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Tetraiodothyroacetic acid (tetrac), integrin $\alpha v \beta 3$ and disabling of immune checkpoint defense

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“An alternative strategy in immune checkpoint-directed treatment of cancer is regulation of the expression of specific genes for checkpoint proteins, avoiding immune system antibody use.”

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Substantial medical and commercial interest exists in the clinical regulation of immune checkpoints that control host lymphocyte immunoprotection against cancer [1–8]. Generated by tumor cells, inhibitory immune checkpoint proteins act to suppress T-cell engagement of cancer cells and may also induce apoptosis of T cells. Clinical manipulation of the immune system in cancer patients is largely via antibodies specifically directed against inhibitory checkpoint molecules that cancer cells express in self-protection. The antibodies developed to date have limited application – that is, they are effective in a minority of patients affected by the tumors – and their use is complicated by substantial adverse immune system side effects. They are also expensive to generate.

Pharmacologic attack of PD-L1 immune checkpoint gene expression

An alternative strategy in immune checkpoint-directed treatment of cancer is regulation of the expression of specific genes for checkpoint proteins, avoiding immune system antibody use. The feasibility of this approach became clear with the recognition that transcription of the *PD-L1* gene in cancer cells could be controlled from a structural protein, $\alpha v \beta 3$, of the plasma membrane of cancer cells [9]. The extracellular domain of $\alpha v \beta 3$ contains a receptor for thyroid hormone at which L-thyroxine (T4) at physiological concentrations and via an initially nongenomic mechanism upregulates expression of *PD-L1*. T4 has been viewed as a prohormone for 3,5,3'-triiodo-L-thyronine (T3), the principal intracellular form of thyroid hormone that mediates genomic effects of the hormone that are initiated at the intranuclear receptors for T3. However, T4 initiates in cancer cells a number of actions that support cancer, including the regulation of the cell cycle, anti-apoptosis and angiogenesis [10]. The action of T4 on *PD-L1* expression is interpreted as another cancer cell support (and defense) mechanism in the euthyroid patient.

The action of T4 on *PD-L1* gene expression in a variety of human tumor cells is blocked at $\alpha v \beta 3$ by a T4 derivative, tetraiodothyroacetic acid (tetrac), which has a number of anticancer properties [11,12]. In the absence of T4, unmodified tetrac or tetrac reformulated as a nanoparticulate (nano-diamino-tetrac) has a modest downregulatory action on basal *PD-L1* transcription. Thus, tetrac formulations may offer a nonimmunologic approach to reduction of regulation of PD-L1 production by tumor cells, particularly in the euthyroid cancer patient. $\alpha v \beta 3$ is a principal product of cancer cells and is present only in small amounts in normal cells, whose proliferation is unaffected by tetrac [13]. Formal toxicology testing of tetrac formulations has not shown adverse effects in subhuman primates [MOUSA SA, UNPUBLISHED OBSERVATIONS].

Can other immune checkpoint genes be managed via integrin $\alpha v \beta 3$

PD-L1 is one example of an inhibitory checkpoint molecule whose antibody targeting has been shown to have clinical significance. The discovery of a regulatory mechanism for PD-L1 production that is based at $\alpha v \beta 3$

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prompted a search for nonimmunologic strategies for control of other inhibitory checkpoint molecules. Tetrac does not appear to affect production of the checkpoint inhibitor, CTLA-4 [LIN HY, UNPUBLISHED DATA], but the stilbene, resveratrol, suppresses production of this checkpoint inhibitor [14], and we have defined a cell surface receptor for resveratrol on $\alpha\text{v}\beta 3$ that underlies the anticancer activity of resveratrol [15]. Antibody to CTLA-4 is in clinical use in tumor management, but we raise the possibility here that $\alpha\text{v}\beta 3$ may offer a pharmacologic approach to control of CTLA-4 generation. In addition to the action of resveratrol, estrogen has been shown to increase transcription of CTLA-4 in regulatory T (Treg) cells involved in autoimmune nervous system disease [16]. While it might be assumed that such an effect would be mediated by nuclear estrogen receptor, we have shown that a cell surface estrogen binding site exists on integrin $\alpha\text{v}\beta 3$ [17]. Thus, both *PD-L1* and *CTLA-4* expression may be regulated pharmacologically by small molecules that act at specific sites on the integrin.

There is also information available about other immune checkpoint inhibitor proteins whose genes may be controlled from $\alpha\text{v}\beta 3$. Indoleamine 2,3-dioxygenase is an enzyme that catabolizes tryptophan, but is known as well to have immune system inhibitory actions that are cancer-relevant. Some of the antitumor activity of resveratrol has been attributed to inhibition of indoleamine 2,3-dioxygenase [18]. While it might be assumed that it is an action of intracellular resveratrol, the rapid metabolism of the intracellular stilbene has confounded attempts to explain biologic actions of the compound, whereas the receptor for resveratrol on $\alpha\text{v}\beta 3$ has been shown to be highly functional.

TIM-3 is an immune checkpoint inhibitor protein that has been shown to be sex hormone-regulated, for example, suppressed in castrated male mice [19]. Again, while this androgen effect may be assumed to be nuclear androgen receptor-mediated, we have described a dihydrotestosterone receptor on $\alpha\text{v}\beta 3$ that is cancer-relevant, in that its activation stimulates estrogen receptor-negative breast cancer cell proliferation [20] and blocks cancer chemotherapeutic activity of resveratrol [21].

Overview

There is substantial and justifiable clinical interest in manipulation of the function of immune checkpoint inhibitor proteins by which cancer cells gain protection from host immune system destruction. Such manipulation to date has involved specific antibody generation, and certain of the antibodies have had important adverse side effects. We have shown that it is pharmacologically feasible *in vitro* to regulate expression of a specific inhibitory checkpoint protein gene, *PD-L1*, thus offering the prospect of avoidance of adverse effects of antibodies to immune checkpoint inhibitory proteins.

We note here that, in addition to *PD-L1*, at least three other inhibitory immune checkpoint genes are apparently subject to regulation by small molecules, such as resveratrol, from the vantage points of discrete receptors on the cell surface protein, integrin $\alpha\text{v}\beta 3$. It is possible to reformulate the small molecules that have targets on this integrin so that their actions are primarily at the extracellular domain of the integrin and do not involve intracellular receptors [21]. Because the expression of $\alpha\text{v}\beta 3$ is generous in tumor cells compared with nonmalignant cells, the effects of these small molecules via the integrin will spare normal cells.

Financial & competing interests disclosure

PJ Davis and SA Mousa hold stock in a small pharmaceutical company, NanoPharmaceuticals LLC, that is developing anticancer drugs, and PJ Davis is Chief Scientific Officer at the company. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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