In tumor cells, thyroid hormone analogues non-immunologically regulate PD-L1 and PD-1 accumulation that is anti-apoptotic

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ABSTRACT

The PD-1/PD-L1 immune checkpoint involving tumor cells and host immune defense lymphocytes is a well-studied therapeutic target in oncology. That PD-1 and PD-L1 may have additional functions within tumor cells that are independent of the checkpoint is indicated by actions of a thyroid hormone analogue, L-thyroxine (T₄), on these checkpoint components. Acting at a cell surface receptor on plasma membrane integrin αvβ3, T₄ stimulates intracellular accumulation of PD-L1 in cancer cells. In these thyroid hormone-treated cells, T₄-induced PD-L1 is non-immunologically anti-apoptotic, blocking activation of p53. Several laboratories have also described accumulation of PD-1 in a variety of cancer cells, not just immune defense lymphocytes and macrophages. Preliminary observations indicate that T₄ stimulates intracellular accumulation of PD-1 in tumor cells, suggesting that, like PD-L1, PD-1 has non-immunologic roles in the setting of cancer. Where such roles are anti-apoptotic, thyroid hormone-directed cancer cell accumulation of PD-1 and PD-L1 may limit effectiveness of immunologic therapy directed at the immune checkpoint.

INTRODUCTION

The programmed death-1 (PD-1)/PD-Ligand 1 (PD-L1) immune checkpoint has been extensively investigated [1-5]. PD-L1 production by tumor cells is a natural defense of cancer cells against host immune system destruction, downregulating the antitumor activity of immune T (killer) cells [1, 6-8]. Antibodies to PD-L1 and to PD-1 have been shown clinically to have important utility in the management of a variety of malignancies [9-15].

A non-antibody-based mechanism by which PD-L1 elaboration by tumor cells can be regulated involves thyroid hormone analogues [16, 17]. L-thyroxine (T₄) is the principal product of the thyroid gland and is viewed as prohormone for the major intracellular thyroid hormone, 3,5,3’-triiodo-L-thyronine (T₃). However, T₄ has a panel of biological actions at a tumor cell surface receptor on plasma membrane integrin αvβ3 [18]. One of these actions downstream of the receptor is upregulation of transcription of PD-L1 [16]. A derivative of T₄, tetraiodothyroacetic acid (tetrac), blocks this action of T₄ initiated at αvβ3. We have proposed that tetrac, modified chemically to limit its actions to the exterior of tumor cells expressing αvβ3 [19], be tested as a non-antibody-based strategy to decrease or eliminate PD-L1 as a cancer cell defense [16].

PD-L1 IN T₄-TREATED TUMOR CELLS

Thyroid hormone as T₄ in physiological free concentrations supports cancer cell proliferation and a
number of survival pathways in tumor cells [20]. These cancer support actions are initiated by \(T_4\) at a receptor site on the extracellular domain of integrin \(\alpha v\beta 3\). While these actions are non-genomic at initiation—that is, they do not directly depend upon the nuclear receptors for thyroid hormone (TRs)—they may culminate downstream in transcription of specific genes [18], certain of which may involve TRs. These downstream effects are mediated by intracellular signal transduction systems, such as MAPK/ERK and PI3K. Unmodified or chemically converted to a nanoparticle, tetrac blocks actions of \(T_4\), including activation of MAPK and PI3K.

The involvement of ERK1/2 and PI3K in the enhancement of PD-L1 gene expression [16, 21] caused us to search for possible involvement of \(T_4\) in the regulation of PD-L1 transcription. Studied in vitro in human breast and colon cancer cell lines, PD-L1 expression was enhanced by \(T_4\) [16] and complimented by accumulation of tumor cell PD-L1 protein by as much as 2.7-fold. Tetrac chemically bound to a poly lactic-co-glycolic acid (PLGA) nanoparticle, (Nanotetrac, nano-diamino-tetrac (NDAT)), substantially reduced the stimulatory effect of \(T_4\) on PD-L1 gene expression and on abundance of cellular PD-L1 protein. In addition to its anti-apoptotic property, PD-L1 may also be a proliferative factor in certain cancer cells [22]. These results support the possibility that circulating host \(T_4\) is contributing to defensive activation in cancer cells of PD-L1.

The NDAT results indicated the feasibility of using a small molecule to modulate the PD-1/PD-L1 checkpoint by reducing the availability of PD-L1 [23]. This approach would also avoid the systemic adverse effects of PD-L1 antibody [24, 25] because actions of NDAT are limited to cancer cells and rapidly dividing endothelial cells that generously express \(\alpha v\beta 3\).

NEWLY RECOGNIZED ROLES OF INTRACELLULAR PD-L1 AND PD-1 IN \(T_4\)-TREATED CANCER CELLS

Against the background described, it is reasonable to ask whether PD-L1 has clinically undesirable intracellular effects that also may be avoided by downregulating the transcription of PD-L1 with NDAT or similar compounds. Resveratrol, a stilbene with anticancer properties, can induce p53-dependent apoptosis in cancer cells by a mechanism that involves nuclear uptake of cyclooxygenase-2 (COX-2) [17, 26]. This was a novel role for COX-2.

Studied in the resveratrol-p53-COX-2 model in tumor cells exposed to \(T_4\), intracellular PD-L1 was found to be complexed with COX-2 in cytoplasm and no nuclear uptake of p53 and COX-2 occurred [17]. Therefore, resveratrol-induced apoptosis was inhibited. Thus, in addition to its function extracellularly as a ligand of T cell PD-1 at the PD-1/PD-L1 immune checkpoint and thus a defense against immune system destruction of cancer cells, PD-1 has an intracellular role as an inhibitor of inducible COX-2/p53-dependent apoptosis. This function is also a cancer cell survival mechanism for PD-L1. The results raise a set of questions that have not yet been addressed. For example, does the interaction of PD-L1 with inducible COX-2 in \(T_4\)-treated tumor cells alter the function of COX-2 and reduce intracellular content of prostaglandins? Does this interaction model other protein-protein interactions in cytoplasm that are relevant to cancer cells and rapidly dividing endothelial cells that generously express \(\alpha v\beta 3\).

Figure 1: \(T_4\) induces PD-1 mRNA expression in human colon cancer (HT-29, HCT116 and breast cancer (MDA-MB-231) cells in vitro. Nano-diamino-tetrac (NDAT) inhibits actions of \(T_4\) that are initiated at plasma membrane integrin \(\alpha v\beta 3\) and has anticancer activity in the absence of \(T_4\). In this study, NDAT inhibited stimulatory activity of \(T_4\) on expression of PD-1 mRNA and also reduced abundance of PD-1 mRNA in the absence of \(T_4\). Materials, including cell lines, and methods used are as previously described [16]. Compared to control, \(* p < 0.05, *** p < 0.001;\) compared to \(T_4\), alone, \(## p < 0.05, ### p 0.001.\)
cancer cell defenses? The proteins could, for example, be signal transducing molecules or hormone-binding proteins such as nuclear thyroid hormone receptors (TRs) or estrogen receptors in cytoplasm.

The possibility that PD-1 has functions in cells other than T and B lymphocytes and macrophages has been suggested in reports from a number of laboratories that PD-1 is expressed by ovarian carcinoma cells [27], melanoma [28], small cell lung carcinoma [29], osteosarcoma cells [30] and murine lung carcinoma cells [31]. Physiological amounts of Tt induce expression of PD-1 mRNA as well as accumulation of PD-1 protein in several human cancer cell lines (HY Lin: unpublished observations). NDAT blocks the action of Tt on the PD-1 axis in these cancer cells (Figure 1). Such preliminary studies provide no functional basis for the PD-1 response in human tumor cells, but it is known that injured, non-tumoral retinal ganglion cells (RGCs) express PD-1 [32], as do mouse RGCs scheduled to undergo apoptosis [33]. Thus, elaboration of PD-1 in cells other than lymphocytes and macrophages may be related to self-defense, e.g., apoptosis. This possibility requires systematic evaluation in other non-cancer cells.

OVERVIEW

That a thyroid hormone analogue such as Tt can regulate intracellular concentrations of PD-1 and PD-L1 by a mechanism that is inhibitable by NDAT indicates that Tt is indeed biologically active and its activity is manifested via the hormone receptor on integrin αvβ3. Tt is the principal ligand of this receptor [20, 34], and NDAT at the concentration used is a specific inhibitor of thyroid hormone actions at αvβ3 [16]. The transcription of a large number of genes is regulated by this cell surface hormone receptor [35], and many of these are relevant to tumor cell proliferation, to tumor cell survival anti-apoptotic pathways [20] and to rapidly dividing endothelial cells and angiogenesis [36, 37]. We suggest that accumulation of intracellular PD-L1 and PD-1 in cancer cells offers another anti-apoptotic defense for tumor cells that is in a compartment inaccessible to clinically used antibodies to PD-1 and PD-L1 [23]. As noted above, accumulation of PD-L1 may occur in non-cancer cells that are at risk of apoptosis. Another role for PD-L1 involves regulation of angiogenesis [38]. Thus, distinct from their synergy in the function of the PD-1/PD-L1 immune checkpoint, these two moieties have functions as independent proteins. At least in part, these functions are regulated by thyroid hormone as Tt.

Another issue is that resistance to apoptosis accompanies activation of the immune checkpoint in tumor cells [39]. Accumulation of PD-L1 and PD-1 within tumor cells exposed to Tt may be a component of the anti-apoptosis encountered in checkpoint activation. Tt is known to have pro-angiogenic and anti-apoptotic properties [18], and the independent control by Tt via αvβ3 of PD-1 and PD-L1 production unrelated to the PD-1/PD-L1 immune checkpoint is consistent with roles already defined for Tt. While further studies are required to determine how substantial the clinical contributions are of Tt to tumor-related angiogenesis and anti-apoptosis, elimination of Tt in patients with advanced cancers has shown stabilization of the disease and extended survival [40].

CONFLICTS OF INTEREST

Co-authors PJ Davis and SA Mousa are stockholders in NanoPharmaceuticals LLC that is commercially developing NDAT, and PJ Davis is an officer of the company. KA Keating is a paid consultant for NanoPharmaceuticals LLC. Co-authors Lin, Chin, Shih, Chen, and Leinung have no conflicts to report.

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