B. Lu manuscript in preparation). These data support the idea that PD-L1/PD-1 pathway plays an important role in many types of cancer in induction of T-cell exhaustion. By the same token, B7-H4 might also be involved in T-cell non-responsiveness in the tumor site. It will be interesting to examine the levels of B7-H4 receptor-positive TILs in the future. The cloning and characterization of B7-H4 receptor will greatly facilitate such studies.

#### **Metabolic competition between cancer cells and anti-tumor T-cell responses**

Tumor cells alter their cellular metabolism, such as glycolysis and protein synthesis, to meet the needs of dividing cells [61]. The PI3K pathway is commonly activated in human cancers. The activated PI3K pathway has profound effects on cancer cell metabolism, such as glycolysis and protein synthesis (Fig. 1) [61]. Similar to cancer cells, T-cell activation initiates a program of cell growth and proliferation that demands higher levels of metabolism such as glycolysis and protein synthesis. Similar increases in metabolism are expected when effector T cells encounter their target cells. PI3K plays an important role in induction of glycolysis and protein synthesis in T cells [62]. Thus, tumor cells and tumor-infiltrating effector T cells are competitors for energy resources in the tumor microenvironment. Tumor cells suppress the T-cell effector function by activating PI3K within themselves which can increase the level of PD-L1 protein [45] (Fig. 1). PD-L1 engages its receptor on T cells to initiate a molecular program that inhibits activation of PI3K in T cells [33]. In this way, expression of PD-L1 allows tumor cells to inhibit the PI3K-driven metabolic program in T cells and thereby inhibiting their effector functions (Fig. 1). Whether B7-H4 has similar functions remains to be seen.

#### **Summary and future perspectives**

Our work supports the idea that tumorigenesis can elicit cellular immune responses aiming at eliminating or containing tumor growth and spread. Both T-bet and Eomes are required for anti-tumor immune responses by CD8<sup>+</sup> T cells. The level of T-bet<sup>+</sup> TILs within tumor tissues correlates with better patient survival. The T cell-intrinsic molecular program regulated by T-bet and Eomes within the tumor tissues need further study. Whether these molecules play important role in CD4<sup>+</sup> TILs and Tregs [63] should also be examined in human upper GI cancers. An important feature of cancer progression is the development

of immune suppressive mechanisms. We show that tumor tissues from advanced stages of upper GI cancer express high levels of PD-L1 and B7-H4. One likely mechanism is to allow tumor to compete efficiently with T cells for limited nutrients. PI3K is a critical player in the molecular network of the tumor immune suppressive microenvironment. Despite a critical role played by TILs in preventing tumor progression, little is known about the intrinsic molecular mechanisms regulating their effector functions. Understanding the signaling and transcriptional regulatory network that are regulated by PI3K and T-bet in TILs will help designing better immunotherapy. Potentiating T cell signaling by either genetically modifying T cells with signaling modulators or stimulating TILs with cytokines and co-stimulatory signals will greatly functional anergy of TILs and improve immunotherapy.

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## References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
- Cohen DJ, Ajani J. An expert opinion on esophageal cancer therapy. *Expert Opin Pharmacother*. 2011;12:225–39.
- Lagergren J, Lagergren P. Oesophageal cancer. *BMJ*. 2010;341:c6280.
- Finn OJ. Cancer immunology. *N Engl J Med*. 2008;358:2704–15.
- Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313:1960–4.
- Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer*. 2005;5:263–74.
- Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol*. 2006;6:295–307.
- Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*. 2008;8:467–77.
- Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol*. 2005;23:515–48.
- Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. *Immunol Rev*. 2009;229:126–44.
- Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. *Clin Cancer Res*. 2007;13:5271–9.
- Martin-Orozco N, Dong C. Inhibitory costimulation and anti-tumor immunity. *Semin Cancer Biol*. 2007;17:288–98.
- Gajewski TF, Meng Y, Blank C, Brown I, Kacha A, Kline J, Harlin H. Immune resistance orchestrated by the tumor microenvironment. *Immunol Rev*. 2006;213:131–45.
- Jiang J, Zhu Y, Wu C, Shen Y, Wei W, Chen L, Zheng X, Sun J, Lu B, Zhang X. Tumor expression of B7–H4 predicts poor survival of patients suffering from gastric cancer. *Cancer Immunol Immunother*. 2010;59:1707–14.
- Chen LJ, Sun J, Wu HY, Zhou SM, Tan Y, Tan M, Shan BE, Lu BF, Zhang XG. B7-H4 expression associates with cancer progression and predicts patient's survival in human esophageal squamous cell carcinoma. *Cancer Immunol Immunother*. 2011;26:26.
- Ju S, Zhu Y, Liu L, Dai S, Li C, Chen E, He Y, Zhang X, Lu B. Gadd45b and Gadd45g are important for anti-tumor immune responses. *Eur J Immunol*. 2009;39:3010–8.
- Zhu Y, Ju S, Chen E, Dai S, Li C, Morel P, Liu L, Zhang X, Lu B. T-bet and eomesodermin are required for T cell-mediated anti-tumor immune responses. *J Immunol*. 2010;185:3174–83.
- Underwood JC. Lymphoreticular infiltration in human tumours: prognostic and biological implications: a review. *Br J Cancer*. 1974;30:538–48.
- Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357:539–45.
- Glimcher LH. Trawling for treasure: tales of T-bet. *Nat Immunol*. 2007;8:448–50.
- Werneck MB, Lugo-Villarino G, Hwang ES, Cantor H, Glimcher LH. T-bet plays a key role in NK-mediated control of melanoma metastatic disease. *J Immunol*. 2008;180:8004–10.
- Garrett WS, Punit S, Gallini CA, Michaud M, Zhang D, Sigrist KS, Lord GM, Glickman JN, Glimcher LH. Colitis-associated colorectal cancer driven by T-bet deficiency in dendritic cells. *Cancer Cell*. 2009;16:208–19.
- Lipscomb MW, Chen L, Taylor JL, Goldbach C, Watkins SC, Kalinski P, Butterfield LH, Wesa AK, Storkus WJ. Ectopic T-bet expression licenses dendritic cells for IL-12-independent priming of type 1 T cells in vitro. *J Immunol*. 2009;183:7250–8.
- Qu Y, Chen L, Pardee AD, Taylor JL, Wesa AK, Storkus WJ. Intraleisional delivery of dendritic cells engineered to express T-bet promotes protective type 1 immunity and the normalization of the tumor microenvironment. *J Immunol*. 2010;185:2895–902.
- Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, Banica M, DiCioccio CB, Gross DA, Mao CA, Shen H, Cereb N, Yang SY, Lindsten T, Rossant J, Hunter CA, Reiner SL. Control of effector CD8+ T cell function by the transcription factor eomesodermin. *Science*. 2003;302:1041–3.
- Lee K, Min HJ, Jang EJ, Hong JH, Hwang ES. In vivo tumor suppression activity by T cell-specific T-bet restoration. *Int J Cancer*. 2010;127:2129–37.
- Groom JR, Luster AD. CXCR3 ligands: redundant, collaborative and antagonistic functions. *Immunol Cell Biol*. 2011;89:207–15.
- Zou W, Restifo NP, Hinrichs CS, Kaiser A, Paulos CM, Cassard L, Sanchez-Perez L, Heemskerk B, Wrzesinski C, Borman ZA, Muranski P. T(H)17 cells in tumour immunity and immunotherapy type 17 CD8+ T cells display enhanced antitumor immunity. *Nat Rev Immunol*. 2010;10:248–56.
- Zou W, Restifo NP. T(H)17 cells in tumour immunity and immunotherapy. *Nat Rev Immunol*. 2010;10:248–56.
- Ishida M, Iwai Y, Tanaka Y, Okazaki T, Freeman GJ, Minato N, Honjo T. Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. *Immunol Lett*. 2002;84:57–62.
- Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JR, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, Freeman GJ. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol*. 2001;2:261–8.
- Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192:1027–34.

33. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev.* 2009; 229:114–25.
34. Sandner SE, Clarkson MR, Salama AD, Sanchez-Fueyo A, Domenig C, Habicht A, Najafian N, Yagita H, Azuma M, Turka LA, Sayegh MH. Role of the programmed death-1 pathway in regulation of alloimmune responses in vivo. *J Immunol.* 2005; 174:3408–15.
35. Dong H, Zhu G, Tamada K, Flies DB, van Deursen JM, Chen L. B7-H1 determines accumulation and deletion of intrahepatic CD8(+) T lymphocytes. *Immunity.* 2004;20:327–36.
36. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med.* 2002;8:793–800.
37. Sabapatha A, Gercel-Taylor C, Taylor DD. Specific isolation of placenta-derived exosomes from the circulation of pregnant women and their immunoregulatory consequences. *Am J Reprod Immunol.* 2006;56:345–55.
38. Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, Greenfield EA, Freeman GJ. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol.* 2003;170:1257–66.
39. Kim PS, Ahmed R. Features of responding T cells in cancer and chronic infection. *Curr Opin Immunol.* 2010;22:223–30.
40. Shin H, Wherry EJ. CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol.* 2007;19:408–15.
41. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med.* 1998;188:2205–13.
42. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature.* 2006;443:350–4.
43. Urbani S, Amadei B, Tola D, Massari M, Schivazappa S, Missale G, Ferrari C. PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. *J Virol.* 2006;80:11398–403.
44. Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V, Subramaniam S, Blattman JN, Barber DL, Ahmed R. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity.* 2007;27:670–84.
45. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, Cachola KE, Murray JC, Tihan T, Jensen MC, Mischel PS, Stokoe D, Pieper RO. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med.* 2007;13:84–8.
46. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell.* 2007;129:1261–74.
47. Hildebrandt MA, Yang H, Hung MC, Izzo JG, Huang M, Lin J, Ajani JA, Wu X. Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. *J Clin Oncol.* 2009;27:857–71.
48. Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem.* 2006;108:19–24.
49. Sun J, Xu K, Wu C, Wang Y, Hu Y, Zhu Y, Chen Y, Shi Q, Yu G, Zhang X. PD-L1 expression analysis in gastric carcinoma tissue and blocking of tumor-associated PD-L1 signaling by two functional monoclonal antibodies. *Tiss Antigens.* 2007;69:19–27.
50. Ohigashi Y, Shio M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, Mizuno T, Yoriki R, Kashizuka H, Yane K, Tsushima F, Otsuki N, Yagita H, Azuma M, Nakajima Y. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clin Cancer Res.* 2005; 11:2947–53.
51. Prasad DV, Richards S, Mai XM, Dong C. B7S1: a novel B7 family member that negatively regulates T cell activation. *Immunity.* 2003;18:863–73.
52. Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath L, Chen L. B7-H4: a molecule of the B7 family, negatively regulates T cell immunity. *Immunity.* 2003;18:849–61.
53. Zang X, Loke P, Kim J, Murphy K, Waitz R, Allison JP. B7x: a widely expressed B7 family member that inhibits T cell activation. *Proc Natl Acad Sci USA.* 2003;100:10388–92.
54. Suh WK, Wang S, Duncan GS, Miyazaki Y, Cates E, Walker T, Gajewska BU, Deenick E, Dawicki W, Okada H, Wakeham A, Itie A, Watts TH, Ohashi PS, Jordana M, Yoshida H, Mak TW. Generation and characterization of B7-H4/B7S1/B7x-deficient mice. *Mol Cell Biol.* 2006;26:6403–11.
55. Zhu G, Augustine MM, Azuma T, Luo L, Yao S, Anand S, Rietz AC, Huang J, Xu H, Flies AS, Flies SJ, Tamada K, Colonna M, van Deursen JM, Chen L. B7-H4-deficient mice display augmented neutrophil-mediated innate immunity. *Blood.* 2009;113:1759–67.
56. Salceda S, Tang T, Kmet M, Munteanu A, Ghosh M, Macina R, Liu W, Pilkington G, Papkoff J. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. *Exp Cell Res.* 2005;306:128–41.
57. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, Brumlik M, Cheng P, Curiel T, Myers L, Lackner A, Alvarez X, Ochoa A, Chen L, Zou W. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med.* 2006;203:871–81.
58. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer.* 2009;9: 798–809.
59. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med.* 2010; 207:2187–94.
60. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, Kirkwood JM, Kuchroo V, Zarour HM. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. *J Exp Med.* 2010;207:2175–86.
61. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer.* 2011;11:85–95.
62. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH, Thompson CB. The CD28 signaling pathway regulates glucose metabolism. *Immunity.* 2002;16: 769–77.
63. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immunol.* 2009;10:595–602.