Published in final edited form as:

Curr Top Microbiol Immunol. 2019 ; 420: 175–210. doi:10.1007/82_2018_124.

Deciphering T Cell Immunometabolism with Activity-Based Protein Profiling

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Abstract

As a major sentinel of adaptive immunity, T cells seek and destroy diseased cells using antigen recognition to achieve molecular specificity. Strategies to block checkpoint inhibition of T cell activity and thus reawaken the patient's antitumor immune responses are rapidly becoming standard of care for treatment of diverse cancers. Adoptive transfer of patient T cells genetically engineered with tumor-targeting capabilities is redefining the field of personalized medicines. The diverse opportunities for exploiting T cell biology in the clinic have prompted new efforts to expand the scope of targets amenable to immuno-oncology. Given the complex spatiotemporal regulation of T cell function and fate, new technologies capable of global molecular profiling in vivo are needed to guide selection of appropriate T cell targets and subsets. In this chapter, we describe the use of activity-based protein profiling (ABPP) to illuminate different aspects of T cell metabolism and signaling as fertile starting points for investigation. We highlight the merits of

ABPP methods to enable target, inhibitor, and biochemical pathway discovery of T cells in the burgeoning field of immuno-oncology.

1 Introduction

1.1 Cellular Interactions that Regulate T Cell Responses

Thymus-educated lymphocytes (T cells) contribute substantially to our overall health: on the one hand, by protecting the body from pathogenic events including infections and cancer; while on the other hand helping potentiate inflammatory events and autoimmune diseases. T cells recognize their targets via their T cell receptors (TCRs), which are exquisitely sensitive and generally very specific for their ligands [a combination of a short 8-20aa peptides derived from protein proteolytic products presented by the major histocompatibility complex (MHC) Class I or Class II molecules at the cell surface; referred to as their “antigen”; Fig. 1]. Mature T cells encounter their ligands initially in secondary lymphoid tissues such as lymph nodes (LNs) and the spleen. “Priming,” or activation, of T cells is initiated by dendritic cells (DCs) that have acquired the proteins that T cells recognize from the extracellular environment. These proteins are acquired by phagocytosis of dying or excreted materials in the periphery (Yatim et al. 2017), which precedes DCs migration via the lymphatics to LN (Randolph et al. 2008). DCs proteolytically degrade proteins to peptides (Alloatti et al. 2016), which then bind to the MHC molecules within DC and are transported out to the surface of the DC where they can be surveilled by T cells (Fig. 1). For tumors, the antigens are commonly over-expressed self-proteins; proteins derived from the viruses that drive tumorigenesis; or “new” neo-antigens that arise due to mutations within the genome. Within the LN, DCs secrete chemokines that attract naïve T cells (Luster 2002), providing the opportunity for the T cells to survey the MHC–peptide complexes on the surface of the DC with their TCRs. Once activated, effector T cells undergo rounds of proliferation and exit the secondary lymphoid organ into the circulation. Trafficking T cells receive signals from chemokines and homing receptor ligands expressed on the surface of endothelial cells at sites of inflammation which cause them to adhere to the endothelial cells, and then extravasate from the vasculature into tissue (Jalkanen et al. 1986). Once in the peripheral tissue, chemokine gradients again cause T cells to move to infected or cancerous cells, where their TCR can be engaged by MHC–peptide complexes on the surface of the target cell. Engagement of the TCR can lead to a variety of functions directly performed by the T cell, including proliferation, production of cell–cell communication molecules called cytokines that can promote or limit inflammation, and the release of lytic granules that can kill target cells. These functions drive the ability of T cells to destroy or modify their target cells directly or cooperate, educate, and regulate other cells of the immune system, such as antibody-producing B cells and myeloid cells that engulf damaged and diseased tissues (Fig. 2). Most of the activated T cells die after a short period in the periphery once the infection and the derived antigen are removed, but some live on as long-lived memory T cells residing in the tissue, draining lymph nodes or circulating in the blood in a quiescent state, awaiting re-exposure to the antigen that originally promoted their expansion (Reading et al. 2018). These interactions underlie the ability of the immune system to counter pathogens and cancer and yet are also the underpinnings of many cardiovascular and neurological diseases,

autoimmunity, and transplant engraftment. Thus, understanding how T cell activation is regulated is anticipated to provide ample opportunities for therapeutic intervention.

In addition to TCR engagement, T cells receive signals from a variety of other receptors on their cell surface. Prominent among these are costimulatory molecules that are members of the immunoglobulin superfamily (IgSF), particularly CD28, and the tumor necrosis factor receptor superfamily (TNFRSF), which supports the cycling and survival of activated T cells without which nascent T cell responses abort. Further, cytokine receptors can have striking influence on T cell fate decisions with respect to both their differentiation into specialized subsets with discrete functions and their long-term survival. On the one hand, pro-inflammatory cytokines such as the type 1 interferon family members or IL-12 can promote the differentiation of helper T cell (CD4+) subsets (Hsieh et al. 1993) and effector function and terminal differentiation in cytotoxic (CD8+) T cells (Cui et al. 2009). Conversely, IL-10 and IL-21 can induce transcription factors that support the ability of T cells to differentiate into long-lived memory T cells (Cui et al. 2011).

1.2 Cell Signaling in T Cell Responses

The initial signals from the TCR are mediated by tyrosine kinases that ultimately activate a collection of serine–threonine kinases that are intrinsically involved in activating/sustaining activities needed for the proliferation and survival of the expanding T cell population (Brownlie and Zamoyska 2013). Like most signaling networks, second messengers play a prominent role in the translation of ligand binding to functional outcomes. In the case of T cells, TCR-mediated activation of the phospholipase C- γ results in the production of inositol-(1,4,5)-trisphosphate (IP3) and diacylglycerols (DAGs). IP3 leads to the activation of protein kinase B/Akt via activating PDK1 (Rosse et al. 2010). Akt phosphorylates many members of the FoxO transcription factor family, leading to their exclusion from the nucleus (Eijkelenboom and Burgering 2013). FoxO transcription factors regulate the expression of cytokine receptors that contribute to T cell survival and, via regulating KLF2, influence the expression of homing receptors and chemokine receptors that direct the trafficking of activating T cells (Kerdiles et al. 2009). Akt additionally regulates the expression of cytolytic effector molecules in cytotoxic T cells and cytokine receptors such as IL12R β (Macintyre et al. 2011), which supports the expression of T-bet and Eomesodermin, transcription factors that engrain effector differentiation. DAG can bind and activate many members of the protein kinase C (PKC) serine–threonine kinase family (Quann et al. 2011), guanine nucleotide exchange factors [RasGRPs (Jun et al. 2013)] and subsequently Erk1 and Erk2 via the mitogen-activated protein kinase (MAPK) pathway (Fischer et al. 2005), and protein kinase D (PKD) members (Spitaler et al. 2006), with ensuing impact on the formation of the immunological synapse that connects T cells to antigen-presenting cells, cell motility, and cytokine production.

1.3 Cell Metabolism in T Cell Responses

The tremendous expansion in T cells after TCR engagement requires substantial alterations to their metabolism to support proliferation and differentiation. This involves the increase in oxidative phosphorylation (OXPHOS) and the induction of glycolysis. Glycolysis is activated by either AKT [in helper T cells (Rathmell et al. 2003)] or Erk-mediated regulation

of c-Myc (cytotoxic T cells) (Wang et al. 2011). mTORC1, the target of the immunosuppressant rapamycin, is critical kinase involved in metabolic regulation via its regulation of p70-S6 kinase and eIF4E-binding protein (contributing to protein transcription and translation) and the SREBP fatty acid synthesis regulators (Zeng and Chi 2017). Furthermore, mTORC1 links to the HIF-1 transcription factor which has a major role in promoting the sustained expression of glucose transporters and the major enzymes of the glycolytic pathway and concomitantly to many genes that are reflected in the “effector” activities of T cells [such as the expression of lytic granules and cytokines (Finlay et al. 2012)]. Conversely, nutrient-sensing serine–threonine kinases, exemplified by the AMP-activated kinase $\alpha 1$ (AMPK $\alpha 1$) which works with liver kinase B1 (LKB1) to sense the availability of nutrients within the T cell, can attenuate mTORC1 and arrest glycolysis once nutrients and ATP become limiting; this allows OXPHOS to become more prominent and elicits a greater use of fatty acids rather than glucose as the T cells transition into a more quiescent state (Tamas et al. 2006, 2010). Thus, glycolysis is commonly linked to the effector functions of T cells, and its induction and sustained activation are regulated by kinases at critical points.

1.4 Fate Decisions in T Cells

As mentioned, once actively proliferating, T cells can be polarized into different effector subsets (Th1, Th2, etc.) that secrete different patterns of cytokines. Further, the capacity for T cells to become long-lived memory cells resides within this primary T cell response, as a subpopulation either that has not received signals that drive to terminal effector differentiation or has received signals that preserve homeostatic functions. These fate decisions are commonly influenced by the amount of antigen and cytokines (interferons; interleukins; colony-stimulating factors) present during the early expansion phases of T cells, and the signals transmitted by their respective receptors (type I and type II) induce chromatin remodeling that durably influences the gene sets transcribed in these subpopulations. Many of the influential cytokine receptors are linked to Janus kinase (JAK) non-receptor tyrosine kinases that auto-phosphorylate and control the STAT family of transcription factors, which in turn promote the expression of genes as homo- or hetero-dimers binding to their target promoters (Yamaoka et al. 2004). For example, interleukin-2, a critical T cell growth factor, binds to the IL-2 receptor complex, activating JAK1 and JAK3, with the ensuing phosphorylation of STAT5A and STAT5B promoting the expression of the IL-4 receptor, which in turn promotes TH2 subset differentiation. However, these signaling pathways are not generally linear, as IL-2 is also a key determinant in the differentiation of immunosuppressive regulatory T cells (Treg). Similarly, IL-12, which activates Jak1 and Tyk2 and STAT4, drives TH1 differentiation and substantially promotes the differentiation of T cells into terminally differentiated effectors by enhancing T-bet expression. Conversely, IL-23 and IL-10, via STAT3, promote memory T cell differentiation at the expense of effectors. How these different outcomes are controlled, from the same receptor kinase–transcription factor complexes, is an area of intense focus, being partially revealed by phosphoproteomic studies (Ross et al. 2016; Hukelmann et al. 2016), and likely involves unequal activity of STATs (and thus JAKs) and feedback loops between STATs in transcriptional regulation (Hirahara et al. 2015). The contribution of this family of kinases to these fate decisions provides substantial opportunity for therapeutic intervention, potentially

ameliorating autoimmune disease by inflating Treg or polarizing vaccine-induced T cell responses to subtypes that are most effective with respect to the purpose of the vaccine. Given that JAKs can also influence chromatin structure, the development of assays that interrogate, and potentially inhibit, the function of these kinases during T cell responses will likely empower much greater understanding of how a relatively constrained family of kinases may drive such complex outcomes.

2 Features of Dysfunctional T Cells

The immune system is a powerful defense against pathogens and tumors, but, as evidenced by autoimmune diseases, toxic shock syndromes and other chronic inflammatory diseases can be deleterious to the host if left unchecked. Consequentially, a multitude of negative feedback mechanisms has evolved to limit immune cell function. This is particularly apparent for T cells in cases of chronic exposure to antigen, such as unresolved pathogen infections (e.g., HIV; HCV; malaria), and these mechanisms for restraining T cell responses are commonly hijacked by tumors.

2.1 Anergy/Tolerance

The dendritic cells that acquire antigen from infected tissues also engulf self-antigens from dying cells. As inflammation from infection is an important element in activating DC to drive T cell responses to a pathogen, it is important that T cells do not also respond to the self-antigens. The majority of self-reactive T cells are culled in the thymus by the processes of negative selection. However, this process is not absolute, and many proteins are expressed late in development and thus avoid “central” tolerance. In the periphery, self-tolerance can be maintained by unactivated DC presenting self-antigen, derived from cells continually undergoing turnover, to T cells in the absence of the costimulatory molecules that are induced by inflammation. Under these circumstances, T cells undergo abortive proliferation and enter a state of anergy, whereby their response to re-exposure to the same antigen is blunted. Anergy is regulated by both ubiquitin ligases and transcription factors that limit the expression of genes that would normally promote T cell expansion and differentiation. Recently, it has become apparent that T cell responses to tumor antigens can be similarly blunted. Although tumors commonly express neo-antigens that would not be the subject of central tolerance, the limited amount of inflammation within the tumor microenvironment can limit DC activation, leading to presentation of antigen in the absence of costimulation.

2.2 Dysfunction/Exhaustion

In many cases of chronic antigen exposure, T cells differentiate but become unresponsive to further stimulation, a situation referred to as exhaustion. This state of exhaustion reflects intrinsic mechanisms of limiting responsiveness to further TCR engagement. T cell receptor activation of tyrosine kinases is highly regulated, and effective signal transduction requires sustained stimulation due to the constitutive activation of protein tyrosine phosphatases (PTPs) that counteract kinase activity. It has been shown in tumor-infiltrating lymphocytes (TILs) that TCR stimulation can be attenuated downstream of antigen engagement (Koneru et al. 2005) by the recruitment of src homology region 2 domain-containing phosphatase (SHP)-1 to the immunological synapse where the T cell engages the target cell (Monu and

Frey 2007). SHP-1 limits further signaling, Ca^{2+} flux, and integrin-mediated binding. This is an active inhibition as removal of TILs from the tumor environment, and brief in vitro culture can restore function. Additionally, after engagement of the TCR, inhibitory molecules are expressed that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and are subsequently phosphorylated on their ITIMs, recruiting and activating PTP such as SHP-1 and SHP-2 (Pauls and Marshall 2017; Srivastava et al. 2013). Activated PTP in turn dephosphorylates proximal substrates: These range from kinases to antigen receptors to adaptor molecules. Supporting this notion, polymorphisms in PTPN22 have been shown to promote autoimmune diseases and anticancer responses due to an inability to constrain TCR signaling (Salmond et al. 2015; Brownlie et al. 2017), and short hairpin RNA (shRNA) screens in vivo identified Ppp2r2d, a regulatory subunit of the PP2A phosphatase, as limiting T cell function in the tumor microenvironment (Zhou et al. 2014). Among the inhibitory molecules that recruit PTPs are those being clinically evaluated with considerable success in immuno-oncology: PD1; CTLA-4; LAG3; TIGIT; TIM3 (Vazquez-Cintron et al. 2010). In addition to SHP recruitment to TCR, signaling can be attenuated by recruiting suppressors of cytokine signaling (SOCS) family member cytokine-inducible SH2-containing protein (CISH), which normally blocks STAT5 activation to support the degradation of PLC- γ 1 (Palmer et al. 2015). Interestingly, RNAi or CRISPR/Cas9 screens have also implicated kinases in tumor cells for their ability to indirectly impede antitumor immunity by desensitizing responsiveness to cytokine signaling (Patel et al. 2017; Manguso et al. 2017).

2.3 Extrinsic Regulation of T Cell Metabolism

A third approach to limiting T cell function in the tumor microenvironment has recently become clear. Tumors can be considered “competitors” for immune cells for the resources that both need to maintain function. For example, studies have implicated the consumption of glucose by tumors (commonly referred to as Warburg metabolism as glycolysis is used even though oxygen may not be limiting) as a mechanism by which T cells can lose their function, given the importance of glycolysis to T cell effector activity (Chang et al. 2013, 2015a). Other essential metabolites have been demonstrated to be critical T cell function, and their availability is attenuated in the TME. The amino acids arginine and tryptophan are actively depleted by myeloid cells expressing arginase-1 [Arg 1 (Vasquez-Dunddel et al. 2013)] and a variety of cells expressing indoleamine 2,3-dioxygenase 1 [IDO-1 (Balachandran et al. 2011; Liu et al. 2010)], respectively. Interestingly, IDO-1 was originally identified as a mechanism of maintaining maternal-fetal tolerance (Munn et al. 1998). The availability of serine has also shown to be critical for T cell effector function (Ma et al. 2017). Thus, these “extrinsic” regulators of T cell metabolism are also current targets for cancer immunotherapy.

2.4 New Opportunities for ABPP Discovery in T Cell Biology

Multiple tyrosine and serine–threonine kinase pathways regulate T cell responses from inception to termination. Conversely, tyrosine phosphatases and ubiquitin ligases play critical roles in attenuating signals that emanate from the TCR and cytokine receptors. Thus, considerable opportunities exist to regulate fate decisions and metabolic activity of T cells, as evidenced by the clinical implementation of rapamycin, the inhibitor of mTOR, and JAK inhibitors. The challenge at hand is that many of these enzymes are active in many different

pathways in many normal cells, so selecting the most appropriate target for the physiological condition is critical to prevent on-target toxicities. Further identifying inhibitors that are truly specific for each of the wide variety of isoforms of different enzymes will be a major thrust of future work. In Sect. 3, we describe examples of using ABPP methods to probe enzymes and pathways that have substantial influence on T cell function.

3 Functional Insights into T Cell Biology Using ABPP Methods

3.1 ABPP Technology

Activity-based protein profiling (ABPP) is a global proteomic method for measuring functional state of enzymes directly in native biological systems (Cravatt et al. 2008; Niphakis and Cravatt 2014). An activity-based probe contains at least two key features: (1) a reactive group that binds and covalently modifies active sites of a large number of enzymes, which share conserved mechanistic and/or structural features and (2) a reporter tag (e.g., fluorophore or biotin) to enable detection, enrichment, and identification of probe-labeled enzyme targets by gel electrophoresis (Jessani et al. 2002) (in-gel analysis) and liquid chromatography–mass spectrometry (LC-MS) (Jessani et al. 2005; Adibekian et al. 2011; Inloes et al. 2014) (LC-MS, Fig. 3a). ABPP probes have been generated for more than a dozen enzymes classes including serine hydrolases (SHs) (Liu et al. 1999), kinases (Patricelli et al. 2011), glycosidases (Vocadlo and Bertozzi 2004; Hekmat et al. 2005), oxidoreductases (Wright and Cravatt 2007; Speers and Cravatt 2005), and cysteine proteases (Greenbaum et al. 2000). ABPP probes label active enzymes but not their inactive forms, enabling detection of changes in enzyme activity independent of alterations in gene or protein expression (Jessani et al. 2002, 2004).

ABPP methods can be configured for inhibitor discovery using a competitive format (Fig. 3b). The potency and selectivity of small molecules can be profiled against many enzymes in parallel by performing competitive ABPP in complex proteomes, where inhibitors compete probe labeling of enzyme targets. Competitive ABPP offers several advantages over conventional substrate assays. First, enzymes can be tested in native proteomes without the need for recombinant protein expression and purification (Adibekian et al. 2011; Long et al. 2009; Hsu et al. 2013a; Inloes et al. 2014; Chang et al. 2015b; Nagano et al. 2013). Second, inhibitors can be developed for uncharacterized enzymes that lack any known substrates (Adibekian et al. 2011; Hsu et al. 2013b; Inloes et al. 2014). Since competitive ABPP is compatible with any enzyme, regardless of its degree of functional annotation, optimized inhibitors can be developed for enzymes that are biologically characterized but devoid of inhibitors, as well as orphan enzymes. In most cases, the compounds developed from these medicinal chemistry programs represent the first pharmacological probes for studying the function of their respective enzyme targets in vivo. Finally, inhibitors are evaluated against many enzymes in parallel, enabling identification of selective agents from non-specific compounds (Bachovchin et al. 2010; Adibekian et al. 2011; Hsu et al. 2013a, b) (Fig. 3b).

3.2 ABPP Discovery of Dimethyl Fumarate Mode of Action in T Cells

ABPP has been used to determine mode of action of immunosuppressive drugs to reveal protein targets important for T cell activation. A recent study used isotopic tandem

orthogonal proteolysis-activity-based protein profiling [isoTOP-ABPP (Weerapana et al. 2010; Wang et al. 2014)] to identify protein targets and site of binding for dimethyl fumarate (DMF) in T cells (Blewett et al. 2016). DMF is an FDA-approved immunosuppressive drug used to treat autoimmune disorders including psoriasis (Mrowietz et al. 2007) and multiple sclerosis (MS) (Linker and Gold 2013). DMF exhibits both neuroprotective and immunomodulatory activities through a poorly understood mechanism of action (Scannevin et al. 2012; Albrecht et al. 2012; Gillard et al. 2015). DMF contains a Michael acceptor functional group that mediates covalent reaction with cysteine residues in proteins (Fig. 4). Based on its cysteine reactivity, several pathways have been proposed to explain DMF activity including Nrf-KEAP1 complex dissociation through cysteine modifications (Taguchi et al. 2011; Scannevin et al. 2012) or perturbation of redox hemostasis by changing cellular GSH concentrations (Mrowietz and Asadullah 2005; Lehmann et al. 2007; Lin et al. 2011; Albrecht et al. 2012). DMF is also proposed to function as a prodrug that is metabolized to monomethyl fumarate (MMF, Fig. 4), which acts as a ligand for the G protein-coupled receptor, hydroxycarboxylic acid receptor 2 (HCAR2) (Chen et al. 2014).

To determine DMF mode of action, the Cravatt Lab applied isoTOP-ABPP (Weerapana et al. 2010; Wang et al. 2014) for global cysteine reactivity profiling to identify and quantify DMF-sensitive cysteine residues in primary human and mouse T cell proteomes (Blewett et al. 2016). In this study, the general cysteine-reactive probe iodoacetamide (IA)-alkyne was used to evaluate the reactivity of DMF across >2400 cysteine residues in a competitive ABPP experiment. Sensitivity of probe-modified sites to DMF treatment was compared with the non-electrophilic analogs MMF and DMS (Fig. 4) to determine DMF-specific covalent reactions with T cell protein targets. The results revealed that DMF, but not MMF or DMS, inhibited T cell activation at an early stage, and the observed cell biology was independent of Nrf2 activation or GSH depletion. The unbiased nature of the isoTOP-ABPP approach revealed that DMF mode of action is via covalent modification of several proteins with known biochemical or genetic links to T cell function including protein kinase C theta (PKC θ). DMF blocked association of PKC θ with the costimulatory receptor CD28 by perturbing a cysteine site in the C2 domain of this kinase (Blewett et al. 2016).

3.3 ABPP Discovery of New Metabolic Regulators of T Cell Proliferation

In contrast to the DMF example above, ABPP can also facilitate studies of protein families without established biology in T cells. Here, we highlight an example of using ABPP methods to discover a new serine hydrolase (SH) target involved in T cell proliferation (Adibekian et al. 2011). SHs represent a large and diverse enzyme class that includes ~200 enzymes or 1% of the genes in the human proteome (Simon and Cravatt 2010; Long and Cravatt 2011). The defining feature of these enzymes is the activated serine nucleophile, as part of a catalytic triad, in their active sites to hydrolyze ester, thioester, or amide bonds in a wide range of substrates including small molecules, peptides, and proteins (Simon and Cravatt 2010; Long and Cravatt 2011). The important biological role of SHs has been exemplified by several clinical drugs targeting individual SHs to treat human diseases including obesity (Henness and Perry 2006), diabetes (Thornberry and Weber 2007), Alzheimer's disease (Birks et al. 2009), and microbial infections (Kluge and Petter 2010). Despite the disease relevance and the successful bench-to-clinic translations, the majority of

the metabolic SHs remain poorly characterized (Simon and Cravatt 2010; Long and Cravatt 2011). In recent years, chemical proteomic methods such as ABPP have emerged as a powerful tool to study these unexplored enzymes.

A case in point is a recent study that identified 1,2,3-triazole ureas (1,2,3-TUs, Fig. 5) as an exceptional chemotype for SH inhibition that offers broad activity against the SH class with tunable selectivity for individual enzymes (Adibekian et al. 2011; Hsu et al. 2012, 2013a, b; Yun et al. 2014; Ogasawara et al. 2016; Shin et al. 2018b). Reporter-tagged fluorophosphonates (FPs) were used as general SH activity-based probes for these studies because these probes detect a large fraction of mammalian SHs with negligible cross-reactivity against other enzyme classes that contain nucleophilic residues (Bachovchin et al. 2010; Jessani et al. 2005; Okerberg et al. 2005). Competitive ABPP using FP probes was used to discover a broad-spectrum 1,2,3-TU inhibitor AA26-9, which showed inhibitory activity against approximately 1/3 of the 40+ SHs detected in immortalized T cell lines. AA26-9 targets included serine peptidases, lipases, amidases, esterases, and thioesterases, which suggests that 1,2,3-TUs represent a versatile starting point for developing inhibitors to probe T cell biology.

Next, a library of 1,2,3-TUs based on AA26-9 was expanded using efficient and accessible click chemistry and screened for novel SH inhibitors. Among those analogs, three inhibitors AA74-1, AA39-2, and AA44-2 stood out with remarkable potency and specificity for their respective SH targets in mouse T cell proteomes (Fig. 5). All three compounds exhibited potent inhibitory activity and high selectivity in live T cells; AA74-1 and AA44-2 blocked more than 95% of acyl peptide hydrolase (APEH) and α,β -hydrolase-11 (ABHD11) activities, respectively, without affecting any of the other 40+ SHs in T cells. AA39-2 displayed similar inhibitory activity against PAFAH2 in T cells and cross-reacted with a single SH off-target, α,β -hydrolase-6 (ABHD6). Furthermore, treatment with AA74-1 resulted in near-complete blockade of APEH activity in brain and heart at doses as low as 0.4 mg kg⁻¹ and exhibited high selectivity against the 44 SHs detected in the mouse brain. Treatment with AA74-1 resulted in accumulation of a panel of N-acetylated proteins important for T cell proliferation, which helped annotate the substrate specificity of APEH and positions this enzyme as a promising T cell metabolic target.

3.4 ABPP Discovery of Proteasome Regulation of T Cell Fate

ABPP can also be used to elucidate underlying mechanisms that control T cell lineage fate. It has been recognized that T cells undergo polarized reorganization of proteins upon activation and this polarization may persist through cell division (Chang et al. 2007; Yeh et al. 2008). Therefore, asymmetric cell division has been suggested in T cells, which enables a single-parent T cell to produce two daughter cells that have distinct fates toward the effector and memory lineages (Chang et al. 2007). It remains poorly understood, however, what critical proteins undergo polarized segregation and unequal partition into progenies for a selected T cell type and what are the underlying mechanisms.

A recent study provided evidence of a lineage-determining transcription factor, T-bet, that was unequally partitioned between the two daughter cells in activated naïve T cells undergoing division (Chang et al. 2011; Lin et al. 2015). This disparity of T-bet is thought to

result from asymmetric destruction during mitosis in the setting of asymmetric distribution of the proteasome, the protein degradation machinery. The progeny that received less proteasome acquired more T-bet, and inhibition of polarized segregation of the proteasome or proteasome-dependent degradation prevented the asymmetric partitioning of T-bet, suggesting that a cell may unevenly deploy cellular activities during mitosis so as to pass a different abundance of cell fate regulators to daughter cells.

To examine whether asymmetric localization of the proteasome is associated with differential rates of T-bet degradation within a mitotic cell, the activity-based probe, MVB003, was used to probe the degradative activity of the proteasome (Fig. 6). The MVB003 probe was designed based on the natural product epoxomicin, which potently inhibits the 20S proteasome via covalent reaction of the amino-terminal threonine with the epoxyketone electrophile (Groll et al. 2000; Meng et al. 1999; Sin et al. 1999). The addition of a BODIPY fluorescent tag converts epoxomicin into a probe for activity-based profiling of the proteasome (Li et al. 2013) (Fig. 6). The authors discovered using MVB003 and immunofluorescence microscopy that activated T cells exhibited unequal proteasome activity within mitotic T cells. Costaining experiments with activated CD8⁺ T lymphocytes indicated that a greater share of T-bet was partitioned into the daughter cell that received less proteasome. The same asymmetric partitioning of T-bet and proteasome was also observed in activated CD4⁺ T lymphocytes.

The findings in this study suggest that both CD8⁺ and CD4⁺ daughter T cells display differential T-bet abundance, which will influence their subsequent fates. By a similar mechanism of distinct localization of the degradation machinery, other biomolecules such as transcription factors and cell proliferation regulators could be inherited by daughter cells with disparity because of asymmetric distribution of the proteasome. The full landscape of the disparities caused by unequal segregation of the proteasome remains to be extensively explored. In this regard, ABPP probes capable of measuring PI3K and mTOR activity [e.g., kinase activity-based probes (Patricelli et al. 2007, 2011; Shin et al. 2018a)] may help further elucidate out how unequal transduction of PI3K/AKT/mTOR signaling during cell division bifurcates transcriptional networks and fates of effector T cells (Lin et al. 2015).

4 Using ABPP to Probe Immunometabolism in the Tumor Microenvironment

The field of cancer immunotherapy has seen great excitement recently, primarily due to the success of immune checkpoint inhibitors, cell therapy with tumor-infiltrating lymphocytes, and chimeric antigen receptor T cell (CAR-T) therapy. As therapies for oncology continue to witness a paradigm shift, small molecules or biological agents that target clinically under-explored immunomodulatory proteins are being actively pursued for potential therapeutic intervention. In the subsequent sections, we focus on metabolic pathways operating in the tumor microenvironment that restrain antitumor immunity via extrinsic and intrinsic mechanisms. Tumors are a metabolically demanding environment that can perturb metabolism and function of infiltrating T cells through competition for substrates required to fuel optimal antitumor effector functions (Buck et al. 2017; Chang and Pearce 2016; Ho et

al. 2015; Nakaya et al. 2014). Metabolites produced from these pathways not only provide energy and building blocks for growth, but also serve as cellular signals to regulate transcriptional programs that tune T cell function and fate. Thus, defining the metabolic pathways and enzymes differentially utilized by cancer cells and tumor-specific T cells could uncover new “checkpoints” for immuno-oncology. ABPP can facilitate these efforts because metabolism is regulated by enzyme chemistry that is highly amenable to activity-based profiling methods.

4.1 Diacylglycerol Signaling as an Intrinsic Regulator of TCR Activation

Studies have demonstrated that the balance between T cell activation and anergy (explained in Sect. 2.1) can be controlled by activity of DGKs. There is compelling evidence in the literature showing that DGKs are key regulators of T cell effector function (Mérida et al. 2015; Riese et al. 2011) due at least in part to regulation of Ras signaling. DGK α expression increases in the absence of costimulation and is high in anergic T cells, and ectopic DGK α overexpression causes an anergic state. Conversely, anergy can be relieved either by ectopic overexpression of a dominant negative DGK α mutant or by pharmacological inhibition of DGK α (Zha et al. 2006; Olenchock et al. 2006). Moreover, T cells from DGK α knockout mice are resistant to induction of anergy and are hyper-responsive to immune stimulation. Blocking the activity of DGK α increases Ras activation in T cells, as measured by GTP loading; overexpression of RasGRP1 or dominant negative Ras overcomes anergy. An alternative isoform, DGK ζ , has also been implicated in restraining effector T cell function directly (Riese et al. 2011), or by promoting Treg development (Joshi et al. 2013), offering an additional target.

4.1.1 Diacylglycerol Kinase Family and Biochemistry—Diacylglycerol kinases (DGKs) are a family of lipid kinases that regulate cell biology through ATP-dependent phosphorylation of diacylglycerol (DAG) to form phosphatidic acid (PA, Fig. 7). Humans express 10 DGK isoforms that are further classified into five subtypes based on the number and type of regulatory domains (Mérida et al. 2008; Sakane et al. 2016; Shulga et al. 2011). All DGKs contain at least two zinc finger domains (C1A and C1B, Fig. 8), which have been shown in DGK γ and β to respond to phorbol ester (DAG mimetics) stimulation (Colón-González and Kazanietz 2006). The role of C1 domains in other DGK isoforms remains poorly understood with respect to catalytic and signaling functions (Shindo et al. 2003).

Type 1 DGKs (α , β , γ) contain a lipid kinase domain split into catalytic (DAGKc) and accessory (DAGKa) regions, a pair of EF-hands, a recoverin homology domain (RVH), and the C1A/B domains (Fig. 8). The EF-hands bind Ca^{2+} and have been shown to activate kinase activity, but various mechanisms of Ca^{2+} -independent activation of DGKs have been demonstrated including lipid binding at the C1 domains (Fanani et al. 2004). Type 1 DGK C1s are classified as typical (DGK β and DGK γ) or atypical (DGK α) based on protein sequence homology with prototypical C1s found in the protein kinase C (PKC) family of Ser/Thr kinases (Merino et al. 2007). DGK α is highly expressed in T cells, DGK β in the caudate nucleus, and DGK γ in cerebellum according to bio-GPS global mRNA expression analysis (Wu et al. 2016).

The type 2 DGKs (δ , η , κ) have the same C1A/B pair, but the DAGKc and DAGKa regions are separated by a short peptide linker of unknown function. They are grouped based on the presence of a pleckstrin homology (PH) domain which bind phosphoinositides (PIP3) and localize proteins to membrane (Hurley and Misra 2000) (Fig. 8). DGK δ also contains a sterile alpha motif (SAM) domain allowing for interactions with other SAM domain-containing proteins (Stapleton et al. 1999). DGK κ has a region of 33 repeats of the amino acid sequence Glu-Pro-Ala-Pro referred to as an EPAP domain of poorly defined function (Shulga et al. 2011). DGK η lacks both the SAM and EPAP domains. DGK δ is highly expressed in T cells as well as B cells and natural killer (NK) cells. DGK κ and DGK η are expressed at low levels in cardiac myocytes.

DGK ϵ is the only type 3 DGK and the smallest of the human DGKs. DGK ϵ contains a combined DAGKc/DAGKa domain, C1A/B, and a hydrophobic domain (HD, Fig. 8). The HD domain at the N terminus is proposed to localize DGK ϵ to the inner membrane of the cell (Jennings et al. 2015). While other DGKs are expressed as soluble proteins that can associate with membranes, DGK ϵ is expressed exclusively in the membrane fraction (Franks et al. 2017). DGK ϵ is highly expressed in the cervical cortical ganglion and the only DGK to show substrate specificity for DAGs that contain arachidonic acid chains (Lung et al. 2009).

Type 4 DGKs (ζ , ι) contain the combined catalytic DAGKc/DAGKa domain, C1A/B, a myristoylated alanine-rich C kinase substrate (MARCKS) domain, a series of four ankyrin repeats, and a PDZ protein–protein interaction domain (Fig. 8). The MARCKS domain can be phosphorylated by PKC, and this domain in DGK ζ also contains a nuclear localization sequence that may facilitate nuclear localization (Aderem 1992). The ankyrin repeats and the PDZ domain mediate protein–protein interactions and are often associated with proteins that function as scaffolds in signaling cascades (Mosavi et al. 2009; Lee and Zheng 2010). DGK ζ is highly expressed in T cells, NK cells, CD33+ myeloid cells, prefrontal cortex, and the amygdala. DGK ι is highly expressed in skeletal muscle, medulla oblongata, and the superior cervical ganglion.

DGK θ is the only member of the type 5 subtype with a combined catalytic DAGKc/DAGKa domain, three C1 domains (C1A/C1B/C1C), PH domain, and a proline-rich domain (Fig. 8). Proline-rich regions can frequently bind proteins containing Src homology 3 domains, but it is unclear whether this domain in DGK θ is mediating protein–protein interactions (Tu-Sekine et al. 2016). DGK θ is expressed ubiquitously throughout normal human tissues.

4.1.2 ABPP Discovery of Novel Druggable Sites of DGKs—Development of DGK inhibitors has been hampered by the lack of crystal structures, chemical scaffolds for medicinal chemistry, and functional assays suited for measuring DGK activity in vitro and in vivo. Recent efforts using ABPP methods are revealing new features of the DGK active site that can be leveraged for development of highly potent and selective inhibitors. Functional insights into ligand binding in DGK active sites can also clarify how DGKs mediate substrate recognition and specificity, which will be critical to elucidate isoform-specific biology in vivo.

To expand knowledge of molecular recognition of DGK active sites, the Hsu laboratory established a chemoproteomic strategy using kinase ATP acyl phosphate activity-based probes to map substrate and inhibitor binding sites of all five DGK subtypes (Franks et al. 2017; McCloud et al. 2018) (Fig. 9). Identification of probe-modified, active-site peptides by quantitative LC-MS indicated that the DAGKc and DAGKa domains are important components of the DGK active-site in complex proteomes (Franks et al. 2017). Competition with free ATP resulted in near-complete blockade of probe binding at both DAGKc and DAGKa domains, designating DAGKc/DAGKa as a primary ATP binding site (Franks et al. 2017). A surprising result from these ABPP studies was discovery of a probe-modified, active-site peptide derived from the atypical C1A domain of DGK α . Competitive ABPP LC-MS using a non-selective DGK inhibitor (ritanserin, inset box, Fig. 9) confirmed that C1A and DAGKa were principal ligand-binding sites for ritanserin and fragments derived from this compound (Franks et al. 2017; McCloud et al. 2018). These findings raised the intriguing question of whether C1A and DAGKc/DAGKa were independent ligand-binding sites or if they interact to form a single contiguous binding site. Competitive ABPP LC-MS using a non-selective DGK fragment inhibitor (RLM001) showed equipotent competition at all three binding sites, providing evidence in support of the C1A, DAGKc, and DAGKa forming a single contiguous binding site (McCloud et al. 2018).

4.1.3 ABPP Strategies for Discovery of DGK Inhibitors—While high-throughput screening (HTS) has been pursued for developing DGK α inhibitors (Liu et al. 2016), one of the challenges facing DGK inhibitor development is optimization of selectivity against the 500+ human kinases that also utilize ATP as a common substrate. An alternative to HTS is the use of fragment-based approaches (Erlanson et al. 2016) for development of DGK inhibitors (Fig. 9). The power of fragment-based lead discovery includes identification of fragment compounds (<300 Da) that possess ligand-efficient interactions with protein binding sites that can produce highly selective compounds (Kuntz et al. 1999). A fragment-based approach for developing DGK inhibitors was first pursued by deconstruction of ritanserin into a hydrophobic fragment (RF001) and a thiazolopyrimidinone (RLM001) (Franks et al. 2017; McCloud et al. 2018). RF001 was discovered to be more selective than ritanserin, which helped identify a minimal pharmacophore for selective DGK α inactivation (McCloud et al. 2018). In contrast, another ritanserin-derived fragment, RLM001, which contained nucleotide-like structural features, functioned as a general binding element to kinase ATP pockets and helps explain why the parent molecule ritanserin shows non-specific binding across the kinome (McCloud et al. 2018). Given the ability of competitive ABPP to rapidly identify fragment leads (Backus et al. 2016; Hacker et al. 2017; Parker et al. 2017), future efforts will benefit from identification of new DGK fragment binders that can be optimized along with RF001 by linking, merging, and/or elaboration strategies to develop potent isoform-selective DGK inhibitors (Erlanson et al. 2016).

4.2 Extrinsic Regulators of T Cell Metabolism

4.2.1 Metabolic Checkpoints in Glycolysis—Activated T cells also undergo a metabolic switch akin to tumor cells and upregulate aerobic glycolysis to allow differentiation into functional effector T cells. In addition to supplying energy and building blocks, the glycolytic metabolite phosphoenolpyruvate (PEP) is required for sustaining

TCR-mediated Ca^{2+} nuclear factor of activated T cells (NFAT) signaling and effector functions by suppressing sarco/ER Ca^{2+} -ATPase (SERCA) activity (Ho et al. 2015). A key glycolytic enzyme that regulates PEP production is the enolase family (EC 4.2.1.11), which comprises a family of metal ion-activated enzymes that catalyze the penultimate step in glycolysis, which is the conversion of 2-phospho-D-glycerate (2PG) to PEP (Liu et al. 2000) (Scheme 1). Humans express three isoforms of enolase, namely non-neuronal enolase (ENO1 or α -ENO) that is expressed in almost all tissues, neuronal enolase (ENO2 or γ -ENO) that is expressed in neuron and neuroendocrine tissues, and muscle-specific enolase (ENO3 or β -ENO) that is predominantly expressed in muscle tissues (Lohman and Meyerhof 1934). Enolases are expressed as homo- or hetero-dimers of α -, β -, or γ -subunits with molecular weights between 82 and 100 kDa (Capello et al. 2011).

Interestingly, ENO1 is also expressed as a shorter isoform (myc-binding protein-1, MBP-1), which localizes to the nucleus and functions as a transcription factor that regulates gene expression of c-Myc (Subramanian and Miller 2000) and FOXP3 (De Rosa et al. 2015). MBP-1 binds to the c-Myc promoter and down-regulates expression of this proto-oncogene (Subramanian and Miller 2000). In contrast, binding of MBP-1 (and potentially other ENO1 isoforms) is required for FOXP3 and specifically the FOXP3-E2 splice variant, expression that drives generation and immunosuppressive activity of human regulatory T cells (Treg cells) (De Rosa et al. 2015). Thus, enolase has adopted both catalytic (glycolysis) and non-catalytic (DNA-binding) functions that will be difficult to distinguish using standard biochemical techniques. Here, the development of activity-based probes that report on ENO1 activity may permit monitoring of active enolase pools in T cell subsets and tumor cells, and guide appropriate strategies to perturb the multifunctional features of this protein. For example, enolase activity-based probes may identify the appropriate ENO1 isoform to selectively inactivate immunosuppressive Tregs in tumors as a potential immunotherapy strategy.

Several enolase inhibitors have been reported (Fig. 10) and serve as starting points for development of enolase activity-based probes. Classical ENO1 inhibitors were designed largely to mimic either the 2PG substrate or its transition state in the dehydration mechanism (Scheme 1). For example, phosphonoacetohydroxamate (PhAH; $K_i = 15 \text{ pM}$) is the most potent ENO1 inhibitor known to date (Anderson et al. 1984), and it is thought to mimic the acid-carboxylate form of the intermediate carbanion in the dehydration reaction. Close analogs of PhAH, (3-hydroxy-2-nitropropyl)phosphonate ($K_i = 6 \text{ nM}$) and (2-phosphonoethyl)nitrolic acid ($K_i = 14 \text{ nM}$), were studied biochemically but not in the context of therapeutic intervention (Anderson et al. 1984). Racemic SF2312, a natural product-based “ring-stabilized” analog of PhAH identified using computational modeling of PEP and 2-PGA in the ENO2 binding site, displays inhibition against both ENO1 (recombinant hENO1 $\text{IC}_{50} = 38 \text{ nM}$) and ENO2 (recombinant hENO2 $\text{IC}_{50} = 43 \text{ nM}$) (Leonard et al. 2016). A deoxy-SF2312 (a deoxy analog of SF2312) displayed poor potency ($\text{IC}_{50} = 2 \text{ }\mu\text{M}$) compared to SF2312, possibly due to the loss of a key hydrogen-bonding interaction with a glutamic acid [Glu 166 in the case of human ENO1 (Leonard et al. 2016)].

As an alternative to designing inhibitors based on substrates, Jung et al. disclosed a novel small molecule named ENOblock (Fig. 10) that was identified through a phenotypic screen

to identify molecules that selectively killed cancer cells in a hypoxic environment (Jung et al. 2013). ENOblock inhibited ENO1 ($IC_{50} = 0.6 \mu M$) and also reduced cancer cell invasion, migration, and metastasis in vivo (Jung et al. 2013) when tested in HCT116 colon cancer cells. Mechanistically, ENOblock treatment decreased the expression of AKT and Bcl-xL (target proteins for cellular responses to hypoxia) and thus reduced the ability of cancer cells to adapt to hypoxic condition. Further investigations using ABPP methods are needed to carefully define the selectivity profile of ENOblock [which has recently been questioned (Satani et al. 2016)] in order to fully understand its target profile and suitability for probing T cell and/or tumor cell metabolism in vivo.

4.2.2 Glutamine Dependence for Effector Function—T cells also rely on amino acids, in addition to glucose, for proper function and survival (Chang and Pearce 2016). Glutamine serves as a reservoir for nitrogen atoms and a key nutrient for intracellular processes including metabolism, generation of ATP, and biosynthesis of biomolecules including proteins (Cluntun et al. 2017). Akin to glucose, competition for glutamine in the tumor microenvironment can block proliferation and cytokine production of T cells. For example, TCR activation of naïve T cells is coupled with enhanced glutamine uptake that is dependent on the amino acid transporter ASCT2. In addition, ASCT2 couples TCR activation with mTORC1 regulation of metabolic signaling, which supports glutamine as an immunomodulatory metabolite (Nakaya et al. 2014).

Glutamine is synthesized from glutamate by glutamine synthetase, whereas the mitochondrial glutaminase (EC 3.5.1.2) catabolizes glutamine to glutamate. A simplified pathway for glutamine metabolism is shown in Scheme 2. In the first step, L-glutamine is hydrolyzed to L-glutamate by glutaminase. Glutamate is subsequently deaminated by glutamate dehydrogenase to α -ketoglutarate (α -KG), a key intermediate that revives the tricarboxylic acid (TCA) cycle (citric acid cycle or Krebs cycle) when the concentrations of citrate fall below the threshold (Lukey et al. 2013). In tumor cells, the glutamine pathway is upregulated by enhanced expression of glutaminase and the c-Myc-regulated transporter SLC1A5 that supplies exogenous glutamine. The net effect is acceleration of the glutamine pathway, which results in excess generation of α -KG (via the formation of glutamate) to drive the TCA cycle and promote cancer cell proliferation by increasing the production of ATP, proteins, lipids, and nucleic acids. Thus, glutaminase through its role as a rate-limiting enzyme in the degradation of glutamine can regulate TCA cycle anaplerosis (Lukey et al. 2013).

Two isoforms of glutaminase exist in mammals: kidney-type glutaminase (KGA; encoded by *GLS*) and liver-type glutaminase (LGA; encoded by *GLS2*). KGA is believed to be an oncogene that is regulated by c-Myc expression, whereas LGA functions as a tumor suppressor and is a target gene for p53 (Lukey et al. 2013). KGA is widely distributed in kidney, brain, intestine, fetal liver, lymphocytes, and transformed cells, whereas LGA is expressed only in periportal hepatocytes of the postnatal liver. Interestingly, a shorter splice variant of KGA, also called GAC, is upregulated in many cancers. A correlation between the expression of GAC and the degree of malignancy, as well as the tumor grade, in breast cancer samples has been established (Cassago et al. 2012; Wang et al. 2010).

First-generation glutaminase inhibitors (Fig. 11) focused on the identification of glutamine-mimetic metabolites; however, the clinical utility of these compounds was limited due to toxicity (Ahluwalia et al. 1990). Compound CB-839 selectively inhibits both the splice variants of *GLS*, namely KGA and GAC (recombinant GAC $IC_{50} = 50$ nM), and is over 1000-fold less active toward *GLS2*. It exerts antiproliferative effects against triple-negative breast cancer (TNBC) cell lines HCC1806 and MDA-MB-231CB-839, as well as against AML and lung cancer cell lines (Gregory et al. 2018; Gross et al. 2014; Momcilovic et al. 2017). Because of its promising in vivo effects, CB-839 is under investigation in clinical trials for possible treatment of solid and hematological tumors. The compound BPTES (Fig. 11) is identified as an allosteric glutaminase inhibitor with an IC_{50} value of ~60 nM in vitro and ~20 μ M in cells (Seltzer et al. 2010). X-ray crystallography data revealed that two molecules of BPTES bind to the glutaminase tetramer and convert it to an inactive conformation (DeLaBarre et al. 2011). Compound 968, a cell-permeable allosteric inhibitor with an IC_{50} of 2.5 μ M, blocks the conversion of inactive glutaminase to active glutaminase by stabilizing the conformation of the former state (Wang et al. 2010; Katt et al. 2012). Molecular docking experiments offered additional insights that this inhibitor binds at the hydrophobic pocket between the N- and the C-termini (Katt et al. 2012).

The reported glutaminase inhibitors provide a good starting point for developing novel glutaminase activity-based probes that can serve as reporters of T cell health in the tumor microenvironment. Given that glutamine is essential for metabolism of both immune and tumor cells, a combination of ABPP and in vivo tumor immunology will be needed to determine how cell metabolism can be tuned in support of T cell effector functions in the complex tumor environment. For example, glutaminase activity-based probes may enable selection of appropriate T cell subsets with metabolic phenotypes suitable for vaccination as well as serving as a biomarker for current immunotherapies.

4.2.3 Immunosuppression by Tryptophan Depletion—Tumors hijack enzymes that contribute to an immunosuppressive tumor microenvironment to evade immune responses (Cheong and Sun 2018). Several lines of study suggest that proliferating tumor cells exploit kynurenine metabolism to support immune evasion (Cheong and Sun 2018; Hornyak et al. 2018). The heme-containing enzyme indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes conversion of L-tryptophan (Trp) to N-formylkynurenine (NFK) in the presence of molecular oxygen as the first and rate-limiting step in formation of L-Kynurenine (Efimov et al. 2011; Cheong and Sun 2018) (Scheme 3). The mechanisms of how kynurenine pathway metabolites contribute to immunosuppression remain an active area of investigation. Two pathways are proposed. In the first mechanism, which is based on Trp depletion, a link between Trp depletion and T cells has been established: a greater Trp local concentration results in greater activity and proliferation of T cells; in contrast, lower Trp levels result in T lymphocyte arrest in the G1 phase of the cell cycle. A second mechanism focused on the role of accumulated Trp catabolites such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, and picolinic acid which induce differentiation of Treg cells and apoptosis of effector T cells (Hornyak et al. 2018).

Several classes of IDO1 inhibitors that elicit antitumor effects are published in the literature (Rohrig et al. 2015) (Fig. 12). Specifically, epacadostat, a hydroxyamidine IDO1 inhibitor

that is orally bioavailable, potently suppresses tryptophan metabolism in vitro in CT26 colon carcinoma and PAN02 pancreatic carcinoma cells (Koblish et al. 2010). In Phase I/II study, this investigational drug showed favorable overall response rate, disease control rate, and progression-free survival (Gajewski et al. 2016). The combination of epacadostat and pembrolizumab (monoclonal anti-PD-1 antibody) was found to be well tolerated, evaluated for efficacy in patients with unresectable or metastatic melanoma (Gajewski et al. 2016), but ultimately failed in the clinic ([NCT02752074](#)). Although the cause of failure is currently under investigation, development of a IDO1-tailored activity-based probe could serve as a valuable biomarker for confirming target engagement with epacadostat in vivo to guide future clinical efforts.

5 Conclusions

In summary, we illustrate how ABPP methods can provide new tools to study T cell metabolism, signaling, and cell fate. The use of ABPP to identify lipid-sensing modules of key protein kinases as a principal mechanism of DMF activity in T cells should help guide development of new immunosuppressive drugs (Blewett et al. 2016). The use of universal serine hydrolase activity-based probes provides opportunities to discover new protein mediators of T cell proliferation (Adibekian et al. 2011). Proteasome activity-based probes are capable of tracking asymmetric distribution of active proteasomes in daughter cells to uncover regulatory mechanisms of T cell fate (Chang et al. 2011). Thus, ABPP enables interrogation of specific biology while exploiting conserved enzyme chemistry for global discovery efforts. Projecting forward, ABPP is poised to tackle the challenge of identifying aberrant metabolic remodeling events that limit antitumor immunity (e.g., metabolic checkpoints in glycolysis and lipid phosphorylation) and the development of corresponding agents to reprogram T cell metabolism for beneficial and ultimately therapeutic purposes.

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Fig. 1. Activation of T cells via MHC–peptide complexes on professional APC triggering the T cell receptor and cascades of tyrosine and serine–threonine kinases

Fig. 2.
Interactivity between immune cell subsets. Helper T cells are activated by DC and in turn provide survival and differentiation signals to cytotoxic CD8+ T cells, B cells, and macrophages

Fig. 3.

a Activity-based protein profiling (ABPP) utilizes active-site directed chemical probes to measure the functional state of large numbers of enzymes in native biological systems (e.g., cells or tissues). Activity-based probes consist of a reactive group (hexagon) for targeting a specific enzyme class and a reporter tag (e.g., fluorophore or biotin denoted by oval) for detection by in-gel fluorescence scanning or by avidin-enrichment coupled with liquid chromatography–mass spectrometry (LC-MS), respectively. **b** For inhibitor discovery, the potency and selectivity of small molecules can be profiled against many enzymes in parallel by performing competitive ABPP in complex proteomes, where inhibitors compete for probe labeling of enzyme targets

Fig. 4.
Chemical structures of dimethyl fumarate (DMF), monomethyl fumarate (MMF), and the non-electrophilic analog dimethyl succinate (DMS)

AA26-9

AA74-1

AA39-2

AA44-2

Fig. 5.
Chemical structures of 1,2,3-TUs that have been optimized by ABPP-guided medicinal chemistry into selective SH chemical probes for in vivo analysis

Epoxomicin

MVB003

Fig. 6. Chemical structure of a proteasome activity-based probe, MVP003 based on epoxomicin, a natural product that functions as a cell-permeable, potent, selective, and irreversible proteasome inhibitor. The epoxyketone functional group enables covalent reaction with the amino-terminal threonine of the 20S proteasome

Fig. 7.
Diacylglycerol kinases (DGKs) phosphorylate DAGs to regulate cellular signaling

Fig. 8.

Domain architecture of the mammalian diacylglycerol kinase (DGK) superfamily. All DGKs contain at least 2 C1 domains (C1A and C1B) and a catalytic domain (divided into DAGKc and DAGKa subdomains). Additional DGK domains permit control of when and where DGKs are active in cell signaling. Only type 3 DGKs are expressed as transmembrane proteins by virtue of a hydrophobic domain (HD). The majority of DGKs are expressed in soluble and membrane fractions and translocated between subcellular locales in an activation-dependent manner. Distinct cell type and tissue expression of DGKs support specific biology regulated by this enzyme family and therapeutic potential for various human diseases

Fig. 9.

ABPP analysis of the DGK superfamily. **a** Mechanism of ATP acyl phosphate probe labeling. Nucleophilic lysines in kinase active sites attack the acyl carbon of the ATP probe. Once ATP has been displaced, the kinase is labeled with a dethiobiotin tag for protein identification by in-gel fluorescence scanning or LC-MS/MS. **b** The binding site of DGK inhibitors can be determined by performing a trypsin digest of proteomes from cells cultured in isotopically heavy (^{13}C , ^{15}N) and light (^{12}C , ^{14}N) lysine and arginine amino acids, and enrichment for probe-modified, active-site peptides by affinity chromatography. Mixed heavy and light samples can be directly compared by their MS1 peaks for quantification. Identification of peptides is achieved by MS2 fragmentation and sequencing using bioinformatics. LC-MS/MS studies using the ATP acyl phosphate probe have uncovered the C1 and DAGKa domains as novel druggable sites. **c** Fragment inhibitors can be screened by competitive ABPP. Screening for DGK inhibitors using ABPP methods provides information on potency and selectivity to identify ligand-efficient small molecule binders. ABPP screening of fragment compounds in complex proteomes can accelerate the medicinal chemistry process by guiding selection of hit compounds based on both potency and selectivity. Inset box shows chemical structure of a non-selective DGK α inhibitor ritanserine that has been used for biochemical and biological studies

Fig. 10.
Chemical structures of enolase inhibitors

Fig. 11.
Chemical structures of glutaminase allosteric inhibitors

Fig. 12.

Representative IDO1 inhibitors published in the literature. For a detailed review, see reference Rohrig et al. (2015)

Scheme 1.

Enzymatic reaction of ENO1. Residues are numbered based on X-ray crystal structure information obtained from yeast variant. The homologous catalytic residues in human ENO1 are Glu 209 and Lys 342

Scheme 2.
Metabolism of L-glutamine

Scheme 3.

IDO1 enzymatic pathway for metabolism of L-tryptophan to produce L-Kynurenine and associated metabolites