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Authors

Marcel, Nimi

Hedrick, Stephen M

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A key control point in the T cell response to chronic infection and neoplasia: FOXO1

Nimi Marcel and Stephen M. Hedrick*

Molecular Biology Section, Division of Biological Sciences

Department of Cellular and Molecular Medicine

TATA Institute for Genetics and Society

University of California, San Diego

La Jolla, CA 92093-0377

*Corresponding author (shedrick@ucsd.edu)

Abstract

T cells able to control neoplasia or chronic infections display a signature gene expression profile similar or identical to that of central memory T cells. These cells have qualities of self-renewal and a plasticity that allow them to repeatedly undergo activation (growth, proliferation, and differentiation), followed by quiescence. It is these qualities that define the ability of T cells to establish an equilibrium with chronic infectious agents, and also preserve the ability of T cells to be re-activated (by checkpoint therapy) in response to malignant cancers. Here we describe distinctions between the forms of inhibition mediated by tumors and persistent viruses, we review the properties of T cells associated with long-term immunity, and we identify the transcription factor, FOXO1, as the control point for a program of gene expression that allows $CD8^+$ T cells to undergo serial reactivation and self-renewal.

Introduction

Cytotoxic T cells specific for an infectious intracellular pathogen (or neoplasia) rapidly diverge toward three major differentiation states characterized as memory precursor cells, that ultimately become central memory cells, short-lived effector cells, and tissue resident memory cells [1–5]. Between the extremes of central memory and cytotoxic effector cells there exists a continuum or at least multiple cell-types in which the differentiated function of cytotoxic T cells is present at the expense of a retained potential for self-renewal and serial reactivation [6, 7]. The appearance and maintenance of these cell populations is strongly influenced by the course and disposition of an infection. With rapid clearance of the recognized foreign agent, the effector cells slowly contract and leave the long-lived memory population in a state of prepared responsiveness—an acute primary response followed by a state of immunity. In those instances where neutralizing antibodies are absent or not protective [8], a secondary infection provokes a rapid expansion of the memory population that once again diverges into effector and long-term memory cells—a secondary or anamnestic response.

When the immune response does not clear the intracellular threat, be it an infection or neoplastic growth, the differentiation of $CD8^+$ cytotoxic T cells is less well delineated. In addition to effector cells and a fate resembling memory cells, antigen-specific $CD8^+$ T cells may enter yet another state of differentiation characterized by reduced responsiveness that has been termed exhaustion [9]. The importance of this state in the progression of cancer or the persistence of intracellular infections, is that T cells which can be effectively reactivated to clear or restrain the inciting principle are those that retain characteristics of central memory [10–13]. Highly differentiated effector cells do not expand further upon reencounter with antigen [14, 15], whereas exhausted T cells, though they continue to possess the potential to mount a response [16], are attenuated in their capacity for dispatching an infection or a tumor mass [9]. In this review, we will highlight the state of T cells present in the face of chronic antigen exposure, and the variable ability of such T cells to continue to mount an effective immune response. This topic has important implications for the failure of immune mechanisms to contain or clear chronic viral infections and neoplastic growth.

Feedback control, evolved under pressure of chronic infections, is disadvantageous to cancer immunosurveillance

Long-lived animal hosts and their parasitic viruses are often characterized as engaging in an arms race, where each is selected to evolve the means to survive and procreate at the expense of the other, an example of Van Valen's "Red Queen Hypothesis" [17]. But this is not quite accurate. The evolution of a virus (and other infectious agents) is based on a generation time that is orders of magnitude shorter than that of its host. Furthermore, viruses are selected to replicate and be transmitted within and between a limited number of host species, and thus they can evolve specifically targeted mechanisms of virulence. Importantly, they are constrained to accomplish transmission before rendering their host unable to spread an infection. In contrast, the host is co-evolving with a plethora of diverse parasitic agents, each displaying distinct sets of virulence mechanisms. So, in addition to a long generation time, the need to simultaneously resist hundreds of different parasitic agents most probably means that host immune mechanisms evolve on a different time scale when compared with the world of parasites. We posit that this arms race is almost entirely one-sided where the parasites, such as persistent viruses, define their niche in a relatively static population of hosts.

This is not the case with neoplasia. With the exception of a few cancers found in Tasmanian devils, domestic canines, and bivalve species [18, 19], cancers themselves are not infectious. The emergence and "success" of one cancer is not inherited by the next. Rather, neoplasia is a "new formation" that is selected for unrestrained growth, without selective pressure to be transmitted or keep its host alive; one cancer resembles another only through convergent evolution [20]. Embedded within this somatic evolution, cancer cells appear to be selected individually and as a population to frustrate mechanisms of immunity that can impede their growth. As such, the forms of immune attenuation and negative feedback control that occur as a result of persistent virus infections and that which occurs coincident with the growth of malignant tumors are based on distinct evolutionary pressures. The former we presume to be advantageous to the long-term survival of the host, the latter favors unrestrained, metastatic tumor growth.

Feedback attenuation displayed by $CD8^+$ T cells is proportional to T cell antigen receptor (TCR) signaling, and it involves expression of receptors connected to inhibitory signaling pathways that activate, for example, tyrosine phosphatases that block signaling cascades based on tyrosine phosphorylation [21]. The prototype for this

feedback pathway is initiated by the inhibitory receptor PDCD1 (PD-1) that is expressed following TCR-mediated signaling on subsets of both $CD4^+$ and $CD8^+$ T cells, and is also expressed by B cells and myeloid cells [22, 23]. This type of control applied to $CD8^+$ T cells would appear to allow a burst of cytotoxic T cell activity, followed by attenuation that limits immunopathology. It is consistent with an evolutionary acknowledgement by pathogen-susceptible hosts that all-out resistance is futile, if not life-threatening, and often unnecessary in that co-evolved, persistent, intracellular infectious agents are not selected to cause premature demise of their hosts [24–26]. It may be a manifestation of the concept of tolerance—the ability of a host to accommodate infectious agents without disease [27].

We speculate that most of the mechanisms we associate with acquired immunity were most strongly selected for resistance to infectious agents. Although immunodeficient mice and human beings can be found to exhibit higher rates of cancer compared with their wildtype brethren, this is most clearly seen under the influence of carcinogens [28]. Furthermore, completely immunodeficient mice housed under standard conditions may experience increased inflammation associated with opportunistic infections, a condition known to favor oncogenesis [29]. We assert that an animal with a severe congenital immunodeficiency in the wild is likely to die of an infection long before metastatic cancer takes hold. The importance of this is that mechanisms of immunity, but also mechanisms of control, have been most strongly selected for relative resistance to infectious agents counterbalanced by regulation that minimizes immunopathology associated with persistent or latent infections. From this we deduce that the immune reaction that arises in response to a cancerous growth is subject to these same regulatory mechanisms, even though they may be disadvantageous in the presence of a life-threatening malignancy. We propose that the vertebrate immune system, although displaying the plasticity to recognize neoplastic growth, has been selected to anticipate a latent or chronic infection, not a rapidly malignant, lethal cancer.

Negative feedback and the regulation of “exhaustion”

A manifestation of negative feedback control in response to persistent infectious agents is ultimately a state of attenuated responsiveness termed exhaustion. Exhaustion in $CD8^+$ T cells was first observed in the context of a chronic lymphocytic choriomeningitis virus (LCMV) infection in mice [30–32], and it has been observed in both mice and human beings subject to several chronic infectious agents as well as the persistent inflammation present in autoimmune pathologies or in the context of cancer [33–41].

Exhaustion has also been reported to occur in $CD4^+$ T cells, although not necessarily characterized by the expression of negative feedback receptors such as PDCD1 [42], and yet, $CD4^+$ T cells play an essential role in the viral-immune equipoise characterizing both chronic and latent infections [43]. Exhausted $CD8^+$ T cells are characterized by the sequential loss of (presumably pathogenic) effector function, and particularly, impairment in IL-2, TNF, and IFNγ production [41]. This altered state is also marked by the sustained induction of inhibitory receptors that, in addition to PDCD1, include LAG3 (Lymphocyte Activation Gene-3), HAVCR2 (TIM3) and TIGIT (T cell immunoglobulin and ITIM domain) [44, 45].

Programs of gene expression associated with $CD8^+$ T cell differentiation

Transcriptional profiling and network analysis of individual T cells has revealed the different programs of gene expression that correlate with memory, effector function, and exhaustion [9]. The transcription factors that appear to be necessary for long-lived memory cell formation and maintenance are: FOXO1, EOMES, and TCF7 (Tcf-1), whereas the alternative $CD8^+$ T cell fate, short-lived effector cells, requires TBX21 (TBET), PRDM1 (Blimp-1), and ID2 (reviewed in [3]). These differences in memory and effector T cells appear to arise with an initial asymmetric division followed by the dynamics of metabolism, proliferation and survival determining the subsequent make-up of the T cell response [46–48]. An interesting concept to emerge, and one that we will discuss below is that most of the highly expressed genes in memory cells are commonly expressed by naïve T cells and many by hematopoietic stem cells. A conclusion is that there are common programs of gene expression found in cells endowed with the capacity for self-renewal [3].

The transcriptional profile of exhausted T cells differs from that of effector and memory cells and extends beyond genes encoding inhibitory feedback circuitry. The profile includes genes encoding transcription factors, metabolic pathways, and signaling intermediates, as well as chemokines, cytokines and their respective receptors [44, 49]. Some of the major transcription factors implicated in the T cell exhaustion include EOMES and TBX21, TCF7, TOX, PRDM1, NFAT, FOXO1, FOXP1, BATF, IRF4 and VHL [50–57]. Most recently, the transcription factor TOX was shown to be required for important aspects of an exhausted phenotype, although one report provided evidence that functional exhaustion could take place without the presence of TOX [58–62]. Interestingly, transcription factors that are key to T cell exhaustion are also important for T effector and memory formation, but they are utilized distinctly in the context of

exhaustion. In chronic infection, the expression of TBX21 and EOMES appears to guide the formation of specific subsets within the T exhausted population, such that elevated expression of TBX21 is associated with a non-terminally exhausted, progenitor, subset (TBET^{hi} PDCD1^{int} EOMES^{lo}), while, contrary to its role in T cell memory, high expression of EOMES is associated with a terminally exhausted T cell subset (EOMES^{hi} PDCD1^{hi}).

Perhaps most importantly for this discussion, TCF7, characterized for its essential role in the induction and maintenance of memory T cells, similarly plays a key role in sustaining long-lived T cells labeled as “exhausted”. It is this sub-population, TCF7^{+} PDCD1^{int} HAVCR2^{lo}, that is credited with mediating a therapeutic response to “checkpoint” immunotherapy [10, 12, 13, 63]. The ability of this subset to revive a functional response when inhibitory signals are blocked is further evidenced by the ability of these TCF7^{+} T cells to clear a viral infection upon transfer to a naïve host [64–66]. scRNA-sequencing analyses reveal that during a chronic infection TCF7 overrides T effector differentiation and skews the differentiation to exhausted T cells via the upregulation of MYB which is a known regulator of BCL2 and EOMES (for persistence of exhaustion phenotype) [67]. We emphasize that PDCD1 is induced under a variety of circumstances in CD4^{+} and CD8^{+} T cells, and alone, it does not signify unresponsive, exhausted T cells.

The origin of responsive TCF7-expressing T cells depends on FOXO1

Studies to date consistently reveal TCF7 to be a nexus of signaling and transcription necessary for T cell survival and self-renewal—and this transcends species (at least applies to human beings and mice) and the inciting chronic immune stimulus [67–69]. In addition to its role in late-stage T cell differentiation, survival and function, TCF7 is important, along with its paralog, LEF, for differentiation and transition through several stages of early T cell development [70]. It also functions to promote the differentiation of precursors to all innate lymphoid cells (ILCs) (reviewed in [69, 71]). The means by which TCF7 accomplishes these varying roles depends upon transcriptional context, but also the many splice forms known for *Tcf7* transcripts. These variously include the N-terminal β-catenin interacting domain mediating Wnt signaling, a 30 amino acid domain with intrinsic HDAC activity [72], and a C-terminal high-mobility-group (HMG) box DNA binding domain that allows TCF7 to directly mediate transcriptional activity [73]. There is presently little understanding of the splice forms that are important for TCF7 activity in

mature T cell differentiation, although reports show its role in establishing a memory precursor cell requires WNT signaling and β-catenin [74–78].

In its role as a central transcription factor in memory and as a linchpin describing $CD8^+$ T cells able to respond to checkpoint therapy, RNA expression studies show that *Tcf7* is often coordinately expressed with *Il7r* (encoding IL7 receptor α-chain, CD127), *Sell* (encoding L-selectin, CD62L), *Ccr7* (encoding CCR7) and in opposition to the expression of *Klrg1*, *Havcr2*, *Cx3cr1* (encoding fractalkine receptor) and effector molecules such as *Gzmb* (encoding granzyme B) [7, 69]. This is a program of gene expression that is directly controlled by the forkhead "O" family member FOXO1.

TCF7 is expressed at high levels in naïve $CD8^+$ T cells—either wildtype or those deleted for *Foxo1*, but with antigen-induced T cell activation, *Tcf7* expression is rapidly lost [79], possibly through the action of inflammatory cytokines [80]. In LCMV gp33-specific T cells it is only reestablished in a minor subset of responding cells that can be observed starting around day 5 post-activation; however, as described above, the dichotomy of memory and effector precursors may be established at the very first asymmetric cell division, one where MYC and FOXO1 are segregated into effector and memory precursors, respectively [47]. At the peak of the T cell response, about day 7, the memory precursor population is characterized as $TCF7^+$ $IL7R^+$ $KLRG1^-$ $HAVCR2^-$ $GZMB^-$, but with the deletion of *Foxo1*, this subset is completely absent [79, 81]. Without *Foxo1*, TCF7 expression is never again reestablished, and the $CD8^+$ T cell response consists entirely of effector cells that lack the ability to undergo a secondary expansion, i. e., an anamnestic response [81–84]. In fact, the characteristic properties of memory cells generated after an acute infection required continuous expression of FOXO1, as late *Foxo1* deletion using tamoxifen-induced CRE recombinase expression in $Foxo1^{f/f}$ T cells, resulted in a reversion to effector phenotype: the gain of KLRG1 and GZMB and loss of BCL2, self-renewal potential, and an ability to proliferate in response to reinfection. Under conditions of chronic or latent virus infection, where we presume T cells continued to receive antigen-mediated signals, deletion of *Foxo1* caused a greatly accelerated loss of memory potential [85, 86].

Control of *Tcf7* transcription and alternate exon usage is likely to be complex, and this may be a focus of future studies. We originally looked at the *Tcf7* gene from $CD4^+$ T cells by analyzing regions of open chromatin, chromosome marks indicative of poised and active enhancers, and binding by FOXO1 by chromatin immunoprecipitation and genomic sequencing (ChIP-Seq) [81]. Subsequently, we have carried out similar studies on $CD8^+$ LCMV-specific T cells, before, and 12 days post infection by LCMV-Armstrong

(data not shown). The results were similar to that of $CD4^+$ T cells in that within a 60 kilobase region that includes the body of the *Tcf7* gene and a 30 kb region upstream of the transcriptional start site, we found 6 distinct regions of open chromatin as detected by ATAC-Seq [87]. Each of these sites was flanked by chromosome marks of H3K27 acetylation (H3K27Ac) indicative of active enhancers [88], and each of these sites was bound by FOXO1. FOXO1 also bound to a 7^{th} site characterized by H3K4 trimethylation (H3K4me3) located at the transcriptional start site (TSS), presumably the promoter [89, 90]. Although these results are correlative, the requirement for FOXO1 in expression post-activation, and the identification of FOXO1 binding to nearby enhancers is consistent with a direct role for FOXO1 in post-activation *Tcf7* regulation; however, we note that other gene elements located much further away may play a role in *Tcf7* transcriptional regulation. For example, loss of *Foxo1* did not affect TCF7 expression in naïve T cells, yet the *Tcf7* proximal sites bound by FOXO1 were identical between naïve T cells and antigen-specific T cells from day 12 post-infection; however, a FOXO1 binding site co-localized with a site of open chromatin 280 kb downstream of the TSS appears to be inaccessible in *Foxo1*-null T cells (data not shown). Without a systematic mutation of these gene elements, alone and in combination, we are presently unable to more exactly identify the mechanism of *Tcf7* regulation by FOXO1.

In addition to *Tcf7* regulation, FOXO1 controls several genes that are coordinately found to be expressed in T cells associated with response to checkpoint therapy. These include *Il7r*, *Sell*, and *Ccr7* [91–94]. In particular, FOXO1 is required for the expression of *Il7r* that encodes the alpha-chain of the receptor for IL7, an important factor in the viability of memory T cells [95, 96]. FOXO1 binds to an enhancer 3.5 kilobases upstream of the *Il7r* TSS [92] in order to displace FOXP1 acting as a transcription repressor [97]. From these studies, FOXO1 emerges as the upstream control point for the program of gene expression that is essential for differentiation and survival of T cells able to control viral or neoplastic parasites.

FOXO1 regulation and the physiology of cell renewal

The role played by FOXO family transcription factors in survival, plasticity and self-renewal has been described previously [98, 98–101]. In particular, FOXO transcription factors have a central role in the ability of metazoans to establish pluripotency and characteristics of stem cells. They were first described as essential to the downstream signaling module necessary for an extended life-span in nematodes or flies subject to diminished insulin-like growth factor signaling [102–104]. More recently, a FOXO

ortholog has been found to be key to the establishment of self-renewal in all three stem cell lineages in hydra, an immortal cnidarian genus diverged from bilateral phyla on the order of 600 million years ago [105]. Closer to home, FOXO1 was found to regulate pluripotency in human embryonic stem cells by binding and activating the promotors of two essential “Yamanaka factors”, *OCT4* (*POU5F1*) and *SOX2* [106, 107]. In addition, genome-wide association studies have revealed *FOXO1* and *FOXO3* to be the most prominent among a small number of genes associated with increased age at death or age at natural menopause [108–114]. As such, it is perhaps not surprising that FOXO factors are prominent in controlling survival and longevity in T cells [115], and a possibility is that the acquired immune system has coopted this ancient pathway for maintaining self-renewal properties as a means of accommodating life-long parasitic infections. We speculate that, while chronic pathogens accommodate FOXO1-expressing long-lived CD8 T cells (selected to maintain host viability), there may be tumors that have evolved mechanisms to impede the activity of FOXO1 in T cells.

To understand the program of gene expression consistent with tumor immunity or control of latent or persistent viral infections, a future task will be to describe how FOXO transcription factors are themselves regulated, and how they regulate downstream gene targets. This challenge is highlighted by the observation that multiple studies using single cell transcriptional profiling for genes that characterize T cells responsive to check-point therapy have identified a common program of gene expression that has not included *Foxo1* [11–13]—even though it is clearly controlling key aspects of the definitive program of gene expression. This is likely due to the fact that FOXO transcription factors, although regulated at the level of gene expression [116], are prominently regulated by most known post-transcriptional mechanisms.

Foxo1 mRNA amounts are altered by multiple microRNAs in different cell types [117–119] including $CD8^+$ T cells via miR-150 [120]. In addition, FOXO factors are potently regulated by post-translational modifications, and most prominent are the inactivating serine-threonine phosphorylations mediated by AKT (but probably not SGK1 [121]). Two pathways converge to activate AKT: PI3K (Phosphoinositide 3-kinase) activation of PDPK1 (3-Phosphoinositide Dependent Protein Kinase 1, PDK1) that phosphorylates AKT at Thr308 [122], and the mTORC2 complex that phosphorylates AKT at Ser473 [123] **(Figure 1)**. Loss of mTORC2 signaling enhances $CD8^+$ memory cell generation [124], presumably by preventing the activation of AKT and thus the inactivation of FOXO1. The outcome of phosphorylation of FOXO1 by AKT is its association with 14-3-3, exclusion from the nucleus, and degradation through ubiquitination [125, 126]. AKT

phosphorylation can also be directly countered by phosphatase 2A [127]. The control of PI3K and mTORC2 signaling in T cells, especially under conditions of chronic infection or neoplasia is complex and has not been studied in detail [128]; however, PI3K delta syndrome is a primary immunodeficiency resulting from a gain-of-function in PI3K-delta, in principle leading to the increased constitutive phosphorylation of FOXO transcription factors among other possibilities. The phenotypic effects of this congenital mutation are varied between affected individuals, even within the same family, but they often include severe and recurrent α, β, and γ herpes family viral infections [129].

Other serine-threonine phosphorylation sites on FOXO1 oppose AKT-mediated nuclear exclusion. 5' adenosine monophosphate-activated protein kinase (AMPK) responds to reduced energy levels and activates FOXO transcription factors indirectly, and through direct phosphorylation [130–132]. Jun N-terminal protein kinase (JNK), responding to oxidative stress, phosphorylates FOXO1, and this enhances its nuclear localization, possibly by dissociation from 14-3-3 [133]. The biological importance of *activating* serine-threonine FOXO1 phosphorylations is exemplified by familial deficiencies in *STK4* (*MST1*). These congenital deficiencies, resulting from consanguineal marriages, arose from *STK4* nonsense mutations that were found to be associated with several combined skin and respiratory infections and multiple herpes virus family infections. Patients experienced a progressive loss of naïve CD4 T cells and central memory T cells that correlated with cellular abnormalities including a loss of FOXO1 expression and its downstream targets, most notably IL7R. Further studies showed that *Stk4* loss-of-function mice showed a very similar phenotype [134] [135].

Additional FOXO1 post-translational modifications mediated by oxidative stress or restricted nutrients include acetylation regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [136], glycosylation catalyzed by O-Linked N-Acetylglucosamine (GlcNAc) Transferase (OGT) [137], methylation carried out by protein arginine methyltransferase (PRMT) [138], and ubiquitination at multiple sites [100, 139–141] **(Figure 1)**. These modifications also affect nuclear vs. cytoplasmic localization, and in addition, stability, turnover, and transcriptional activity.

A large caveat is that most of the studies characterizing signaling pathways important for FOXO1 regulation have not been carried out in T cells, but rather in other differentiated cell types especially those that regulate metabolism such as liver, fat and muscle. We do not understand the progression of FOXO1 posttranslational modifications in detail in any T cell subset, and certainly not in T cells found under conditions of chronic virus infection or neoplasia. On top of this, all of these signaling

modules, and especially those involving mTOR are entangled such that drawing linear pathways is mainly uninformative. The means by which FOXO1 regulates physiology in each differentiated cell-type may need to be studied from a systems analysis approach that would begin by correlating FOXO protein modifications, intracellular localization, chromatin binding and gene expression in T cell subsets (or individual cells) at different times subsequent to viral infection or tumor growth. If the goal is to understand the biology underlying long-term stem-like activity and a continuing capacity for immune activation, a reasonable approach is to compare wildtype and *Foxo1*-deficient cells.

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Figure Legend

Figure 1. Opposing influences on FOXO1 cellular localization and transcriptional activity. PI3K, Phosphoinositide 3-kinase; PTEN, Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase; PDPK1, 3-Phosphoinositide Dependent Protein Kinase 1 (PDK1); mTORC2, mTOR complex 2 (consisting of 7 components); AKT, (Protein Kinase B); PP2A, Protein phosphatase 2A; HDAC, Histone deacetylase; AMPK, 5' adenosine monophosphate-activated protein kinase; STK4, Serine/Threonine Kinase 4 (MST1); OGT, O-Linked N-Acetylglucosamine (GlcNAc) Transferase; PRMT1, protein arginine methyltransferase; JNK, Jun N-terminal protein kinase.

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A series of papers showed that the transcription factor, TOX, is important or essential in the establishment of exhaustion. This particular study shows that TCR signaling induces NFAT, and even in the absence of its partner AP1 factors (FOS-JUN), there occurs the induction of *Nr4a* family members, *Tox,* and *Tox2*. Deletion of both *Tox* and *Tox2* or all three *Nr4a* paralogs facilitated a CAR-T response and survival of tumor-bearing mice. This was the only study to analyze the genetics of all the *Tox* and *Nr4a* paralogs in the induction of exhaustion.

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This study showed that deletion of *Tox* was sufficient to diminish the expression of inhibitory receptors, e. g., PD1, and yet *Tox*-deleted T cells remained dysfunctional. The authors assert that this uncouples exhaustion from inhibitory receptor expression. A possible complication is the potential for redundancy in between the two *Tox* paralogs: Tox and Tox2

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This study purports to show that TOX acts via endocytic recycling of PD1 between the cell surface and the endosome. The contention is that TOX acts directly to enhance cell surface expression of PD1, and not necessarily as a transcription factor.

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Here Tox was studied in chronic viral infections, and an important point was that the DNA binding domain is important for TOX function. Thus, TOX most likely acts as a transcription factor to promote exhaustion. Consistent with the selective advantage of the exhaustion response, T cells with a *Tox* DNA binding domain deletion showed increased effector function and caused more immunopathology; yet, these T cells exhibited a massive decline in the self-renewing subpopulation expressing TCF7.

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TOX was shown here to be essential for exhaustion, and participate in the regulation of its own expression, an example of positive-feedback control (as opposed to feed-forward control that is independent of the parameter in question, in this case PD1 expression). They showed that strong expression of TOX results in a more permanent commitment to the exhaustion state.

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Experiments demonstrated the requirement for TCF7 in T cells able to carry out an anti-tumor response upon treatment with checkpoint therapy.

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Figure

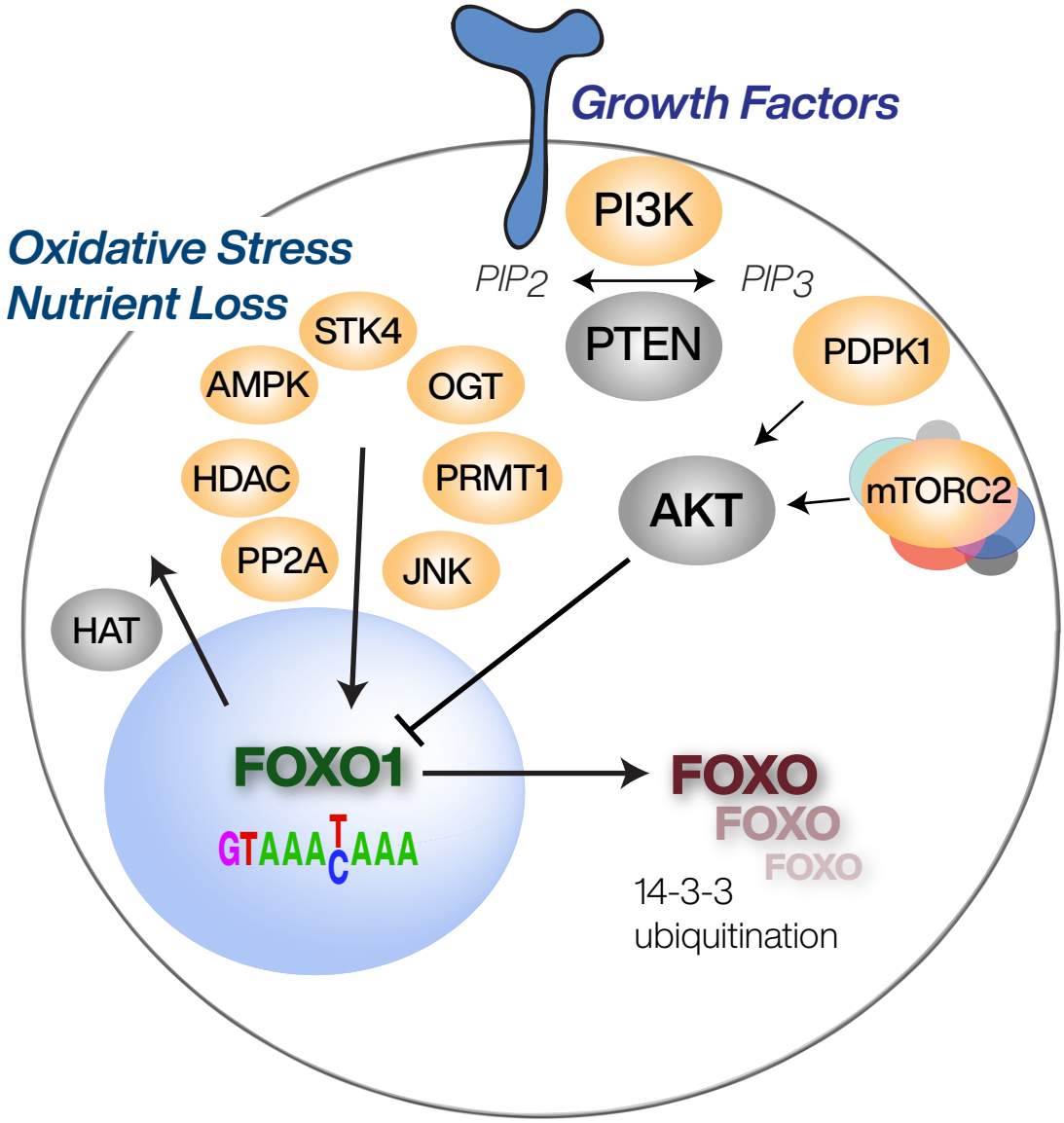

Figure 1. Opposing influences on FOXO1 cellular localization and transcriptional activity. PI3K, Phosphoinositide 3-kinase; PTEN, Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase; PDPK1, 3-Phosphoinositide Dependent Protein Kinase 1 (PDK1); mTORC2, mTOR complex 2 (consisting of 7 components); AKT, (Protein Kinase B); PP2A, Protein phosphatase 2A; HDAC, Histone deacetylase; AMPK, 5' adenosine monophosphate-activated protein kinase; STK4, Serine/Threonine Kinase 4 (MST1); OGT, O-Linked N-Acetylglucosamine (GlcNAc) Transferase; PRMT1, protein arginine methyltransferase; JNK, Jun N-terminal protein kinase.