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Cytokines and metabolic factors regulate tumoricidal T-cell function during cancer immunotherapy

Recent advances in cancer biology and genetics have fostered precision therapies targeting tumor-specific attributes. Immune-based therapies that elicit cytolytic T cells (CTL) specific for tumor antigens can provide therapeutic benefit to cancer patients, however, cure rates are typically low. This largely results from immunosuppressive mechanisms operating within the tumor microenvironment, many of which inflict metabolic stresses upon CTL. Conversely, immunotherapies can mitigate specific metabolic stressors. For instance, dual costimulation immunotherapy with CD134 (OX40) plus CD137 (4-1BB) agonists appears to mediate tumor control in part by engaging cytokine networks that enable infiltrating CTL to compete for limiting supplies of glucose. Future efforts combining modalities that endow CTL with complimentary metabolic advantages should improve therapeutic efficacies.

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Dysregulation in any of a relatively small number of oncogenic signaling pathways is responsible for driving cancer in a wide variety of organs, and common oncogenic driver mutations often arise in a high proportion of patients with a particular type of cancer [1]. Small-molecule drugs that selectively inhibit oncogenic tyrosine kinases, for instance, can be effective in treating patients whose tumors harbor the corresponding common mutation [2,3]. Although tumor regression tends to be only temporary due to the outgrowth of resistant clones that have acquired compensatory mutations that bypass the targeted oncogenic kinase [4], these therapeutic responses nevertheless highlight that it is possible to selectively target tumor-specific determinants and thereby minimize collateral damage.

The adaptive immune system evolved to distinguish self from nonself in order to detect and eliminate pathogens while avoiding autoimmunity. In the case of T cells whose antigen receptors (TCRs) recognize

processed peptide epitopes in the context of MHC presented on target cells, this capacity also facilitates the detection of tumor cells expressing mutated self-proteins. Immune-based cancer therapeutics seek to exploit the genetic instability of tumors by targeting these neoantigens. Indeed, the absence of T cells or their critical effector molecules can increase cancer incidence or rates of progression, inferring that clinically detectable tumors in immune-competent individuals have been selected by the immune system for low immunogenicity [5–8]. Accordingly, immunotherapies must overcome a variety of immunosuppressive mechanisms employed by these ‘edited’ tumors.

Tumors utilize multiple mechanisms to avoid immune elimination

T-cell tolerance mechanisms that prevent autoimmunity can negatively impact T cell mediated tumor immunity. The majority of potentially self-reactive T cells undergo

negative selection during thymic development [9,10]. This central tolerance process cannot directly shape the repertoire of effector T cells specific for mutated tumor epitopes, since they are not encoded in the germline genomes of thymic antigen presenting cells (APCs). Nevertheless, it can impact tumor immunity through positive selection of tissue-specific Foxp3⁺ Tregs capable of suppressing tumor-specific effector T cells [11].

Thymic deletion of self-reactive conventional T cells is incomplete [12], thus necessitating peripheral tolerance mechanisms [13]. Dendritic cells play a critical role in regulating priming versus tolerization of mature T cells in peripheral lymphoid organs [14]. For instance, during viral infections they acquire antigen from infected cells, then migrate to draining lymph nodes where they cross-present processed epitopes to cognate CD4⁺ helper T cells and CD8⁺ cytolytic T cells (CTL). Importantly, pathogen-associated molecular patterns (PAMPs) and associated inflammatory signals induce these dendritic cells to express costimulatory ligands that provide a critical second signal that enables the TCR-stimulated T cells to undergo expansion and gain the capacity to migrate into peripheral tissues and elaborate effector functions [15]. During steady-state conditions, dendritic cells continue to cross-present self-antigens acquired from peripheral tissues. The absence of PAMPs results in suboptimal costimulation and hence cognate self-reactive T cells undergo an abortive response culminating in deletion, anergy or the development of suppressive function [16–19]. Tumor-specific T cells can also undergo peripheral tolerization through this pathway [20,21]. This tolerization reflects that tumors often lack sufficient inflammatory signals to convert steady-state tolerogenic dendritic cells into immunogenic APCs capable of programming and expanding tumoricidal effector T cells. In sum, tolerization impedes the activity of T cells capable of recognizing tumor epitopes [22–24].

Despite the potential for tumors to tolerize cognate T cells, in some settings tumor-specific T cells undergo productive priming. Thus, tumor-specific effector T cells have a propensity to infiltrate several types of human tumors [25–27], and tumor progression in some mouse models is associated with immunogenic (rather than tolerogenic) T-cell responsiveness [28–30]. This may result from the turnover of tumor cells undergoing specific forms of programmed death that involve release of damage-associated molecular patterns that can function in an analogous manner as PAMPs in activating dendritic cells [31].

Once effector T cells infiltrate tumors, however, a variety of immunosuppressive cells and soluble factors conspire to block the elaboration of tumoricidal

functions. For instance, Tregs suppress tumor-specific CD8⁺ CTL by releasing cytokines such as TGF- β , IL-10 [32] and IL-35 [33] or by removing costimulatory ligands from the surface of dendritic cells [34]. Additionally, tumor-associated macrophages can secrete factors that promote tumor growth and metastasis [35] and myeloid-derived suppressor cells can induce TCR nitration and hence reduce CTL recognition of cognate tumor epitopes [36].

Tumors suppress T-cell function by creating metabolic challenges

Dysregulated whole-body metabolism impacts tumor development. An emerging example is the rise in obesity and its connection to T-cell biology [37], especially Tregs [38]. While obesity is a risk factor for a broad range of metabolic disorders, 3.6% of all new cancer cases worldwide are attributable to obesity [39]. A signature feature of metabolic syndrome is chronic low-level inflammation, including circulating peptides and hormones, such as increased IGF-1 and leptin, but decreased adiponectin, and formation of inflammatory cytokine networks [40–44]. The impact of this response is likely an acceleration of cancerous transformation and/or tumor growth. Specifically, activation of inflammatory signaling pathways such as NF- κ B and c-Jun N-terminal kinase 1, along with increased IL-1 β , TNF and IL-6 have been linked with tumorigenesis [45–47]. Second, adipocyte-secreted adiponectin has the potential to influence tumor growth by affecting the mTOR and AMP-activated protein kinase pathways [48,49].

Tumors in turn create metabolic stressors for infiltrating CTL that suppress their tumoricidal activity. For instance, immunoregulatory plasmacytoid dendritic cells residing in the tumor microenvironment can secrete the enzyme indoleamine-2,3-dioxygenase that catabolizes the essential amino acid tryptophan causing tumor infiltrating lymphocytes (TIL) to undergo the GCN2 stress response and become anergic [50]. Additionally, tumor cells consume copious amounts of glucose [51], causing a state of glucose deprivation in the microenvironment that starves TIL [52,53]. This poses a significant therapeutic hurdle since CTL must actively undergo glucose-fueled aerobic glycolysis to secrete the tumoricidal cytokine IFN- γ [54].

Immunotherapies overcome tumor-induced immunosuppression

Since clinically detectable tumors have been edited to evade naturally arising antitumor immune responses, immunotherapies need to reverse or overcome these immunosuppressive hurdles. Tumoricidal T-cell functions are regulated by a series of potentiating and

inhibitory signals delivered initially by dendritic cells in draining lymph nodes and subsequently by tumor cells and supporting stroma. Upon initial recognition by the TCR of cognate MHC–peptide complexes on dendritic cells, interaction of the T-cell costimulatory receptor CD28 with its ligands CD80 (B7–1) or CD86 (B7–2) provides a critical second signal that facilitates T-cell activation and cell cycle progression [55]. This initial activation also induces T-cell surface localization of the checkpoint receptor CTLA-4 that also binds B7–1/2 to temper the response and prevents excessive inflammation [56,57]. A monoclonal antibody specific to CTLA-4 that antagonizes its inhibitory signal elicits antitumor immunity in mice [58], and a humanized anti-CTLA-4 (ipilimumab) was approved by the US FDA to treat patients with metastatic melanoma [59]. The ability of anti-CTLA-4 to potentiate T-cell priming in conjunction with its antitumor therapeutic potential led to the notion that checkpoint inhibitors remove the brakes on tumor-specific effector T cells. Subsequent studies have revealed that anti-CTLA-4 may augment tumor immunity by acting at multiple sites. Thus, in addition to activated conventional T cells, CTLA-4 is expressed on Foxp3⁺ Tregs and anti-CTLA-4 can induce Treg deletion in the tumor microenvironment via a mechanism that appears to involve FcR-mediated antibody-dependent cellular cytotoxicity or phagocytosis [60].

Another inhibitory checkpoint molecule whose activity can be blocked to augment therapeutic tumor immunity is PD-1. Its ligands PD-L1 and PD-L2 can be expressed on dendritic cells and, similar to CTLA-4, PD-1/PD-L1 interaction can regulate initial T-cell priming [61], but importantly PD-L1 is also expressed on a variety of tumors and can thus induce TIL to become functionally exhausted [62]. Furthermore, PD-L1 expression on tumor cells is induced to even higher levels by IFN- γ secreted from CD8⁺ CTL [63,64], suggesting that tumors utilize PD-L1 as a countermeasure against antitumor immunity. Disrupting the PD-1/PD-L1 axis using either anti-PD-1 or anti-PD-L1 thus enables tumor-specific CD8⁺ TIL to regain tumoricidal function, and extends survival in metastatic melanoma patients [65,66]. PD-1 is also expressed on melanoma cells, and its binding to PD-L1 augments glucose uptake and glycolysis through activation of the mTOR signaling pathway [53,67]. Intriguingly, PD-1 ligation on T cells has the opposite effect of blocking glycolysis [68], and thus impeding glycolysis-dependent effector functions [54]. Thus, PD-1/PD-L1 checkpoint blockade not only terminates an inhibitory signal on TIL but also indirectly boosts the activity of these T cells by increasing intratumoral glucose availability as a consequence of limiting glucose consumption by the tumor cells.

The third promising checkpoint target is LAG-3, an immunoglobulin superfamily member and structural homolog of CD4 expressed on activated T cells, plasmacytoid dendritic cells, NK cells and regulatory CD4 T cells that binds MHC class II with a higher affinity than CD4 and limits T-cell expansion and antitumor effector functions [19,69–72]. Clinical trials are currently evaluating anti-LAG-3 blocking monoclonal antibody given to cancer patients alone or in combination with anti-PD-1 (NCT02061761, NCT01968109). Additionally, a LAG-3-immunoglobulin fusion protein that promotes DC activation has shown promising activity in early-stage clinical trials [73,74]. Besides CTLA-4, PD-1 and LAG-3, inhibitors of several other checkpoint molecules such as TIM-3, TIGIT and VISTA are being developed as cancer therapeutics [75–77].

T-cell expansion, effector differentiation, migration and survival are potentiated by signals from costimulatory receptors belonging to the TNFR superfamily whose expression are induced following initial antigen stimulation and CD28 costimulation [78–80]. The ligands for these receptors are typically expressed on PAMP-activated dendritic cells, and agonists to these costimulatory receptors thus provide critical signals for programming T-cell effector functionality that are otherwise lacking during steady-state antigen presentation [81]. Costimulatory agonists and checkpoint inhibitors thus act in reciprocal, but potentially synergistic manners [82–84]. Currently, agonists to the TNFR family members CD134 (OX40) [85], CD137 (4–1BB) [86], GITR [87] and CD27 [88] have demonstrated therapeutic activity in preclinical mouse models and are at various stages of clinical testing. Several of these TNFR members are expressed on cells besides activated conventional T cells, in particular Foxp3⁺ Tregs. GITR agonist, for instance, destabilizes Foxp3 expression in intratumoral Tregs leading to a loss of their suppressor function [89]. OX40 agonist can also impede Treg-suppressor activity [90,91], and similar to anti-CTLA-4 depletes intratumoral Tregs [92]. Finally, 4–1BB agonist can program Tregs to gain cytotoxic potential [93], but can also elicit CD8 T cell based suppression [94–96].

Combining modalities can boost therapeutic response

A strategy to avoid the outgrowth of therapy-resistant tumors is to combine modalities that target distinct tumor attributes. For instance, treating metastatic melanomas harboring the common BRAF(V600E) mutation with a small-molecule inhibitor leads to tumor regression followed by recurrence of tumors capable of bypassing BRAF to activate downstream mitogenic MAPK signaling, but importantly these

variant tumors can be responsive to cotreatment with an MEK inhibitor [4]. Similarly, KRAS-mutant lung tumors develop resistance to MEK inhibition via compensatory mitogenic signals received through FGFR1 and thus become susceptible to FGFR1 inhibition [97]. Given the reciprocal effects, checkpoint inhibitors and costimulatory agonists have in regulating T-cell responsiveness by relieving negative and potentiating positive signals, respectively, it was perhaps logical that combining biologics from each class do enhance therapeutic efficacy [82–84]. Nevertheless, even though nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) are both checkpoint inhibitors, combination therapy is clinically superior compared with the monotherapies [98]. This beneficial effect likely results from the nonoverlapping roles of CTLA-4 and PD-1 in regulating T-cell function at distinct stages of the antitumor response [62].

Immunotherapies can also be effectively combined with nonimmune-based modalities. Therapies that reduce tumor burden could reasonably be expected to augment the efficacy of immunotherapies simply by reducing the number of tumor cells that need to be targeted. For instance, androgen deprivation has been a standard treatment for advanced prostate cancer owing to the dependence of prostate tumor cells on the androgen receptor signaling axis [99], and thus subsequently administered immunotherapies might effectively target residual tumor cells to prevent the recurrence of castration-resistant tumors [100]. Some ‘nonimmune’ therapies have unexpected immunological effects that can potentiate antitumor T-cell responsiveness. For instance, imatinib that targets a mutant oncogenic kinase to block mitogenic signaling in tumor cells can also diminish tumor production of indoleamine-2,3-dioxygenase, thereby boosting CD8⁺ TIL activity [101]. Additionally, a BRAF inhibitor induces BRAF (V600E) mutant melanoma cells to increase presentation of tumor antigens [102,103] and potentiates the responsiveness of T cells expressing WT BRAF to boost the therapeutic effect of anti-CTLA-4 [104]. Castration also has immune-potentiating effects such as reviving thymic production of naive T cells [105], mitigating peripheral tolerization of prostate-specific T cells [106], accentuating IL-12-mediated Th1 differentiation [107] and increasing intratumoral T-cell migration [108]. Notably, surgical castration of mice prior to immunotherapy can potentiate T-cell mediated control of prostate tumors, although pharmacologic castration using certain androgen receptor antagonists can blunt efficacy via off-target effects that impact T cells [109]. Taken together, these studies highlight the potential of combination therapies to boost therapeutic efficacy, but emphasize the importance of

understanding the mechanisms by which the individual modalities work in order to select precise combinations that are more likely to be synergistic rather than additive or even mutually inhibitory.

CD134 plus CD137 dual costimulation elicits a multipronged antitumor therapeutic response

Just as different checkpoint inhibitors can be effectively combined [98], in murine tumor models costimulatory agonist combinations can also be more efficacious than the individual monotherapies [110]. For instance, combining three separate agonists to DR5, CD40 and CD137 (4-1BB) can elicit CD8⁺ CTL-mediated therapeutic tumor immunity [111]. Combinations targeting only two costimulatory receptors can also be effective. Thus, two recent studies have demonstrated efficacy in combining CD40 plus CD137 [112] and inducible T-cell co-stimulator (ICOS) plus CD134 (OX40) [113].

Over a decade ago, we found that combining agonists to CD134 plus CD137 can elicit robust CD8⁺ CTL response and therapeutic tumor immunity in a fibrosarcoma model [114,115]. Subsequent studies by other groups and ourselves have confirmed and extended this finding that ‘dual costimulation’ is therapeutic in breast cancer [116], melanoma [117] and lymphoma models [118].

Besides eliciting potent CD8⁺ CTL, dual costimulation programs unusual CD4 T-cell functions that can augment the antitumor therapeutic effect. First, dual costimulated CD4 T cells differentiate into cytotoxic Th1 effectors that have the capacity to produce copious amounts of IFN- γ , TNF- α and IL-2, and importantly can also directly kill MHC class II⁺ tumor cell targets presenting cognate peptide [118]. This may be beneficial not only in treating tumors that normally express MHC class II (e.g., B-cell lymphomas) but also tumors such as melanoma [119,120] that have a propensity to downregulate MHC class I [121] but can be induced by IFN- γ to express MHC class II [122]. Indeed, dual costimulated CD4 T cells can control the highly aggressive B16-F10 melanoma in the absence of CD8 T cells [118]. These observations in conjunction with the previous findings that 4-1BB agonist activates dendritic cells [123,124] and tumoricidal NK cells [125] suggest that dual costimulation programs at least a tripartite cytotoxic antitumor response.

The second unusual feature of dual costimulated CD4 T cells is that they can provide help not only to CD8 T cells responding to antigen presented by the same APC (linked help [126–128]) but also elicit CTL function in T cells not actively responding to cognate antigen (nonlinked help [118,129,130]). These helper effects are at least partially mediated through the secre-

tion of IL-2 and IFN- γ by the dual costimulated CD4 T cells [118,129,130]. Given that antigen cross-presentation by dendritic cells is biased toward highly abundant antigens [30,131], this nonlinked helper pathway might engage CD8⁺ CTL specific for less abundant tumor epitopes and thus increase the overall breadth of the antitumor response, and perhaps even target tumor variants that lack dominant epitopes. To test the ability of dual costimulated CD4 T cells capable of providing both linked and nonlinked help to boost therapeutic efficacy, mice bearing B16 melanomas were immunized with an MHC class II-restricted helper peptide along with dual costimulation [130]. Importantly, the helper peptide was foreign and not expressed by the tumor, thus bypassing the issue that tumor-specific CD4 T cells are often tolerant [106,132,133]. These tumor-unrelated CD4 helper T cells not only substantially boosted dual costimulation-elicited control of the aggressive melanoma but also migrated into the tumors where they provided help to support CD8⁺ TIL and thereby diminished the frequency of intra-tumoral Foxp3⁺ Tregs [130]. Given their lack of tumor specificity, this result raises the intriguing question of how these dual costimulated CD4 T cells are triggered within tumors to elaborate help.

As mentioned earlier, tolerance and immunosuppressive mechanisms operating within the tumor microenvironment limit high-avidity CD8⁺ TIL recognition of tumor epitopes. Strategies that could boost weak TCR–tumor peptide interactions (or even elicit TCR-independent elaboration of effector functions) should thus enhance the efficacy of immunotherapies. A notable attribute of dual costimulated CD8 T cells in this regard is their ability to be triggered by cytokines in the absence of TCR stimulation. Specifically, stimulation with either IL-12 or IL-2 followed by either of the IL-1 family members IL-33 or IL-36 induces IFN- γ secretion, subsequent induction of MHC class I and II on tumor cells, and degranulation of the CD8⁺ CTL [134,135]. The underlying mechanism of this TCR-independent process was further studied with the IL-2 plus IL-36 combination, where it was found that IL-2 activates the JAK-STAT pathway that induces transcription of the *Il1rl2* gene that encodes the IL-36 receptor [135].

CD8⁺ CTL must actively undergo aerobic glycolysis to secrete IFN- γ because GAPDH binds to the IFN- γ mRNA 3'UTR to block translation when it is not catalyzing glycolysis [54]. Correspondingly, the ability of dual costimulated CD8 T cells to be triggered by cytokines to secrete IFN- γ tracks with their glycolytic potential that is robust at the early effector stage but later diminishes as they begin transitioning into memory cells [135]. This is critical since TIL must compete with glycolytic tumor cells for limited supplies of

glucose [52,53]. Importantly, dual costimulated CD8⁺ effectors appear to be worthy competitors due to their robust expression of the glucose transporter Glut1 [135].

Based on the findings described thus far, we constructed the following model to explain the dual costimulation therapeutic response (Figure 1). Prior to therapy (Figure 1A), tumor-specific CD8⁺ CTL accumulate within tumors but weakly kill tumor cells due to several mechanisms that include: first, TCRs tend to have low avidity for cognate tumor epitopes, and tumor cells express low amounts of MHC class I; second, tumor cells consume large amounts of glucose, thus limiting availability to the CD8⁺ CTL and impeding glycolysis-dependent effector functions such as IFN- γ secretion and third, CD8⁺ CTL receive insufficient CD4 T-cell help, while being suppressed by Foxp3⁺ Tregs. Dual costimulation appears to overcome each of these therapeutic hurdles. First, IL-2 (possibly supplied by tumor-unrelated CD4 helper T cells) and/or IL-12 (possibly supplied by mature dendritic cells or macrophages) prepares CD8⁺ TIL to transcribe IFN- γ mRNA in response to the IL-1 family cytokines IL-33 and IL-36 that may derive from live or necrotic skin or tumor cells [136–138]. Furthermore, dual costimulation-mediated induction of the glucose transporter Glut1 on the CD8⁺ TIL enables them to internalize glucose that sustains glycolysis, thus fostering translation and secretion of IFN- γ protein (Figure 1B). Finally, IFN- γ induces MHC class I expression and hence presentation of tumor epitopes, and the continuous stimulation with IL-1 family cytokines facilitates TCR-mediated cytotoxicity directed against otherwise low-avidity tumor epitopes (Figure 1C).

Future studies will critically test the various aspects of this model, and also address several related questions. For instance, how are dual costimulated tumor-unrelated CD4 T cells triggered within tumors to deliver therapeutic help, and are Foxp3⁺ Tregs reprogrammed to aid or impede the therapeutic response. Lastly, control of T-cell metabolism within the tumor microenvironment may prove paramount for effective immunotherapy. Understanding this process and enhancing Glut1 or other means to increase glycolysis in T cells should help antitumor responses. Given the potential of insulin to impact T-cell function [139,140], it will also be critical to determine whether obesity, metabolic syndrome and insulin resistance impact the ability of T cells to become glycolytic during immunotherapy. IL-33 may play a particularly important role during dual costimulation since it cross-regulates immunity, obesity and cancer [141], and as we propose in Figure 1 may stimulate T cells within the tumor microenvironment in a TCR-independent manner. While the impact of CD134 and CD137 costimulated T cells during the intersection of these responses is

Figure 1. Hypothesized mechanism of the dual costimulation antitumor therapeutic response. (A) Prior to therapy tumor-infiltrating CD8⁺ CTL (tumor infiltrating lymphocyte) inefficiently kill tumor cells due to weak presentation and recognition of tumor epitopes, competition with tumor cells for limiting glucose, insufficient support from CD4⁺ helper T cells and suppression by Foxp3⁺ Tregs. **(B)** Dual costimulation therapy elicits IL-2 and IL-12 from intratumoral CD4⁺ helper T cells and APC that increases expression of Glut1 on the CD8⁺ tumor infiltrating lymphocyte and primes them to respond to IL-33 and/or IL-36 in a TCR-independent manner leading to IFN- γ release. Specifically, Glut1 fosters glycolysis that opens the availability of IFN- γ mRNA through the release of the 3'UTR by GAPDH. **(C)** The presence of IFN- γ induces MHC class I on the tumor cells that then facilitate TCR-mediated cytotoxicity. APC: Antigen presenting cell; CTL: Cytotoxic T cell; TCR: T-cell receptor; UTR: Untranslated region.

Figure 2. Hypothesized mechanism of PD-1/PD-L1 blockade plus dual costimulation therapeutic synergy. (A & B) In addition to releasing the brakes on tumor-specific T cells [62], PD-1/PD-L1 checkpoint therapy decreases tumor cell glycolysis and glucose consumption, thus increasing glucose availability to tumor infiltrating lymphocyte (TIL) and enabling them to fuel aerobic glycolysis and secrete IFN- γ [52–54,67]. **(C)** Based on their robust glycolytic potential and expression of the glucose transporter Glut1 [135], dual costimulated CD8 T cells should be competitive in the tumor microenvironment. **(D)** We propose that PD-1/PD-L1 blockade and dual costimulation will be therapeutically synergistic because they confer distinct, complimentary metabolic advantages to the CD8⁺ TIL. Specifically, dual costimulation would enhance the ability of TIL to absorb the glucose made available through disruption of the PD-1/PD-L1 axis. CTL: Cytotoxic T cell.

unknown, it is possible that by influencing inflammation costimulated T cells alter whole-body metabolism. Perhaps this might be best visualized in adipose tissue where costimulated T cells could receive IL-33R triggering followed by release of cytokines in a TCR-independent manner. Overall, much needs to be uncovered regarding cellular and whole-body metabolism to overcome hurdles posed on immunotherapeutic strategies.

Rational designing of combination therapies that incorporate dual costimulation

Although dual costimulation is itself a combination therapy, it should be possible to achieve even greater therapeutic benefit by further combining dual costimulation with other modalities that operate via complimentary mechanisms. For instance, certain checkpoint inhibitor plus costimulatory agonist combinations have already been shown to have synergistic efficacy [82–84].

As an example of how our mechanistic understanding of dual costimulation's tumoricidal actions might inform the rational design of effective combination therapies we hypothesize below how PD-1/PD-L1 checkpoint blockade and dual costimulation might synergize by providing complimentary metabolic benefits to T cells (Figure 2).

Conclusion

Tumors inflict a variety of metabolic stresses on infiltrating T cells, and thus effective immunotherapeutic strategies must incorporate means to restore proper metabolic function in these T cells to enable them to fully elaborate their tumoricidal potential. Indeed, some modalities such as PD-1/PD-L1 blockade work in part by enhancing glycolytic metabolism in tumor-infiltrating T cells. A better understanding of the metabolic interplay between tumor cells and T cells should, thus, facilitate new avenues for improving the efficacy of immunotherapies.

Executive summary

Tumors utilize multiple mechanisms to avoid immune elimination

- Tissue-specific Foxp3⁺ Tregs develop in the thymus that can suppress T cell mediated antitumor immunity.
- Tumor-specific effector cytolytic T cells (CTL) can be induced to become functionally tolerant by steady-state dendritic cells that cross-present tumor antigens.
- The tumor microenvironment contains multiple cell types (Tregs, tumor-associated macrophages and myeloid-derived suppressor cells) and cytokines (TGF- β , IL-10 and IL-35) that suppress infiltrating tumor-specific CTL (tumor-infiltrating lymphocyte [TIL]).
- Tumors adversely impact the function of TIL through metabolic effects that involve limiting the availability of glucose and essential amino acids.

Immunotherapies overcome tumor-induced immunosuppression

- Inhibitors (antagonists) of checkpoint receptors such as CTLA-4, PD-1 and LAG-3 elicit therapeutic tumor immunity by disrupting inhibitory signals in tumor-specific CTL and depleting intratumoral Tregs.
- Costimulatory agonists elicit therapeutic tumor immunity by boosting pathways that program T-cell expansion, migration and acquisition of tumoricidal effector functions.

Combining modalities can boost therapeutic response

- Combining modalities that target distinct tumor attributes can boost therapeutic efficacy.
- Effective combinations can involve immune-based plus 'nonimmune' modalities, a checkpoint inhibitor plus a costimulatory agonist or even two immune biologics within the same class that work via complimentary mechanisms.

CD134 plus CD137 dual costimulation elicits a multipronged antitumor therapeutic response

- Dual costimulation CD134 (OX40) plus CD137 (4-1BB) agonist combination immunotherapy favorably impacts the function of multiple immune cell types such as CD8 and CD4 T cells, NK cells and Tregs.
- Dual costimulation programs unusual but beneficial T-cell functions that include: first, CD4 T-cell cytolytic function; second, the ability of CD4 T cells to help CD8⁺ CTL via both antigen-linked and nonlinked pathways and third, the capacity of CD8⁺ CTL to be triggered through a T-cell receptor-independent mechanism.
- Dual costimulation programs CD8⁺ CTL to effectively compete for limiting glucose.

Rational designing of combination therapies that incorporate dual costimulation

- An example is illustrated whereby dual costimulation and PD-1/PD-L1 checkpoint blockade therapies might be effectively combined based on their potentially synergistic effects in providing distinct metabolic advantages to CD8⁺ TIL.

Future perspective

- Developing mechanistic understandings of how individual modalities work will aid in the selection of combinations that have the greatest potential for enhanced efficacy.
- Dual costimulation may serve as an effective foundation onto which other modalities can be added based on their potential to elicit complimentary therapeutic effects.

Future perspective

Given the plethora of T cell intrinsic and extrinsic pathways that impact antitumor activity and the corresponding modalities being developed to target these pathways, there will be many potential permutations of combination therapies that could be tested. As illustrated in the example outlined above for dual costimulation plus PD-1/PD-L1 checkpoint combination therapy (Figure 2), detailed mechanistic understandings of how individual modalities work will be instrumental in the selection of combinations that have the greatest potential for enhanced efficacy.

Dual costimulation is already capable of favorably altering the function of multiple cell types that impact tumor immunity (CD8 and CD4 T cells, Tregs, NK cells and dendritic cells), and programs unexpected functional properties in CD8 and CD4 T cells that

can counter immunosuppressive mechanisms that normally impede their antitumor activity. We propose that dual costimulation can thus serve as a foundation onto which other modalities can be added based on their potential to compliment these effects.

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