Clinical & Cellular Immunology

Yamagishi and Watanabe, J Clin Cell Immunol 2012, S12
http://dx.doi.org/10.4172/2155-9899.S12-007

Review Article Open Access

New Paradigm of T cell Signaling: Learning from Malignancies

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Abstract

T cells are key mediators of cell-mediated immunity. Their functions and proliferation result from T cell-specific receptor signaling (TCR/CD28) that activates the NF-κB, NFAT, Ras-MAPK, and PI3K-Akt pathways. Their development and activation also involve a complex array of signaling pathways that regulate gene expression networks, including signaling of mTOR, Notch, Wnt, Hedgehog, TGF-β, and toll-like receptors. Furthermore, recent discoveries have provided two molecular hallmarks of potential generality: miRNA patterns and polycomb-mediated epigenetic reprogramming, which can strongly coordinate the balance between molecular networks in lymphocytes. Their deregulation apparently causes T cell disorders, such as T cell acute lymphoblastic leukemia (T-ALL), and human T cell leukemia virus (HTLV-1)-induced adult T cell leukemia (ATL). This review continues with a description of our understanding of crosstalk among the signaling pathways, which contribute to the highly orchestrated development of T cell fate specification under both normal physiological and pathological conditions.

Introduction

T cells use diverse genetic programs to direct the development of distinct lineages and the generation of several effector functions required for innate and adaptive immunity. The molecular networks in T cells are strictly controlled by the input from intercellular and extracellular signals. T cell activation is basically achieved by antigen-presenting cells (APCs). Antigen recognition by and signaling through the T cell receptor (TCR) are crucial processes for T cells and provide the molecular underpinning for the specificity and execution of immune system responses.

TCR-mediated signaling pathways are the bare bones of T cell activation. However, several other signaling pathways are intimately associated with T cell development, activation, and homeostasis. In addition, newly emerging molecular characteristics have been proposed on the basis of studies of normal T cells and T cell malignancies. This new paradigm includes miRNA-mediated gene regulation and epigenetic reprogramming.

In this review, by dissecting each signaling pathway, we summarize the molecular hallmarks of T cells. We first briefly introduce the fundamental molecular mechanisms involved in T cell activation and gene expression related to T cell functions [1,2]. These signaling pathways are also involved in T cell development and disorders. We next discuss the newly emerging signaling pathways involved in T cell biology. Genetic and physiological studies have indicated that the pathways primarily identified in the areas of development, regeneration, and innate immunity are also closely associated with T cell functions and their appropriate development. Finally, we discuss regarding a conceptual advance, i.e., crosstalk between several signaling pathways.

The complexity of signaling networks within T cells confers robustness to specific and diverse gene expressions and biological functions. The complex signaling regulation that is involved within different environments has been suggested from investigations in immature and mature T cell malignancies, including T cell acute lymphoblastic leukemia (T-ALL) and adult T cell leukemia/lymphoma (ATL).

Proximal Signaling of TCR-mediated T cell Activation

T cells are instructed to use their developmental and effector programs through stimulation of the TCR complex and costimulatory molecules, which are presented by professional APCs, e.g., macrophages, dendritic cells, and B cells. TCR signaling is initiated by CD3 phosphorylation. The CD3 proteins comprise a series of dimers, including γε, δε, and ζζ, which are associated with a single TCRαβ heterodimer (Figure 1). After the recognizing cognate complexes of foreign peptide and self major histocompatibility complex (MHC) class II (pMHC) molecules, TCR/CD3 affects signaling cascades, including phosphorylation of proximal TCR components, Ras–mitogen-activated protein kinase (MAPK) signaling, activation of nuclear factor κB (NF-κB) by protein kinase C θ (PKCθ), and Ca^{2+} flux-mediated signaling [1]. In response to stimulation, signaling through these mediators is integrated with input from other signaling pathways. This provides the biological output after TCR recognition of a ligand.

CD3 phosphorylation is regulated by the local balance of the tyrosine kinase Lck, which is associated with the monomorphic coreceptors CD4 and CD8 and phosphatases in the TCR signaling complex. Functional key motifs within CD3ε, γ, δ, and ζ chains, designated immunoreceptor tyrosine-based activation motifs (ITAMs), are phosphorylated by Lck after TCR ligation, which is an early, prerequisite step for TCR-directed T cell activation. Importantly, conformational changes in CD3 molecules are directly induced by CD3 phosphorylation after pMHC–TCR ligation. These conformational changes in CD3ε and CD3ζ make ITAMs more accessible for phosphorylation and are required for efficient T cell activation.

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Received August 30, 2012; **Accepted** October 29, 2012; **Published** November 05, 2012

Citation: Yamagishi M, Watanabe T (2012) New Paradigm of T cell Signaling: Learning from Malignancies. J Clin Cell Immunol S12:007. doi:10.4172/2155-9899.S12-007

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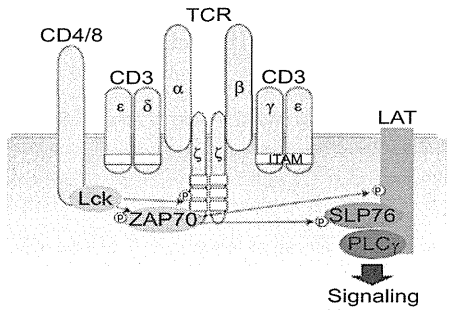

Figure 1: TCR/CD3-mediated proximal signaling. After engagement of TCR/CD3 complex, conformation change and following phosphorylation cascade is induced. Activated PLCγ coordinates the signaling required for T cell activation.

Phosphorylated CD3ζ (and other ITAM-containing proteins) provides recruitment sites of the 70-kDa phosphoprotein ZAP-70, a Syk kinase family member. That is, TCR engagement by pMHC leads to the activation of Src family PTK, such as Lck, which results in ITAM phosphorylation and ZAP-70 recruitment (Figure 1). The tandem SH2 domains of ZAP-70 are engaged by the phosphorylated ITAMs of CD3ζ, where in turn ZAP-70 is activated by Lck-mediated phosphorylation.

Among the most important ZAP-70 targets are the transmembrane adapter protein linker for the activation of T cells (LAT) and the cytosolic adapter protein Src homology 2 (SH2) domain-containing leukocyte phosphoprotein of 76 kDa (SLP-76). Phosphorylated LAT in turn serves as a docking site to which a number of signaling proteins can bind. These two adapters form the backbone of a complex that includes several effector molecules to allow for the activation of multiple signaling pathways. Proteins that are incorporated in these assemblies include other scaffold molecules, such as Grb2, Gads, and enzymes like phospholipase C γ1 (PLCγ1), and phosphoinositide 3-kinase (PI3K). The coordination of these interactions and the ensuing signaling result in a stable, but dynamic, zone of contact between APCs and T cells; this zone has been designated the immunological synapse (IS). IS has become a paradigm for studying the signals exchanged between the two cell types.

A lipid raft is a microdomain within the plasma membrane that is rich in cholesterol, glycosphingolipids, and sphingomyelin. Lipid rafts accumulate at IS. Initial TCR activation and an early phosphorylation cascade precedes the formation of IS, which not only provides the sustained signaling required for gene regulation in T cells but may also control the eventual halt of the signaling pathways. It is important that the ability of a kinase to support TCR signaling critically depend on its lipid modification. Indeed, the accumulation of lipid rafts and Lck in these areas can accelerate increased phosphorylation. Within a lipid raft, the signaling backbone established by LAT and SLP-76 coordinates downstream signaling by controlling PLCγ1 activity. However, a large amount of data suggests that the formation of this complex is more complicated. For its optimal activity, PLCγ1 directly binds to SLP-76, LAT, and Vav1 as well as to its activating kinase Itk. Lck, which binds to CD4/8 and activates ZAP-70, also phosphorylates Itk. Itk in turn phosphorylates PLCγ1. Activated PLCγ1 then hydrolyzes the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2), producing the second messengers inositol trisphosphate (IP3) and diacylglycerol (DAG), that are essential for T cell function (Figure 2).

TCR-mediated Gene Regulation via NFAT, NF-κB, and MAPK Cascades

TCR engagement results in the induction of expression of numerous genes required for T cell activation by triggering several signaling pathways. These pathways are described follows. Each of them acts specifically, but collaboratively, during T cell activation process.

Ras–MAPK pathway

TCR-induced DAG production results in the activation of two major key molecules: Ras and PKCθ. DAG recruits both RasGRP and PKCθ to the plasma membrane. Activated Ras, a guanine nucleotide-binding protein, initiates the MAPK phosphorylation and activation cascade by activating of the serine/threonine kinase Raf1 [1]. Raf1 is a MAPK kinase kinase (MAPKKK) that phosphorylates and activates MAPK kinases (MAPKKs), including MEK. This in turn phosphorylates and activates MAPK extracellular signal-regulated kinase 1 (Erk1) and Erk2 (Figure 2) [3]. Erk kinase activity is regulated by its phosphorylation. The transcription factor Elk1 is one of downstream molecule of Erk1 and induces Fos expression. Consequently, the activation of the activator protein-1 (AP1) complex constituted by Jun and Fos is sustained by the DAG–Ras pathway. Jun is activated via the Vav1–Rac pathway. In addition, Erk activation results in phosphorylation of signal transducer and activator of transcription 3 (STAT3) and Lck.

PKCθ-dependent NF-κB and AP1 pathways

Increased cellular concentrations of DAG also activates NF-κB signaling through PKCθ, a PKC family member that contains a lipid-binding domain specific for DAG. This domain is required for recruiting PKCθ to the lipid raft after TCR engagement. Moreover, Lck-mediated PKCθ phosphorylation appears to contribute to conformational changes required for binding of PKCθ to the lipid membrane. This concentration of PKCθ within the lipid raft enhances binding of PKCθ to DAG and results in PKCθ activation.

NF-κB plays its most important and evolutionarily conserved role in the immune system by regulating genes involved in inflammatory and immune responses as well as in some aspects of cell growth, survival and differentiation [4]. Both canonical (classical) and noncanonical (alternative) activation of this pathway in T cells are intimately involved in TCR-mediated T cell activation. PKCθ-mediated NF-κB induction has been shown to be selectively mediated by IKKβ that is

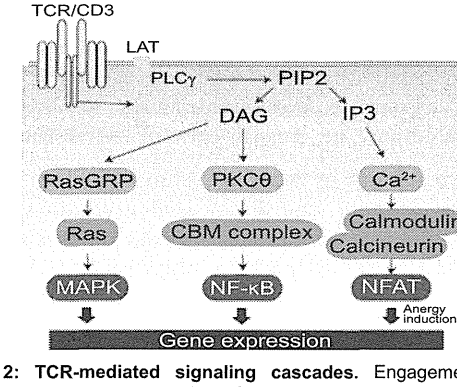

Figure 2: TCR-mediated signaling cascades. Engagement of TCR consequently induces expression of numerous genes required for T cell activation by triggering several signaling pathways, including Ras–MAPK pathway, NF-κB pathway, and NFAT pathway. Each of them specifically but collaboratively acts during T cell activation or anergy induction processes.

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associated only with the canonical pathway, whereas noncanonical activation in T cells remains elusive. One study showed that MAP3K14 (also called NF-κB-inducing kinase; NIK), which is an essential factor for the noncanonical NF-κB cascade, was required for complete T cell activation [5].

Following TCR stimulation, PKCθ regulates the assembly of a CBM complex (CARMA1/Bcl10/MALT1) through its phosphorylation of CARMA1, which is required for CARMA1 oligomerization and association with Bcl10 [6]. MALT1 physically binds to Bcl10 and induces polyubiquitination of IKKγ, the regulatory subunit of the IKK complex, via activation of the E3 ubiquitin ligase TRAF6. K63 polyubiquitination appears to have a role in IKKγ activation, which results in IκB phosphorylation by IKK catalytic subunits and subsequent IκB degradation. Nuclear localization of NF-κB heterodimers induces gene activation (Figure 2). NF-κB activation is also regulated by costimulatory signaling (described below).

The original analysis of PKCθ knockout mice revealed that two transcription factors, NF-κB and AP1, are targets of PKCθ in TCR/CD28-costimulated T cells [7-9]. The pathway leading from PKCθ to AP1 activation is less clearly understood. One study has reported that SPAK, a Ste20-related MAP3K, is a direct substrate of PKCθ in the pathway leading to AP1, but not NF-κB, activation [10]. SPAK is most likely a mediator of PKCθ signals leading to AP1 activation, but intermediates downstream of SPAK in AP1 activation remain to be identified and functionally characterized.

Ca^{2+}-dependent NFAT pathway

PLCγ1-mediated IP3 generation stimulates Ca^{2+} permeable ion channel receptors (IP3R) located on the endoplasmic reticulum (ER). This leads to the release of ER Ca^{2+} stores into the cytoplasm. Because Ca^{2+} ions are universal second messengers in eukaryotic cells, TCR-induced increases in intracellular Ca^{2+} levels result in the activation of Ca^{2+}- and calmodulin-dependent transcription factors, including phosphatase calcineurin and the Ca^{2+}/calmodulin-dependent kinase (CaMK). Activated calcineurin then dephosphorylates members of the nuclear factor of activated T cells (NFAT) family, which results in their translocation to the nucleus. In the nucleus, NFATs form cooperative complexes with various other transcription factors, and this results in differential gene expression patterns and functional outcomes, depending on the context in which the TCR signal is delivered [11]. AP1 proteins are the main transcriptional partners of NFAT during T cell activation. Cooperation between NFAT and AP1 integrates two of the main signaling pathways i.e. the Ca^{2+} signaling and RAS-MAPK pathway. The NFAT-AP1 cooperation during T cell activation is responsible for a specific pattern of gene expression, which induces the functional changes that characterize an activated T cell. In the absence of AP1, NFAT proteins activate a distinct program of gene expression. The products of these genes inhibit T cell function at different levels and induce a status of T cell unresponsiveness, which is one of processes to induce the immune tolerance [12]. Thus, NFAT proteins control two opposing aspects of T cell function i.e. activation and anergy [13] (Figures 2 and 3).

Costimulation Signaling

A most important gene induced by TCR-mediated signaling is *IL-2*, which can activate STAT pathways. However, full induction of IL-2 expression is not achieved by TCR signaling alone, i.e., a nonresponsive state (anergy) exists in which T cells are refractory to restimulation. Additional engagement of other cell surface receptors provides and integrates signals required for anergy avoidance and productive T cell activation. Although many cell surface receptors can participate in costimulation signaling, CD28 provides more robust signals than other costimulatory molecules.

Weak TCR ligation does not lead to cell proliferation and differentiation; rather, it results in anergy or cell death. However, if engaged, CD28 strongly amplifies a weak TCR signal. In contrast, triggering of CD28 alone results in the transient expression of only a few genes and has no obvious biological consequences. A question has arisen whether TCR and the coreceptors induce separate signaling pathways (qualitative model) or whether the signaling pathways triggered by both receptor systems are entirely overlapping (quantitative model) [14].

Many studies have indicated that the signals generated by CD28 ligation have no unique effects. A microarray study showed that TCR-induced expression of thousands of genes in primary T cells was amplified (or suppressed) to varying degrees by CD28 costimulation, but that no new gene was induced by CD28 costimulation [15]. Indeed, all of the factors identified as components of the CD28 signaling pathway are those that are implicated in TCR-mediated signaling. These include PI3K, the Tec family of PTKs, such as Itk, Vav1, and the serine/threonine kinase Akt (also known as PKB), and NF-κB signaling.

CD28-mediated PI3K–Akt pathway

PI3K recruitment is a key event for coupling CD28 to several signaling pathways. Following engagement of CD28 with its ligands CD80 or CD86 expressed on APCs, the p85 regulatory subunit of PI3K associates with the phosphorylated cytoplasmic tail of CD28. This regulatory subunit recruits the p110 catalytic subunit of PI3K, which can convert PIP2 to PIP3 in the cell membrane. Then, the locally generated PIP3 serves as a docking site for the PH domains of PDK1 and its target Akt (Figure 3).

Akt phosphorylates multiple proteins involved in numerous cellular responses. Activated Akt enhances the nuclear translocation of NF-κB by associating with CARMA1 and facilitating the assembly of the CBM complex, a step critical for NF-κB activation [16]. In addition, one well-known Akt target is GSK-3, a serine/threonine kinase that influences the nuclear export of NFAT as well as the Wnt pathway. Thus, Akt-mediated GSK-3 inactivation might be a pathway responsible for prolonged NFAT nuclear localization and thus *IL-2* transcription following CD28 costimulation (Figure 3).

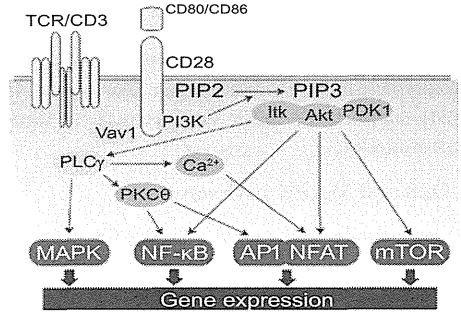

Figure 3: Costimulation signaling. Additional engagement of cell surface receptors such as CD28 provides and integrates signals required for anergy avoidance and productive T cell activation. The signaling generated by CD28 ligation has no unique effects but enhances the T cell signaling, including pathways of PI3K–Akt–mTOR, NF-κB, NFAT-AP1, and MAPK.

CD28/PI3K-generated PIP3 also provides a docking site for the PH domain of Itk. As described above, Itk associates with the TCR downstream complex consisting of LAT/SLP-76/Gads/PLCγ1 (Figure 3). Importantly, the localization and activation of this signaling complex depends on PI3K-generated PIP3.

A novel signaling mechanism has been suggested where by CD28 regulates cAMP degradation in T cell rafts through the recruitment of an Akt/β-arrestin/PDE4 complex [17]. CD28/PI3K-mediated PIP3 production recruits this supramolecular complex to lipid rafts, with recruitment occurring through the Akt PH domain.

Thus, although many of these pathways are activated by TCR ligation alone, the magnitude and maintenance of T cell activation signaling required for an appropriate response appear to be considerably regulated by costimulatory signals. This suggests that CD28 engagement primarily functions in a quantitative rather than a qualitative change in T cell activation.

CD28-mediated NF-κB pathway

Another important CD28 mediator is NF-κB signaling. CD28/PI3K generates the second messenger PIP3 that binds to various proteins harboring PH domains, including PDK1 and Akt. At present, we know that Akt is not an essential component of NF-κB signaling, at least in T cells. Akt appears to function as a rheostat; Akt can gently modulate the strength of the NF-κB cascade through its interaction with CARMA1 (Figure 3) [16]. Of note, PDK1, which is recruited to newly generated PIP3, can efficiently bind to both PKCθ and CARMA1 [18]. CD28 facilitates NF-κB activation by regulating the recruitment and phosphorylation of PDK1, which are necessary for the efficient binding of PDK1 to PKCθ and CARMA1 and thus for NF-κB induction. Furthermore, TCR/CD28 confers the noncanonical NF-κB activation in naïve T cells [19]. Taken together, NF-κB signaling is one of the major signaling pathways regulated by costimulation. Full activation of the NF-κB pathway requires CD28-mediated costimulatory signals in T cells.

CD28–Vav1 pathway

Vav1 is a guanosine exchange factor (GEF) for several small GTPases, including RAC1, RAC2, and RHOG. Vav1 controls several biochemical processes, such as those involved in cytoskeletal rearrangements. In addition, Vav1 strongly amplifies CD28-mediated costimulation-dependent activation of NFAT, NF-κB, CD28 response element, and JNK [20,21].

T cell development is markedly reduced in Vav1-deficient mice [22]. Of note, a CD28 mutant, which is unable to activate Vav1, does not alter TCR-directed ZAP-70 and LAT phosphorylation, but it does affect SLP-76, PLCγ1, and Itk phosphorylation and Akt activation [23]. This phenotype, which is remarkably similar to that of Vav1-deficient T cells [24], supports a role for Vav1 as a crucial signaling effector of CD28 costimulation.

TCR/CD28 and MAPK pathways

TCR engagement activates the ERK, JNK, and p38 cascades in T cells. With regard to costimulation effects, one study demonstrated that T cell activation involves these three MAPK cascades [25]. pMHC ligation directly activates the Ras–MEK–ERK pathway, as described above. JNK activation stimulated by TCR engagement requires CD28 coligation in T cell clones. However, JNK activation is not observed in mouse primary T cells. TCR and CD28 synergize after coligation to elicit enhanced p38 MAPK activation. p38 MAPK, but not JNK, is involved in signal integration during costimulation of naive mouse primary T cells. Indeed, the p38 MAPK inhibitor SB203580 blocks CD28-dependent proliferation and IL-2 production in human T cells [26]. We currently do not know how p38 modulates TCR signaling and IL-2 production.

Studies using both pharmacological and genetic manipulations have provided evidence that p38 is a crucial mediator of T cell development, activation, and inflammation [27]. In addition, p38α function in T and B cells has been addressed using the RAG-deficient blastocyst complementation method. Surprisingly, p38α-deficient T and B cells developed in normal numbers and proliferated normally in response to various stimuli, such as antigen–receptor ligation [28]. Moreover, mice that lack p38β appear to be completely normal with no obvious defects in T cell development or LPS-induced cytokine production [29]. To reconcile the genetic and pharmacological data with the paucity of observable abnormalities in p38 isoform-specific knockouts, further characterizations and detailed analyses of the p38 pathway in both normal T cells and T cell malignancies will be necessary.

Signaling Pathways Linking to T cell Activation, Development, and Disorder

In addition to TCR/CD28 engagements, several other signaling inputs participate in the regulation of T cell fates. These comprise pathways responsible for the development and differentiation of several cell types. Of note, deregulation of these signaling pathways lead to T cell disorders.

NF-κB signaling

In addition to TCR engagement-mediated NF-κB activation, pathological studies have strongly demonstrated that NF-κB activation is often sustained in T cell disorders. ATL cells show strong, constitutive NF-κB activation that contributes to their prolonged survival, proliferation, and invasiveness [30]. In particular, human T cell leukemia virus type 1 (HTLV-1) Tax is an intracellular stimulator of IKK. This stimulation is based on the physical interaction between Tax and IKK, which leads to the persistent activation of NF-κB-mediated transcription. Formation of the Tax/IKK complex relies on the physical interaction between Tax and the IKK regulatory subunit IKKγ. The Tax–IKKγ interaction is required for recruiting Tax to IKK catalytic subunits and for Tax-mediated IKK activation (Figure 4) [31].

In leukemic cells in which Tax is not expressed, a noncanonical NF-κB cascade appears to be important for the cellular characteristics of ATL. Both the canonical and noncanonical NF-κB pathways are persistently activated because NIK is aberrantly expressed in ATL cells [32]. NIK plays a central role in noncanonical NF-κB signaling through IKKα phosphorylation [33], and its constitutive expression leads to aberrant NF-κB activation in various malignancies, including B cell lymphoma, multiple myeloma, breast cancer, pancreatic cancer, and ATL [30].

Recently, we identified a novel molecular link between NF-κB activation and miRNA deregulation. Comprehensive gene expression analysis and *in vitro* experiments showed that miRNA-31 (miR-31) could regulate NIK expression through the 3′ untranslated region (UTR) in several cell types. In ATL cells, miR-31 expression was genetically and epigenetically silenced, which in turn induced constitutive NF-κB activation through NIK expression (Figure 4) [34]. Because current evidence clearly indicates that miR-31 dominates NF-κB activity in T cells, manipulating cellular miR-31 levels may be a novel molecular approach to reduce NF-κB activity and induce cellular apoptosis.

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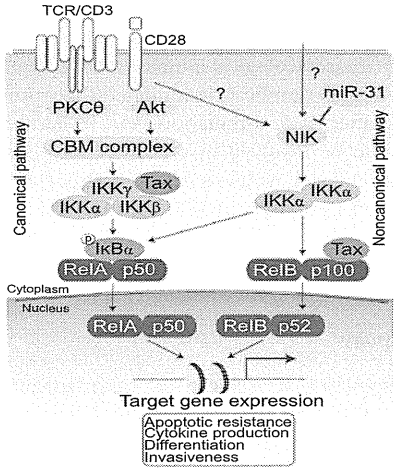

Figure 4: NF-κB signaling in T cell. Canonical and noncanonical pathways contribute to gene expression required for apoptotic resistance, inflammatory cytokine production, appropriate differentiation, and invasiveness. In HTLV-1 infected cells, viral protein Tax binds and drastically activates both pathways. In transformed leukemic cells, loss of miR-31 leads to NIK accumulation and persistent NF-κB activation.

Why is NF-κB signaling important for ATL cell survival? One of the target genes is Bcl-xl, which is expressed when NF-κB is activated. In HTLV-1-infected cell lines, Bcl-xl is expressed through the Tax–NF-κB pathway. Interestingly, fresh ATL samples exhibit Bcl-xl overexpression. Given that Bcl-xl is a principal anti-apoptotic protein as a Bcl-2 family member, persistent Bcl-xl expression is one of the molecular means to resist apoptosis, which may contribute to clinical chemoresistance. Indeed, inhibition of the NF-κB pathway by NIK depletion leads to impaired Bcl-xl expression and apoptotic death of ATL cells [34].

Because prevention of NF-κB activation showed good results in a xenograft model of cell lines with and without Tax [30], molecular targeting therapy based on the NF-κB pathway is a promising new treatment for ATL. Of note, we found that specific inhibition of NF-κB by DHMEQ could also remove virus-carrying cells from carrier peripheral blood mononuclear cell (PBMC) samples [35].

mTOR pathway

The signaling pathway target of rapamycin (TOR) is at the intersection between cell growth and starvation. The evolutionarily conserved kinase mammalian TOR (mTOR; officially known as the mechanistic target of rapamycin) controls cell growth and metabolism-related response to environmental inputs by regulating gene expression, which has been implicated in disease states, such as cancer, metabolic diseases and ageing. In T cell regulation, mTOR signaling is also involved in immune signals and metabolic cues for the proper maintenance and activation of T cells. Under resting conditions, mTOR signaling is strictly controlled by multiple inhibitory mechanisms, which enforces normal T cell homeostasis. T cell activation through antigen recognition triggers mTOR activation, which in turn influences the differentiation patterns of these cells.

mTOR, which belongs to the family of PI3K-related protein kinases (PIKK), is a conserved serine/threonine kinase and plays a central role in the regulation of cell growth and metabolism [36]. Diverse environmental signals, such as nutrients and growth factors, deliver their signaling inputs to the PI3K–Akt axis. Many upstream signals activate the mTOR complex 1 (mTORC1) via the GTP-loaded small GTPase RHEB. The activated PI3K–Akt pathway phosphorylates the Thr1462 residue of tuberous sclerosis 2 (TSC2), which in turn inhibits the TSC1/2 complex. Because RHEB is tightly regulated by the TSC1/2 complex, TCR/CD28 engagement activates RHEB/mTORC1 via the PI3K–Akt pathway. Recent studies have shown that RHEB deficiency in T cells reduced mTORC1 activation in response to TCR stimulation [37]. In addition, loss of TSC1 disrupts the TSC1/2 complex and enhances basal and TCR-induced mTORC1 activity [38,39]. Therefore, these results suggest a crucial role for the TSC–RHEB axis in T cell responses.

TCR engagement can activate both mTORC1 and mTORC2 within minutes. The strength of mTOR activation appears to be directly correlated with the duration of T cell–APC interaction and the cognate antigen dose. It should also be noted that mTOR activity is further conferred by costimulatory signals. CD28-mediated costimulation is a major driving force for the PI3K–AKT axis (described above), which in turn upregulates TCR-induced mTOR activity to facilitate productive T cell activation (Figure 5) [40,41]. In addition to CD28, another costimulatory receptor, OX40, which is a member of the tumor necrosis factor receptor (TNFR) family, assembles a signaling complex by recruiting PI3K–AKT to augment TCR-dependent Akt signaling; this may also affect mTOR signaling in T cells [42].

Several genetic studies have strongly suggested that the mTOR pathway is closely associated with T cell differentiation and homeostasis [43]. In particular, $CD8^+$ T cell differentiation is strictly regulated by mTOR signaling [44]. In addition, mTOR signaling appears to be involved in T helper (Th) cell differentiation and proliferation [43]. In sum, mTOR dictates the cell fate decisions of effector and regulatory T cells.

A common hematological malignancy, T-ALL, frequently harbors activating mutations in *NOTCH1* and/or loss-of-function mutations in a gene encoding phosphatase and tensin homolog deleted on chromosome 10 (PTEN). PTEN plays critical roles in cell growth, migration, and death because it can inhibit the PI3K–Akt pathway by catalyzing PIP3 dephosphorylation. It is noteworthy that both of these mutations can activate mTOR signaling, which suggests a pivotal role for mTOR in T-ALL development. Taken together, mTOR-mediated T cell fate decisions are of particular importance because of the unique functions of the mTOR pathway. Recent experimental advances have established that mTOR is a fundamental determinant of T cell homeostatic and functional fates. In addition, therapeutic targeting of mTOR may have beneficial effects for treating T cell disorders.

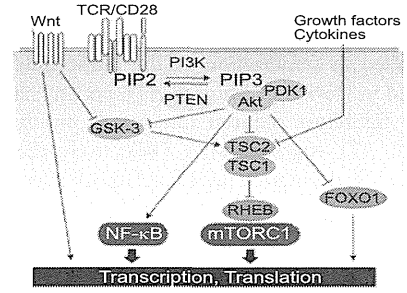

Figure 5: PI3K–Akt–mTOR axis. After TCR/CD28 stimulation, activated PI3K enhances phosphorylation of PDK1 and Akt. The Phosphorylated Akt in turn inhibits tumor suppressor TSC2, resulting in activation of mTORC1. In addition, Akt supports NF-κB signaling through CBM complex.

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Notch pathway

Notch proteins are cell-surface receptors and their expression is widely conserved in numerous species. It has been shown that Notch signaling is essential for T cell lineage development, thymocyte survival, and proliferation of T cell progenitors [45]. In addition, Notch signaling has been extensively studied in T-ALL because several lines of genetic evidence highlight the Notch signaling cascade as a central player in T-ALL pathogenesis [46].

Notch proteins are synthesized as a single polypeptide (approximately 300 kDa). After intracellular modifications that include fucosylation, glysocylation, and cleavage, a Notch heterodimer associates with the plasma membrane. Signaling is triggered by the interaction of Notch with Delta-like ligands (DLLs) or Jagged ligands on the surface of instructing cells. This ligation induces proteolytic cleavage of the Notch receptor by disintegrin and metalloproteinase (ADAM) and mono-ubiquitination of the intracellular portion of the transmembrane Notch fragment. This final cleavage leads to the release of the Notch intracellular domain from the plasma membrane and its translocation to the nucleus.

CBF1/suppressor of hairless/Lag1 (CSL) represses transcription of Notch target genes. Following activation by Notch, CSL is converted into a transcriptional activator and activates the transcription of the same genes. Intracellular Notch displaces the corepressors from CSL and recruits coactivators, such as Mastermind-like 1 (MAML1), to activate the expression of target genes (Figure 6). Intracellular Notch is then degraded by a polyubiquitination–proteasome pathway.

A landmark study published in 2004 changed our perspectives dramatically by showing that more than 50% of T-ALL cases harbored Notch1 mutations, which resulted in the hyperactivation of the Notch pathway. This finding implicated Notch1 has a very important role in the pathogenesis of T-ALL [47]. This suggests that Notch likely interacts with several important cellular pathways and can cooperate with other oncogenes during leukemogenesis.

Although the importance of Notch signaling activation in T-ALL is well established, the detailed molecular mechanisms by which NOTCH1 induces T cell transformation remain unclear. The best-characterized direct target genes are the bHLH transcriptional repressor Hes1 and the transcription factor c-Myc. Recently, Hes1 was shown to be a key regulator in the induction and maintenance of T-ALL. Notch-mediated c-Myc expression is also crucial for maintaining T-ALL.

Recently, activating mutations in Notch were identified in more than 30% of ATL patients [48]. Of note, compared with the activating frameshift mutations observed in T-ALL, ATL showed single substitution mutations activating the Notch pathway. These gene mutations could reduce CDC4/Fbw7-mediated degradation, which resulted in stabilization of the intracellular NOTCH1. They also resulted in Hes1 expression in ATL patient samples. In addition, γ-secretase inhibitors reduced tumor cell proliferation and tumor formation in ATL-engrafted mice. Collectively, these findings suggest that activated Notch may be important for ATL leukemogenesis.

There is mounting evidence that Notch signaling is also an important pathway for mediating cell fate decisions in developing thymocytes and peripheral T cells. Strong Notch signaling restricts the multilymphoid progenitors of the T cell lineage and promotes thymocyte development [49]. Results from several studies support a role for Notch in the generation of single-positive cells from double-positive precursor thymocytes [50].

Notch proteins are now emerging as potentiators of TCR/CD28 signaling in mature T cells. Some studies have confirmed the roles for Notch receptors in T cell activation, proliferation, and cytokine production. Pharmacological inhibition of the Notch pathway in $CD4^+$ and $CD8^+$ T cells significantly inhibited their proliferation and IFNγ production after TCR stimulation [51]. In addition, Notch signaling was shown to be involved in a positive-feedback loop of IL2–IL2R that regulated T cell proliferation [52]. Important pathways activated through Notch1 signaling include the PI3K–Akt and mTOR signaling cascades. In addition, NF-κB signaling, which can be activated through TCR/CD28 signaling, appears to be sustained by the Notch pathway. These details are discussed in a later chapter.

Wnt pathway

Wnt signaling is subject to strict molecular control. Thus, deregulated Wnt signaling has been implicated in the development of several malignancies, including the leukemias and lymphomas [53]. To date, at least three different Wnt pathways have been identified: the canonical Wnt pathway, which involves β-catenin and the T cell factor (TCF)/lymphocyte-enhancer-binding factor (LEF) family; the planar cell polarity pathway; and the Wnt/Ca^{2+} pathway. Among these, the canonical (Wnt/β-catenin) pathway is primarily involved in T cell development and proliferation [54].

One important study demonstrated that a T cell-specific defect in Wnt signaling by deletion of β-catenin impaired T cell development at the β-selection checkpoint, which resulted in a decrease in splenic T cells. In addition, β-catenin appeared to be a target of TCR signals in thymocytes and mature T cells. These results indicate that β-catenin-mediated signals are required for normal T cell development [55].

TCF1 is highly expressed in T cells. A recent study showed that β-catenin expression was stabilized after TCR-mediated T cell stimulation, resulting in the upregulation of TCF1-dependent gene expression in T cells [56]. It also suggested that TCF1 initiated Th2 differentiation of activated $CD4^+$ T cells by promoting GATA-3 expression and suppressing IFN-γ expression. In addition, during T cell development, TCR signaling was shown to stabilize β-catenin and regulate the response to αβTCR engagement [57]. In sum, several genetic studies have suggested that the canonical Wnt pathway participates in T cell development. In $CD8^+$ T cells, activation of Wnt/

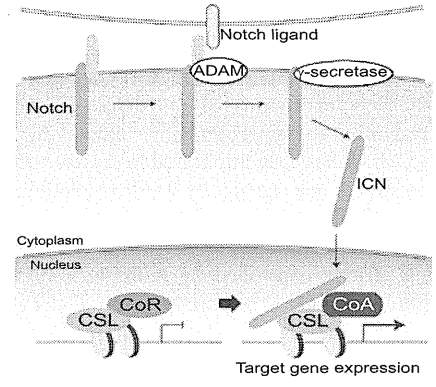

Figure 6: Notch signaling. After intracellular modifications, the Notch heterodimer associates with the plasma membrane. Signaling is triggered by the interaction with Notch ligands, which induces proteolytic cleavage of the Notch and leads to the nuclear translocation of the Notch intracellular domain (ICN). The intracellular Notch displaces the co-repressors (CoR) from CSL and recruits co-activators (CoA) to activate the expression of target genes.

β-catenin signaling inhibited effector differentiation and promoted the generation of self-renewing $CD8^+$ memory stem cells and central memory T cells. In $CD4^+$ T cells, Wnt/β-catenin signaling facilitated Th2 polarization and enhanced the survival of naturally occurring regulatory T cells (nTreg). Canonical Wnt signaling has also been suggested as important for the self-renewal of hematopoietic stem cells (HSCs) and progenitor compartments. β-catenin deletion perturbs HSC survival, self-renewal, and their subsequent development into T cells [58,59].

Because the Wnt pathway is important for normal T cell development and signaling pathways, constitutively active Wnt signals can lead to T cell acute malignancies. An experimental model of Wnt activation demonstrated that the constitutively active form of β-catenin led to development of an aggressive T cell lymphoma that could invade the bone marrow and was transplantable into irradiated recipient mice [60]. These data suggest that Wnt-dependent c-Myc expression is required for lymphomagenesis. Interestingly, this appears to be independent of Notch signaling that has been thought to be an analog of the Wnt pathway. Crosstalk between these pathways will be discussed in a later chapter.

Interestingly, modest transgenic expression of β-catenin in thymocytes enhances the generation of $CD8^+$ thymocytes and accelerates thymic involution [61]. Some evidence indicated that oncogenic β-catenin induced p53-independent oncogene-induced senescence and p53-dependent apoptosis, which protected developing thymocytes from transformation [62]. The molecular mechanisms induced by the Wnt pathway in T cells remain to be elucidated.

Hedgehog (Hh) pathway

The Hh family of secreted intercellular molecules, including Sonic, Indian, and Desert Hh, regulates target gene transcription through GLI transcription factors. This family is of pivotal importance in embryogenesis and the homeostasis of adult tissues, as well as cell differentiation, cell cycle progression, and cell survival [63].

Hh signaling is important in T cell development in both humans and mice [64].The generation of mature functional T cells in the thymus requires sonic Hh (Shh) signals from the thymic epithelium. Shh-induced signaling is important for the differentiation and proliferation of early thymocyte progenitors. Shh also modulates TCR signaling during repertoire selection.

Hh signaling is involved in the conversion of immature DP cells to mature SP thymocytes and can affect TCR repertoire selection and CD4/8 lineage choices. Depletion of Hh signaling promotes CD4 lineage commitment and results in an increased CD4:CD8 ratio. In contrast, increased Hh signaling results in a reduced CD4:CD8 ratio, suggesting that Hh signaling is an inducer of $CD8^+$ T cell development [64,65].

Hh is also involved in peripheral T cell activation. Additional Shh supports TCR/CD28 signaling and enhances T cell activation and subsequent proliferation [66-68]. In contrast, Hh activation by the expression of the constitutive active form of Gli2 results in inhibition of TCR/CD28-mediated T cell activation. In this case, Hh appears to inhibit TCR-dependent Erk phosphorylation [65]. Modulation of TCR signal strength by Hh has profound implications for immunity. Thus, the precise molecular involvement of Hh in T cell signaling needs to be examined.

Hh signaling is implicated in several hematological malignancies, including chronic lymphocytic leukemia (CLL), plasma cell myeloma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), ALK-positive anaplastic large cell lymphoma (ALCL), chronic myelogenous leukemia (CML), and acute leukemias (AML). ALK+ ALCL is an aggressive type of non-Hodgkin's lymphoma of the T cell/null lineage. One study demonstrated that Hh ligands and GLI1 were highly expressed in ALK+ ALCL [69]. NPM-ALK activates the PI3K–Akt pathway, which contributes to GSK-3β inactivation. Because GLI1 and GLI2 are the targets of GSK-3-mediated proteasomal degradation, NPM-ALK activates Hh signaling through the PI3K–Akt–GSK-3 cascade. Of note, we have demonstrated that the NPM-ALK abrogates CD30 signaling and constitutive NF-κB activation in ALCL cells [70]. The reciprocal relationship between Hh and NF-κB signaling in T cells remains unclear.

TGF-β-mediated signaling

The TGF-β superfamily regulates many cellular functions, including cell growth, differentiation, migration, and apoptosis. TGF-β-mediated signaling is essential for embryonic development, embryogenesis, and cell fate decisions [71].

TGF-β is synthesized in an inactive form consisting of a TGF-β dimer in association with the latency-associated protein (LAP). Signaling is initiated by a conformational change in a tetrameric complex consisting of TGF-β receptor II (TGF-βRII), TGF-β receptor I (TGF-βRI), and activated TGF-β dimer. Activated TGF-βRI then phosphorylates the transcription factors Smad2 and Smad3 and triggers their translocation into the nucleus. Smads then bind to the specific regulatory sequences of target genes and regulate gene expression by recruiting transcription cofactors.

TGF-β regulates T cell development, homeostasis, tolerance, and immunity [72]. TGF-β signaling promotes the differentiation of thymic T cells into natural killer T cells, nTreg cells, and $CD8^+$ T cells. TGF-β signaling is likely to be pivotal for the survival of peripheral $CD4^+$ and $CD8^+$ T cells with low affinity. In contrast, it inhibits the proliferation and differentiation of $CD4^+$ and $CD8^+$ T cells with high affinity.

In particular, TGF-β plays central roles in Treg cell regulation. It inhibits proliferation of Treg cells, although it supports their maintenance in peripheral lymphoid organs, which can control Treg cell survival. TGF-β promotes the differentiation of induced Treg (iTreg) cells, which is potentiated by IL-2 and retinoic acid (RA). In the presence of IL-6, TGF-β drives the differentiation of Th17 cells and supports their maintenance.

The interplay between TCR and TGF-β signaling has been implicated in the regulation of several genes, including FoxP3, which is important for Treg functions [73]. However, because most studies have mainly performed phenotypic analyses, the exact molecular signaling pathway induced by TGF-β in T cells is not completely understood.

Signalings from receptors of innate immune cells

Innate and adaptive immune responses have many interactions that are regulated by the balance of signals initiated by several activatory and inhibitory receptors. Toll like receptor (TLR) expression has been generally assigned to professional APCs, such as dendritic cells. However, several recent studies have demonstrated that the low expression levels of TLRs could also be detected on naïve and memory T cells [74,75]. In addition, TLR agonists can directly target TLRs expressed on T cells and function as signal inducers.

Engagement of the TLR1/2 complex was associated with T cell

proliferation, survival, and functions [76]. Ligation of TLR3 on effector $CD8^+$ T cells increased IFN-γ secretion by these cells. Interestingly, the effects of CpG (TLR9 ligand) on $CD4^+$ T cells were mediated by MyD88 and PI3K-dependent mechanisms [77]. Another study showed that ligands for TLR3 and TLR9 directly enhanced T cell survival, which was sustained by NF-κB signaling [78]. Collectively, these findings suggest that TLR agonists can directly target TLR signaling in effector $CD4^+$ and $CD8^+$ T cells. We do not understand the molecular mechanisms by which TLR signaling can affect the other sustained T cell signaling.

NKG2 receptors are expressed by natural killer (NK) cells and a subset of $CD8^+$ T cells. They regulate the development and function of NK cells by inhibiting and activating cytotoxicity and promoting cell survival. The CD94/NKG2 heterodimers consist of one CD94 molecule and one NKG2 family member, including NKG2A and its splice variant NKG2B, which form inhibitory receptors, as well as NKG2C, NKG2E, and NKG2H, which form activating receptors [79]. NKG2D is unique in that it does not dimerize with CD94; instead, it associates with the adaptor molecules DAP10 and DAP12 for its activating function [80]. Among these, the acquisition of CD94/NKG2A expression by $CD8^+$ T cells could play a role during infection by pathogens. This has been observed in mice infected with a variety of pathogens, including lymphocytic choriomeningitis virus, herpes simplex virus (HSV)-1, influenza virus, polyomavirus, and *Listeria monocytogenes* [81]. Furthermore, HIV-1 infection has also been implicated in the upregulation of CD94/NKG2A [82,83]. The expression of CD94/NKG2A, which is acquired early during the infection and persists after clearance of the pathogen, does not interfere with the activity of $CD8^+$ T cells. However, the CD94/NKG2A expression inhibits antiviral $CD8^+$ T cell responses [84]. In addition, high level of CD94/NKG2A expression on $CD8^+$ T cells protects them from apoptosis, thereby perhaps inducing in the generation and maintenance of memory cells.

Emerging Regulatory Factors: Learning from Malignancies

Recent experimental evidence indicates the presence of additional molecular hallmarks of T cells. miRNAs regulate gene expression patterns in all cell types. Epigenetic gene regulation is based on chromatin regulation. Both of these are pivotal for development, differentiation, proliferation, and other cellular processes in hematopoietic lineage. Their deregulation is frequently observed in T cell disorders, which strengthens the fact that miRNA-mediated gene regulation and epigenetic mechanisms may be important for maintaining T cell homeostasis.

miRNA in T cells

One of the most significant recent advances in biomedical research has been the discovery of the 22-nt-long class of non-coding RNA designated miRNA that post-transcriptionally regulates gene expression by binding to the target mRNAs. miRNA is expressed by all metazoans and plants, as well as by several DNA viruses; it regulates cellular processes such as development, differentiation, growth, homeostasis, stress responses, apoptosis, and immune activation [85].

Some recent extreme studies have collectively suggested several concepts. First, miRNAs have unique roles as time-sensitive gene regulators. Second, miRNAs have distinct functions in distinct cell types. Third, miRNAs regulate gene expression by incompletely repressing their targets. These specific functions of miRNAs as "fine-tuners" are apparently involved in immune systems as well as hematological cancers. It is not surprising that miRNA deregulation appears to play important roles in human cancers.

miRNAs provide modest changes in gene expression [86]. However, it is also true that gene expression patterns can occasionally be changed drastically by a change in a single miRNA that regulates a key transcription factor. For example, a decrease in a miRNA, miR-150, leads to drastic deregulation of the B cell transcriptome by regulating c-MYB [87]. Furthermore, given that miRNAs are upstream of gene regulation systems, miRNAs confer robustness to several biological processes by controlling several feed-forward and feed-back loops [86]. Several recent reports including our own have demonstrated crosstalk between miRNA-mediated gene regulation and several signal transduction pathways in T cells.

Specific knockout of *Dicer*, which is essential for processing miRNAs and siRNAs in the T cell lineage, leads to impaired T cell development and aberrant Th cell differentiation and cytokine production [88,89]. In addition, peripheral $CD8^+$ T cell development was severely blocked after *Dicer1* deletion in the thymus. These cells were defective in miRNA processing, and after stimulation, they proliferated poorly, which resulted in their apoptosis. Dicer-deficient Th cells preferentially expressed INFγ. The levels of secreted IL-2 in *Dicer*-deficient and control cultures were comparable after primary stimulation, and addition of exogenous IL-2 to the growth media did not rescue the proliferative defect of *Dicer*-deficient T cells, which suggested that Dicer might be involved in IL-2 receptor (CD25) signaling in T cells [89].

An early study revealed that miR-181a acted as an intrinsic antigen sensitivity "rheostat" during T cell development [90]. miR-181a expression enhanced the basal activation of TCR signaling and IL-2 production because miR-181a repressed multiple negative regulators of TCR signaling (Figure 7). Thus, several miRNAs probably participate in each step of T cell regulation. This in turn suggests that miRNA deregulation may cause catastrophic gene regulation in T cells.

Individual miRNAs have been implicated in T-ALL. miR-19b is

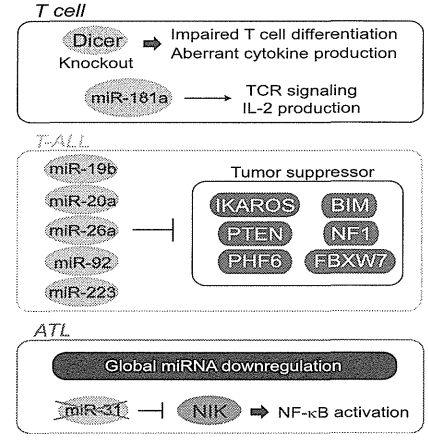

Figure 7: miRNA in T cell biology. Specific knockout of *Dicer* in T cell lineage leads to impaired T cell development and aberrant T helper cell differentiation and cytokine production. miR-181a expression enhances the basal activation of TCR signaling and IL-2 production (top box). In T-ALL cells, upregulation of five miRNA leads to repression of tumor suppressor genes implicated in T-ALL (middle box). Global downregulation of miRNA is the one of molecular hallmarks of ATL. In particular, miR-31 expression is genetically and epigenetically silenced, which is prerequisite condition of ATL cells. The loss of miR-31 consequently contributes to NIK over expression.

a member of the oncogenic miR-17-92 cluster, which is targeted by the t(13;14)(q32;q11) translocation in T-ALL [91]. Comprehensive methods have been used to address the miRNA candidates associated with abnormal gene expression and leukemogenesis. Recently, one study reported on miRNA signature in T-ALL [92]. A total of 430 types of miRNAs were analyzed in 50 clinical T-ALL and 18 T-ALL cell lines by quantitative RT-PCR, revealing that a small set of miRNAs was responsible for the cooperative suppression of several tumor suppressor genes. Cross comparisons of miRNA expression profiles in human T-ALL with the results of an unbiased miRNA library screen identified five miRNAs (miR-19b, miR-20a, miR-26a, miR-92, and miR-223), which were capable of promoting T-ALL development in a mouse model. Furthermore, these miRNAs produced overlapping and cooperative effects on tumor suppressor genes implicated in T-ALL, including *IKAROS, PTEN, BIM, PHF6, NF1,* and *FBXW7.* Thus, a comprehensive and unbiased analysis of miRNA actions in T-ALL reveals a striking pattern of miRNA–tumor suppressor gene interactions (Figure 7).

For ATL, some studies have reported several miRNA aberrations identified in HTLV-1-infected cells and ATL samples [93-95]. Very recently, we established global gene expression analysis in a large cohort ATL study that included analyses of mRNA expression, miRNA levels, and genomic copy numbers [34]. A strict threshold ($p < 1 \times 10^{-5}$) and two-dimensional hierarchical clustering analysis revealed 61 miRNAs with significantly altered expression levels in ATL cells (n = 40) compared with control $CD4^+$ T cells (n = 22). It is most important that primary ATL samples show global miRNA downregulation, similar to observations in other cancer researches [96,97]. Of the 61 miRNAs, 59 (96.7%) exhibited decreased expression in ATL (Figure 7).

Among these, miR-31 was the most profoundly repressed miRNA in all ATL individuals (fold change of 0.00403). This is a known tumor suppressor that may also be associated with metastatic breast cancer [98]. Other downregulated miRNAs found in ATL patients may also be involved in the hallmark capabilities of ATL because they were uniformly decreased in tested ATL samples and each miRNA may regulate a large number of genes.

Several predictions and experimental approaches have defined a novel miR-31 target gene, NIK, which acts as a persistent NF-κB activator in various malignancies (described above). Manipulation of the miR-31 level clearly indicated that the miR-31 level was negatively correlated with cellular NF-κB activity. Importantly, enforced miR-31 expression in B cells attenuated both BAFF- and CD40L-mediated NIK accumulation and subsequent canonical and noncanonical NF-κB signaling. Induced miR-31 expression or NIK knockdown reduces apoptotic resistant proteins such as BCL-XL and XIAP, which results in significant apoptosis among ATL cell lines as well as among primary leukemic cells from ATL patients [34]. Several lines of evidence definitively support two notions: (1) miR-31 acts as a tumor suppressor in T cells and (2) NIK-regulated NF-κB is of pivotal importance to cancer cell survival (Figure 7).Taken together, based on the results of genetic studies and those on T-ALL and ATL, abnormal miRNA expression has a critical impact on the development of hematological malignancies.

Epigenetic regulation

Technological advances in genomics and epigenomics have provided new methods to distinguish one cell type from another. The epigenetic code consists of the combined on–off states of hundreds of genes, which coordinately dictate cellular identity and function. Increasing attention is being paid to global regulatory factors and molecular mechanisms by which gene transcription control is regulated. Within these environments, genome programming operates fundamentally through DNA methylation, histone chemical modification, and protein complex binding.

The appropriate control of gene expression must be maintained in normal T cells by utilizing transcriptional networks as well as epigenetic controls. Indeed, several specific, comprehensive studies have demonstrated the epigenetic regulation of genes encoding for cytokines that are required for T cell development, differentiation, and activation [99]. Collectively, several global analyses have proposed that naïve T cell activation, differentiation, and lineage commitment result in epigenetic changes and that a fine balance between different histone modifications is required. In contrast, memory T cells are already poised and do not require epigenetic changes for short-term activation. It has been suggested that transcriptional memory, particularly epigenetic marks on chromatin, forms the underpinning of immunological memory [100-102].

Integrated histone modifications consequently decide the degrees of chromatin condensation and subsequent transcriptional sensitivity. Trimethylation of the histone H3 Lys27 (H3K27) mark plays a central role in repressing transcription, primarily in the euchromatin region. The polycomb family is a master regulator of the H3K27me3 level by inducing and maintaining the histone mark. Polycomb repressive complex 1 (PRC1) and PRC2 are recognized as essential molecular machines involved in polycomb-mediated gene silencing [103].

The polycomb family participates in the control of T cell activation. Enhancer of zeste homolog 2 (EZH2) serves as the catalytic subunit in PRC2 and mediates gene silencing by catalyzing H3K27 trimethylation in the promoters of target genes. One study showed that triggering of TCR and costimulation signaling in *Ezh2*-deficicint T cells resulted in an impaired proliferative response [104]. The mechanism by which Ezh2 can affect T cell signaling is not completely understood.

Deregulation by the polycomb family confers a specific gene expression pattern that is responsible for chronic proliferation, survival, peculiar development, and cancer-associated stemness in various cancer types, including ATL [105]. The involvement of the polycomb family in ATL was first revealed by global gene expression analysis. Significantly higher levels of EZH2 as well as RING1- and YY1-binding protein (RYBP) transcripts with enhanced H3K27me3 levels were found in ATL cells compared with normal $CD4^+$ T cells [106]. EZH2 is highly expressed in many cancer types, including breast and prostate cancers and lymphomas, and its expression is often correlated with advanced stages of tumor progression and a poor prognosis. We recently identified a notable gene silenced by polycomb. A human gene that encodes for miR-31, *hsa-miR-31,* is located at 9p21.3, which is adjacent to clusters of the *CDKN2* and *IFNA* families. In addition to loss of this gene (12.5% of ATL cases), transcription of the miR-31 precursor was completely lost in ATL cells. Computational predictions and experimental evidence clearly demonstrated that an assembly of YY1-binding motifs upstream of the miR-31 region was responsible for the occupancy of the polycomb family at the target region, which resulted in H3K27me3-dependent transcriptional repression. Of note, given that miR-31 is a master regulator of the ATL-specific gene expression pattern described above, polycomb-mediated loss can influence gene expression downstream of miR-31in T cells and other cell types (Figure 8) [34].

Intriguingly, several recent lines of evidence have shown two faces

of polycomb. It was surprising that these were some somatic mutations in the gene encoding EZH2 in some sets of mature B cell malignancies, including germinal center-type DLBCL (GCB-DLBCL) and follicular lymphoma [107]. However, subsequent studies have demonstrated that the mutations observed in B cell lymphomas sustained strong activity of histone methyltransferases.

Very recently, however, additional experimental evidence suggested that polycomb acted as a tumor suppressor in a cell type-specific manner. In disorders of HSCs or progenitors, such as myelodysplastic syndromes (MDS), myeloproliferative disorder (MPD), and AML, homozygous and heterozygous *EZH2* deletions or inactivating mutations are commonly observed [108], thus suggesting that loss of PRC2 contributes to oncogenesis in malignant myeloid diseases. Interestingly, inactivating mutations in H3K27 demethylase *UTX* have also been identified in myeloid malignancies. In immature T-ALL, EZH2 is suggested to be a tumor suppressor. Several experimental approaches and clinical observations have indicated that a loss of *EZH2* contributes to the development of aggressive T-ALL in mice and humans [109-111].

Collectively, several studies of hematological disorders, including ATL and T-ALL, have indicated that the polycomb family acts as an oncogene in differentiated cells, such as mature T and B cells, and as a tumor suppressor in undifferentiated cells, such as hematopoietic stem/progenitors, which is an origin of acute leukemia (Figure 8). Importantly, the differentiation of these immune cells is directly regulated by polycomb-dependent epigenetic mechanisms [99,105]. The epigenetic background may be involved in distinct outputs. It must also be noted that the polycomb family regulates large numbers of genes in an epigenetic manner and by other routes (discussed below).

Novel Concept: Signal Cross Talking in T cell Regulation

Each of the signaling pathways described above contributes to T cell biology. However, physical and functional interactions between these pathways may be involved in complex T cell regulation. Based on this concept, we discuss the possible molecular interconnections that affect T cell functions and fates (Figure 9).

Notch and PI3K–Akt–mTOR

Several genetic lines of evidence and studies on T-ALL have demonstrated that the Notch pathway has central roles in T cells. Complex gene regulation through Notch may be sustained by signal crosstalk. As described above, the PI3K–Akt and mTOR pathways are connected to the Notch pathway through PTEN regulation. Notch-mediated Hes1 expression results in the transcriptional repression of PTEN [112]. PTEN negatively regulates the PI3K–Akt–mTOR pathway. In addition, a linkage between the Notch and mTOR pathways has been implicated. Treatment with a γ-secretase inhibitor

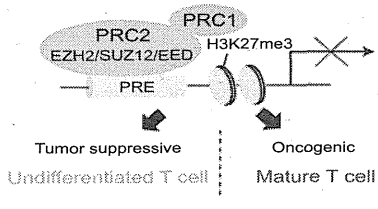

Figure 8: Mode of polycomb-dependent epigenetic gene regulation. Polycomb family is likely to act as an oncogene in differentiated cells such as mature T and B cells and as a tumor suppressor in undifferentiated cells such as hematopoietic stem/progenitors, which is an origin of acute leukemia.

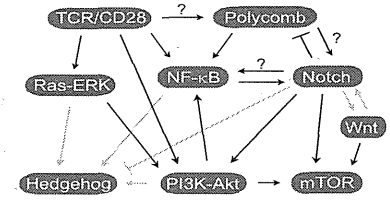

Figure 9: Crosstalk within signaling pathways. Each element contributes to regulation of gene required for T cell development, differentiation, and proliferation, respectively. However, substantially, they can interplay with each other, enabling T cell to from a more complicated regulatory network. The black lines represent relationship demonstrated in T cell. The gray ones show possible relationships suggested from non-T cell studies.

to inhibit the Notch pathway in T-ALL cell lines suggested a model in which Notch stimulated mTOR activity through a pathway that was independent of PI3K and Akt but dependent on c-Myc [113].

Notch and NF-κB

Crosstalk between Notch and NF-κB signalings has also been suggested. First, NF-κB2 (p100/p52) and IκBα have been identified as NOTCH1-targeted genes [114,115]. Second, the NF-κB component of the Rel family promoted the upregulation of Jagged1 expression and its functional interaction with Notch receptors on cocultured T cells [116]. Third, NF-κB subunits functioned as possible nuclear binding partners of intracellular Notch proteins. A complex containing Notch1, NF-κB1 (p50), and RelA could bind to DNA from the promoter region of the gene encoding IFNγ [117]. This study suggested that although an initial TCR-dependent NF-κB activation did not require the Notch signaling, intracellular Notch protein appeared to be necessary for sustained NF-κB activation in T cells. Finally, results of a recent study suggested that Hes1, which is positively regulated by Notch, appeared to sustain the NF-κB activation by repressing the expression of deubiquitinase CYLD, a negative regulator of the IKK complex, in T-ALL cells [118]. In contrast, some reports have indicated that Notch signaling could inhibit NF-κB activation by physical blocking mechanisms [119,120]. Delineation of the exact molecular mechanism involved in the crosstalk between Notch and NF-κB is urgently needed.

Notch and Wnt

The Notch and Wnt pathways, which were first discovered in *Drosophila*, are evolutionary conserved signaling pathways and control the earliest steps of T cell development. It would seem that the Wnt and Notch pathways have opposing functions in the thymus. Wnt signaling serves cell proliferation, whereas Notch signaling regulates cell differentiation. These processes are mutually exclusive. However, gain-of-function mutations in the positive effectors of the Notch (NOTCH1) and Wnt (β-catenin) pathways lead to cancer development, which indicates that both may participate in cell proliferation.

One report has suggested the molecular cooperation between the Notch and Wnt pathways in HSC self-renewal [121]. Notch signaling has a dominant function in inhibiting HSCs differentiation, in which Wnt signaling mediates cell proliferation. It is also suggested that Notch signaling is required for Wnt-mediated maintenance of undifferentiated HSCs but not for their survival or entry into the cell cycle. With regard to lymphoma development, β-catenin-dependent development of lymphoma appears to be independent of Notch signaling [60].

Molecular crosstalk between the Wnt and Notch pathways has

been described, including genetic interactions in *Drosophila*, physical interaction between Notch and β-catenin, and their associations with common cofactors. In mammalian cells, GSK-3β directly phosphorylates Notch protein and modulates its transcriptional activity [122]. Moreover, β-catenin activates Jagged1 transcription thus leading to Notch activation in colorectal cancer [123]. Inhibition of Wnt and Notch results in downregulation of a common gene program. Conversely, in different types of tumor cells, Notch activates the Wnt pathway and stabilizes β-catenin by unknown mechanisms in melanoma [124] or by the transcriptional activation of Slug [125] in breast cancer. More intensive physiological and molecular studies regarding the associations between Notch and Wnt signaling should be conducted.

Notch and the polycomb family

A recent study suggested that NOTCH1 activation specifically induced the loss of the repressive H3K27me3 by antagonizing the PRC2 activity [111]. This suggests a tumor suppressor role for PRC2 in human acute leukemia and a novel concept regarding the dynamic interplay between oncogenic NOTCH1 and PRC2 function for the regulation of gene expression and cell transformation. In immature lymphoid and myeloid cells, the polycomb family might act as tumor suppressors [109-111]. Conversely, in mature T lymphocytes, the polycomb family clearly functions as oncogenes. In ATL, both the polycomb and Notch pathways are strongly activated, which contributes to the expression of several genes and signaling pathways [30,34,48]. The two faces of the polycomb family need to be addressed in the near future.

Wnt and mTOR

In response to Wnt activation, GSK-3 inhibition appears to result in the inactivation of the tumor suppressor complex TSC1/2, followed by activation of the mTOR signaling pathway [126]. GSK-3 can phosphorylate and stimulate the TSC2 in a coordinated manner, which results in TORC1 inactivation and reduced protein synthesis. Therefore, Wnt activation can inhibit the TCS1/2 complex and activate mTOR signaling through GSK-3. In T cells, both the Wnt and mTOR pathways are involved in T cell development, as described above. Thus, the crosstalk between these pathways might shed some light on the molecular basis of T cell development. In addition, from a pathological viewpoint, this provides a novel therapeutic concept for Wnt signaling pathway-dependent cancers. Rapamycin may prove to be efficacious for treating tumors with activated Wnt.

Hedgehog and other signaling pathways

Hh signaling is indeed physiologically interconnected with Ras–Raf–MEK, PI3K–Akt, TGF-β, Notch, and NF-κB signaling in several types of cells [127]. In a complicated interaction, the Ras–Raf–MEK and PI3K–Akt pathways modulate the amount and activity of GLI, which results in the transactivation of Hh signaling-dependent gene expression. TGF-β also activates Hh signaling through GLI2 induction and binding with GLI family. In contrast, in mammalian skin, Notch deficiency increases GLI2 levels, which causes Hh activation and development of basal cell carcinoma-like tumors. This suggests opposing roles of Notch and Hh [128].

NF-κB activation is one of the mechanisms underlying Shh overexpression in pancreatic cancer. It has been suggested that proliferation of pancreatic cancer cells is accelerated by NF-κB activation, in part, through Shh overexpression [129]. These interconnections between numerous oncogenic pathways have important implications for T cell regulation. These connections associated with Hh signaling have not yet been reported in studies of T cells. Complex, yet hierarchical, gene regulation may be achieved during appropriate T cell development and homeostasis.

Polycomb and NF-κB

As described above, the polycomb family regulates the expression of a large number of genes by epigenetic molecular mechanisms. In turn, this suggests that gene expression fluctuations induced by the polycomb family may be involved in other signaling pathways that participate in T cell regulation.

Using ATL as a model of a mature T cell disorder, we have demonstrated that the cellular amounts of PRC2 components, EZH2 and SUZ12, indirectly affected the NF-κB signaling pathway [34]. Expression levels of EZH2 and SUZ12 directly strengthened the cellular miR-31 depletion, which in turn activated the NF-κB pathway through NIK induction and conferred anti-apoptotic features to T cells. The dynamic regulation of miR-31 by polycomb-dependent epigenetic machinery is critical for mature T cell-derived leukemic cell survival and other hallmark capabilities, such as promoting cell cycle, invasiveness, chronic proliferation, replicating immortality, and drug resistance, because both miR-31 and NF-κB signaling can predominantly control the expression of genes responsible for these processes [30]. It is also noteworthy that the molecular and biological interconnections between polycomb–miR-31–NF-κB are conserved in breast cancer cells and B lymphocytes. By organizing this new principle, various cell types may realize the more complex gene regulatory networks required for maintaining and executing various cellular functions. An imbalance in this network probably switches the cell fate from one to another.

Our findings on ATL study also provided new ideas: YY1 is one of the recruiters of PRC2 in T cells. In humans, polycomb response elements (PREs) have not been definitively identified. A good candidate for a mammalian recruiter of PRC2 is YY1, which is the homolog of *D. melanogaster* PHO and is abundantly expressed in almost all cell types [130]. The detailed mechanism by which YY1-mediates the recruitment of the polycomb family may be important with regard to epigenetic regulation of orchestrated gene expression and T cell functions. Identification of targets of polycomb, such as miR-31, will facilitate our understanding of the molecular mechanisms of T cell regulation.

Interconnections between the polycomb and NF-κB pathways have been reported in other cell types. Epigenetic silencing of the RasGAP gene *DAB2IP* is a key mechanism by which EZH2 activates Ras and NF-κB and triggers metastasis in prostate cancer [131]. In addition, physical interactions between EZH2 and RelA/RelB were suggested in basal-like breast cancer cells [132]. Thus, our findings and molecular mechanisms introduced from other cell types suggest that polycomb-mediated epigenetic changes modulate NF-κB signaling activity in mature T cells.

miRNA control of signaling transduction

After the identification of hundreds of functional miRNAs, the challenge now is to understand their specific biological functions. Signaling pathways are ideal candidates for miRNA-mediated regulation owing to their robustness, which is the capacity of biological systems to generate invariable phenotypes even when encountering genetic or environmental perturbations. miRNAs have been proposed to contribute to robustness by regulating signaling pathways, including TGF-β, Wnt, Notch, Hh, and pathways driven by receptor tyrosine kinases [133]. A clear indication from current studies is that miRNAs

participate in signaling networks, both as backups for transcriptional control and as feed-forward or feedback devices that confer robustness to the output of cell signaling (Figure 10). Thus, the effects of a miRNA can be the result of the net effects of opposing regulations or of the activities against mutually inhibiting factors. Therefore, new miRNA functions may be revealed as we refine our understanding of the networks in which they operate.

Some recent T cell studies have provided support for this proposition. In T-ALL, miR-19 upregulation results in the enhancement of the activities of PI3K–Akt and mTOR pathways by regulating Bim (Bcl2L11), AMP-activated kinase (Prkaa1), and the phosphatases Pten and PP2A [91].

NF-κB signaling is also linked to miRNAs. One study focused on the functional roles of miR-146a in T cell-mediated immune responses [134]. miR-146a was induced by TCR stimulation in naïve T cells. Moreover, NF-κB- and c-ETS-binding sites were required for inducing miR-146a transcription after TCR engagement. Interestingly, miR-146a could impede both AP-1 activity and IL-2 production induced by TCR engagement. In contrast, miR-146a targeted TRAF6 and IRAK1, two key protein adapters that activate a spectrum of signaling pathways from NF-κB to ERK [135]. It was also noted that HTLV-1 Tax could induce miR-146a expression through NF-κB activation [93].

As described above, our comprehensive study revealed novel interconnections between miR-31 and noncanonical NF-κB pathways in T cells [34]. The dynamic regulation of miR-31 by polycomb is critical for sustaining anti-apoptosis and proliferation capabilities in ATL cells. This polycomb–miR-31–NF-κB linkage provides speculation that, in addition to controlling transcription, the polycomb group can modulate translation via miRNA regulation (Figure 10). This signaling circuit permits the regulation of multiple genes. Another comprehensive study revealed that lymphocyte-specific miRNAs were either tightly controlled by polycomb group-mediated H3K27me3 or maintained in a semi-activated epigenetic state prior to their full expression [136].

Concluding Remark

Gene expression control ultimately regulates T cell fate and activation. In reality, very complex signaling pathways appear to be involved in the biology of T cell commitment. Using mouse models, in vitro studies, as well as patient's samples, a large number of studies have revealed novel conceptual advances that explain the complex regulation of genes in T cells. However, some discrepancies have also been shown. It should be noted that although each of the signaling pathways described in this review must contribute to gene expression, crosstalk between these pathways is required for realizing the complex gene regulatory network required for the complete functional activity in T cells. It also should be noted that the tumorigenic precursor T cells (i.e. T-ALL) are not terminally differentiated and their molecular setting does not appear to be directly associated with the mature TCR/CD28 signaling. It is required to further investigate physiological signaling pathways in terminally differentiated T cells to those observed in malignant T-ALL and ATL. To further our understanding of the precise molecular mechanisms involved in T cell activation and regulation in the immune system and disorders, the biological significance and interference with each other should be considered in future signaling research.

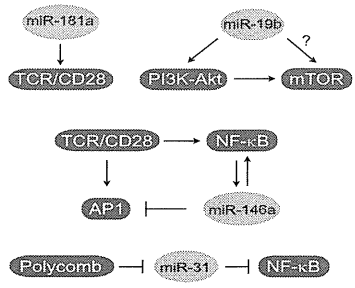

Figure 10: miRNA control of signal transduction. miRNAs participate in signaling networks, both as backups of transcriptional control and as feed-forward or feedback devices that confer robustness to the output of cell signaling.

Acknowledgement

This work is supported by JSPS KAKENHI Grant Number 24790436 (M.Y.), 23390250 (T.W.), NEXT KAKENHI Grant Number 221S0001 (T.W.), Grants-in-Aid from the Ministry of Health, Labour and Welfare H24-G-004 (M.Y. and T.W.) and H24-AIDS-008 (T.W.).

References

1. Smith-Garvin JE, Koretzky GA, Jordan MS (2009) T cell activation. Annu Rev Immunol 27: 591-619.
2. Koch U, Radtke F (2011) Mechanisms of T cell development and transformation. Annu Rev Cell Dev Biol 27: 539-562.
3. Kolch W (2005) Coordinating ERK/MAPK signalling through scaffolds and inhibitors. Nat Rev Mol Cell Biol 6: 827-837.
4. Hayden MS, Ghosh S (2008) Shared principles in NF-kappaB signaling. Cell 132: 344-362.
5. Matsumoto M, Yamada T, Yoshinaga SK, Boone T, Horan T, et al. (2002) Essential role of NF-kappa B-inducing kinase in T cell activation through the TCR/CD3 pathway. J Immunol 169: 1151-1158.
6. Weil R, Israël A (2006) Deciphering the pathway from the TCR to NF-kappaB. Cell Death Differ 13: 826-833.
7. Isakov N, Altman A (2002) Protein kinase C(theta) in T cell activation. Annu Rev Immunol 20: 761-794.
8. Hayashi K, Altman A (2007) Protein kinase C theta (PKCtheta): a key player in T cell life and death. Pharmacol Res 55: 537-544.
9. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, et al. (2000) PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. Nature 404: 402-407.
10. Li Y, Hu J, Vita R, Sun B, Tabata H, et al. (2004) SPAK kinase is a substrate and target of PKCtheta in T-cell receptor-induced AP-1 activation pathway. EMBO J 23: 1112-1122.
11. Jain J, McCaffrey PG, Valge-Archer VE, Rao A (1992) Nuclear factor of activated T cells contains Fos and Jun. Nature 356: 801-804.
12. Macián F, García-Cózar F, Im SH, Horton HF, Byrne MC, et al. (2002) Transcriptional mechanisms underlying lymphocyte tolerance. Cell 109: 719-731.
13. Macian F (2005) NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol 5: 472-484.
14. Acuto O, Michel F (2003) CD28-mediated co-stimulation: a quantitative support for TCR signalling. Nat Rev Immunol 3: 939-951.
15. Diehn M, Alizadeh AA, Rando OJ, Liu CL, Stankunas K, et al. (2002) Genomic expression programs and the integration of the CD28 costimulatory signal in T cell activation. Proc Natl Acad Sci U S A 99: 11796-11801.
16. Narayan P, Holt B, Tosti R, Kane LP (2006) CARMA1 is required for Akt-mediated NF-kappaB activation in T cells. Mol Cell Biol 26: 2327-2336.
17. Bjørgo E, Taskén K (2010) Novel mechanism of signaling by CD28. Immunol Lett 129: 1-6.
18. Park SG, Schulze-Luehrman J, Hayden MS, Hashimoto N, Ogawa W, et al. (2009) The kinase PDK1 integrates T cell antigen receptor and CD28

coreceptor signaling to induce NF-kappaB and activate T cells. Nat Immunol 10: 158-166.

19. Ishimaru N, Kishimoto H, Hayashi Y, Sprent J (2006) Regulation of naive T cell function by the NF-kappaB2 pathway. Nat Immunol 7: 763-772.
20. Turner M, Billadeau DD (2002) VAV proteins as signal integrators for multi-subunit immune-recognition receptors. Nat Rev Immunol 2: 476-486.
21. Hehner SP, Hofmann TG, Dienz O, Droge W, Schmitz ML (2000) Tyrosine-phosphorylated Vav1 as a point of integration for T-cell receptor- and CD28-mediated activation of JNK, p38, and interleukin-2 transcription. J Biol Chem 275: 18160-18171.
22. Acuto O, Michel F (2003) CD28-mediated co-stimulation: a quantitative support for TCR signalling. Nat Rev Immunol 3: 939-951.
23. Michel F, Attal-Bonnefoy G, Mangino G, Mise-Omata S, Acuto O (2001) CD28 as a molecular amplifier extending TCR ligation and signaling capabilities. Immunity 15: 935-945.
24. Reynolds LF, Smyth LA, Norton T, Freshney N, Downward J, et al. (2002) Vav1 transduces T cell receptor signals to the activation of phospholipase C-gamma1 via phosphoinositide 3-kinase-dependent and -independent pathways. J Exp Med 195: 1103-1114.
25. Zhang J, Salojin KV, Gao JX, Cameron MJ, Bergerot I, et al. (1999) p38 mitogen-activated protein kinase mediates signal integration of TCR/CD28 costimulation in primary murine T cells. J Immunol 162: 3819-3829.
26. Ward SG, Parry RV, Matthews J, O'Neill L (1997) A p38 MAP kinase inhibitor SB203580 inhibits CD28-dependent T cell proliferation and IL-2 production. Biochem Soc Trans 25: 304S.
27. Ashwell JD (2006) The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol 6: 532-540.
28. Kim JM, White JM, Shaw AS, Sleckman BP (2005) MAPK p38 alpha is dispensable for lymphocyte development and proliferation. J Immunol 174: 1239-1244.
29. Beardmore VA, Hinton HJ, Eftychi C, Apostolaki M, Armaka M, et al. (2005) Generation and characterization of p38beta (MAPK11) gene-targeted mice. Mol Cell Biol 25: 10454-10464.
30. Yamagishi M, Watanabe T (2012) Molecular hallmarks of adult T cell leukemia. Front Microbiol 3: 334.
31. Sun SC, Yamaoka S (2005) Activation of NF-kappaB by HTLV-I and implications for cell transformation. Oncogene 24: 5952-5964.
32. Saitoh Y, Yamamoto N, Dewan MZ, Sugimoto H, Martinez Bruyn VJ, et al. (2008) Overexpressed NF-kappaB-inducing kinase contributes to the tumorigenesis of adult T-cell leukemia and Hodgkin Reed-Sternberg cells. Blood 111: 5118-5129.
33. Thu YM, Richmond A (2010) NF-kappaB inducing kinase: a key regulator in the immune system and in cancer. Cytokine Growth Factor Rev 21: 213-226.
34. Yamagishi M, Nakano K, Miyake A, Yamochi T, Kagami Y, et al. (2012) Polycomb-mediated loss of miR-31 activates NIK-dependent NF-kappaB pathway in adult T cell leukemia and other cancers. Cancer Cell 21: 121-135.
35. Watanabe M, Ohsugi T, Shoda M, Ishida T, Aizawa S, et al. (2005) Dual targeting of transformed and untransformed HTLV-1-infected T cells by DHMEQ, a potent and selective inhibitor of NF-kappaB, as a strategy for chemoprevention and therapy of adult T-cell leukemia. Blood 106: 2462-2471.
36. Zoncu R, Efeyan A, Sabatini DM (2011) mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 12: 21-35.
37. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, et al. (2009) The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 30: 832-844.
38. Yang K, Neale G, Green DR, He W, Chi H (2011) The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. Nat Immunol 12: 888-897.
39. Wu Q, Liu Y, Chen C, Ikenoue T, Qiao Y, et al. (2011) The tuberous sclerosis complex-mammalian target of rapamycin pathway maintains the quiescence and survival of naive T cells. J Immunol 187: 1106-1112.
40. Colombetti S, Basso V, Mueller DL, Mondino A (2006) Prolonged TCR/CD28 engagement drives IL-2-independent T cell clonal expansion through signaling mediated by the mammalian target of rapamycin. J Immunol 176: 2730-2738.
41. Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, et al. (2007) A role for mammalian target of rapamycin in regulating T cell activation versus anergy. J Immunol 178: 2163-2170.
42. Song J, So T, Cheng M, Tang X, Croft M (2005) Sustained survivin expression from OX40 costimulatory signals drives T cell clonal expansion. Immunity 22: 621-631.
43. Chi H (2012) Regulation and function of mTOR signalling in T cell fate decisions. Nat Rev Immunol 12: 325-338.
44. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, et al. (2009) mTOR regulates memory CD8 T-cell differentiation. Nature 460: 108-112.
45. Osborne BA, Minter LM (2007) Notch signalling during peripheral T-cell activation and differentiation. Nat Rev Immunol 7: 64-75.
46. Koch U, Radtke F (2011) Notch in T-ALL: new players in a complex disease. Trends Immunol 32: 434-442.
47. Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, et al. (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306: 269-271.
48. Pancewicz J, Taylor JM, Datta A, Baydoun HH, Waldmann TA, et al. (2010) Notch signaling contributes to proliferation and tumor formation of human T-cell leukemia virus type 1-associated adult T-cell leukemia. Proc Natl Acad Sci U S A 107: 16619-16624.
49. MacDonald HR, Radtke F, Wilson A (2001) T cell fate specification and alphabeta/gammadelta lineage commitment. Curr Opin Immunol 13: 219-224.
50. Robey EA, Bluestone JA (2004) Notch signaling in lymphocyte development and function. Curr Opin Immunol 16: 360-366.
51. Palaga T, Miele L, Golde TE, Osborne BA (2003) TCR-mediated Notch signaling regulates proliferation and IFN-gamma production in peripheral T cells. J Immunol 171: 3019-3024.
52. Adler SH, Chiffoleau E, Xu L, Dalton NM, Burg JM, et al. (2003) Notch signaling augments T cell responsiveness by enhancing CD25 expression. J Immunol 171: 2896-2903.
53. Staal FJ, Luis TC, Tiemessen MM (2008) WNT signalling in the immune system: WNT is spreading its wings. Nat Rev Immunol 8: 581-593.
54. Staal FJ, Clevers HC (2005) WNT signalling and haematopoiesis: a WNT-WNT situation. Nat Rev Immunol 5: 21-30.
55. Xu Y, Banerjee D, Huelsken J, Birchmeier W, Sen JM (2003) Deletion of beta-catenin impairs T cell development. Nat Immunol 4: 1177-1182.
56. Yu Q, Sharma A, Oh SY, Moon HG, Hossain MZ, et al. (2009) T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-gamma. Nat Immunol 10: 992-999.
57. Kovalovsky D, Yu Y, Dose M, Emmanouilidou A, Konstantinou T, et al. (2009) Beta-catenin/Tcf determines the outcome of thymic selection in response to alphabetaTCR signaling. J Immunol 183: 3873-3884.
58. Zhao C, Blum J, Chen A, Kwon HY, Jung SH, et al. (2007) Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell 12: 528-541.
59. Cobas M, Wilson A, Ernst B, Mancini SJ, MacDonald HR, et al. (2004) Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. J Exp Med 199: 221-229.
60. Guo Z, Dose M, Kovalovsky D, Chang R, O'Neil J, et al. (2007) Beta-catenin stabilization stalls the transition from double-positive to single-positive stage and predisposes thymocytes to malignant transformation. Blood 109: 5463-5472.
61. Xu Y, Sen J (2003) Beta-catenin expression in thymocytes accelerates thymic involution. Eur J Immunol 33: 12-18.
62. Xu M, Yu Q, Subrahmanyam R, Difilippantonio MJ, Ried T, et al. (2008) Beta-catenin expression results in p53-independent DNA damage and oncogene-induced senescence in prelymphomagenic thymocytes in vivo. Mol Cell Biol 28: 1713-1723.
63. Ng JM, Curran T (2011) The Hedgehog's tale: developing strategies for targeting cancer. Nat Rev Cancer 11: 493-501.
64. Crompton T, Outram SV, Hager-Theodorides AL (2007) Sonic hedgehog signalling in T-cell development and activation. Nat Rev Immunol 7: 726-735.

65. Rowbotham NJ, Hager-Theodorides AL, Cebecauer M, Shah DK, Drakopoulou E, et al. (2007) Activation of the Hedgehog signaling pathway in T-lineage cells inhibits TCR repertoire selection in the thymus and peripheral T-cell activation. Blood 109: 3757-3766.

66. Lowrey JA, Stewart GA, Lindey S, Hoyne GF, Dallman MJ, et al. (2002) Sonic hedgehog promotes cell cycle progression in activated peripheral CD4(+) T lymphocytes. J Immunol 169: 1869-1875.

67. Stewart GA, Lowrey JA, Wakelin SJ, Fitch PM, Lindey S, et al. (2002) Sonic hedgehog signaling modulates activation of and cytokine production by human peripheral CD4+ T cells. J Immunol 169: 5451-5457.

68. Chan VS, Chau SY, Tian L, Chen Y, Kwong SK, et al. (2006) Sonic hedgehog promotes CD4+ T lymphocyte proliferation and modulates the expression of a subset of CD28-targeted genes. Int Immunol 18: 1627-1636.

69. Singh RR, Cho-Vega JH, Davuluri Y, Ma S, Kasbidi F, et al. (2009) Sonic hedgehog signaling pathway is activated in ALK-positive anaplastic large cell lymphoma. Cancer Res 69: 2550-2558.

70. Horie R, Watanabe M, Ishida T, Koiwa T, Aizawa S, et al. (2004) The NPM-ALK oncoprotein abrogates CD30 signaling and constitutive NF-kappaB activation in anaplastic large cell lymphoma. Cancer Cell 5: 353-364.

71. Schmierer B, Hill CS (2007) TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol 8: 970-982.

72. Li MO, Flavell RA (2008) TGF-beta: a master of all T cell trades. Cell 134: 392-404.

73. Maruyama T, Konkel JE, Zamarron BF, Chen W (2011) The molecular mechanisms of Foxp3 gene regulation. Semin Immunol 23: 418-423.

74. Salem ML (2011) Triggering of toll-like receptor signaling pathways in T cells contributes to the anti-tumor efficacy of T cell responses. Immunol Lett 137: 9-14.

75. Mills KH (2011) TLR-dependent T cell activation in autoimmunity. Nat Rev Immunol 11: 807-822.

76. Cottalorda A, Verschelde C, Marçais A, Tomkowiak M, Musette P, et al. (2006) TLR2 engagement on CD8 T cells lowers the threshold for optimal antigen-induced T cell activation. Eur J Immunol 36: 1684-1693.

77. Gelman AE, LaRosa DF, Zhang J, Walsh PT, Choi Y, et al. (2006) The adaptor molecule MyD88 activates PI-3 kinase signaling in CD4+ T cells and enables CpG oligodeoxynucleotide-mediated costimulation. Immunity 25: 783-793.

78. Gelman AE, Zhang J, Choi Y, Turka LA (2004) Toll-like receptor ligands directly promote activated CD4+ T cell survival. J Immunol 172: 6065-6073.

79. Gunturi A, Berg RE, Forman J (2004) The role of CD94/NKG2 in innate and adaptive immunity. Immunol Res 30: 29-34.

80. González S, Groh V, Spies T (2006) Immunobiology of human NKG2D and its ligands. Curr Top Microbiol Immunol 298: 121-138.

81. Borrego F, Masilamani M, Marusina AI, Tang X, Coligan JE (2006) The CD94/NKG2 family of receptors: from molecules and cells to clinical relevance. Immunol Res 35: 263-278.

82. Tarazona R, DelaRosa O, Casado JG, Torre-Cisneros J, Villanueva JL, et al. (2002) NK-associated receptors on CD8 T cells from treatment-naive HIV-infected individuals: defective expression of CD56. AIDS 16: 197-200.

83. Costa P, Rusconi S, Fogli M, Mavilio D, Murdaca G, et al. (2003) Low expression of inhibitory natural killer receptors in CD8 cytotoxic T lymphocytes in long-term non-progressor HIV-1-infected patients. AIDS 17: 257-260.

84. Moser JM, Gibbs J, Jensen PE, Lukacher AE (2002) CD94-NKG2A receptors regulate antiviral CD8(+) T cell responses. Nat Immunol 3: 189-195.

85. Esquela-Kerscher A, Slack FJ (2006) Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 6: 259-269.

86. Ebert MS, Sharp PA (2012) Roles for microRNAs in conferring robustness to biological processes. Cell 149: 515-524.

87. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, et al. (2007) MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell 131: 146-159.

88. Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, et al. (2005) T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. J Exp Med 201: 1367-1373.

89. Muljo SA, Ansel KM, Kanellopoulou C, Livingston DM, Rao A, et al. (2005) Aberrant T cell differentiation in the absence of Dicer. J Exp Med 202: 261-269.

90. Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, et al. (2007) miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell 129: 147-161.

91. Mavrakis KJ, Wolfe AL, Oricchio E, Palomero T, de Keersmaecker K, et al. (2010) Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. Nat Cell Biol 12: 372-379.

92. Mavrakis KJ, Van Der Meulen J, Wolfe AL, Liu X, Mets E, et al. (2011) A cooperative microRNA-tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL). Nat Genet 43: 673-678.

93. Pichler K, Schneider G, Grassmann R (2008) MicroRNA miR-146a and further oncogenesis-related cellular microRNAs are dysregulated in HTLV-1-transformed T lymphocytes. Retrovirology 5: 100.

94. Yeung ML, Yasunaga J, Bennasser Y, Dusetti N, Harris D, et al. (2008) Roles for microRNAs, miR-93 and miR-130b, and tumor protein 53-induced nuclear protein 1 tumor suppressor in cell growth dysregulation by human T-cell lymphotrophic virus 1. Cancer Res 68: 8976-8985.

95. Bellon M, Lepelletier Y, Hermine O, Nicot C (2009) Deregulation of microRNA involved in hematopoiesis and the immune response in HTLV-I adult T-cell leukemia. Blood 113: 4914-4917.

96. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, et al. (2005) MicroRNA expression profiles classify human cancers. Nature 435: 834-838.

97. Gaur A, Jewell DA, Liang Y, Ridzon D, Moore JH, et al. (2007) Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res 67: 2456-2468.

98. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szász AM, et al. (2009) A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 137: 1032-1046.

99. Cuddapah S, Barski A, Zhao K (2010) Epigenomics of T cell activation, differentiation, and memory. Curr Opin Immunol 22: 341-347.

100. Roh TY, Cuddapah S, Cui K, Zhao K (2006) The genomic landscape of histone modifications in human T cells. Proc Natl Acad Sci U S A 103: 15782-15787.

101. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, et al. (2009) Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity 30: 155-167.

102. Barski A, Jothi R, Cuddapah S, Cui K, Roh TY, et al. (2009) Chromatin poises miRNA- and protein-coding genes for expression. Genome Res 19: 1742-1751.

103. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G (2007) Genome regulation by polycomb and trithorax proteins. Cell 128: 735-745.

104. Su IH, Dobenecker MW, Dickinson E, Oser M, Basavaraj A, et al. (2005) Polycomb group protein ezh2 controls actin polymerization and cell signaling. Cell 121: 425-436.

105. Sparmann A, van Lohuizen M (2006) Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 6: 846-856.

106. Sasaki D, Imaizumi Y, Hasegawa H, Osaka A, Tsukasaki K, et al. (2011) Overexpression of Enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy. Haematologica 96: 712-719.

107. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, et al. (2010) Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 42: 181-185.

108. Hock H (2012) A complex Polycomb issue: the two faces of EZH2 in cancer. Genes Dev 26: 751-755.

109. Simon C, Chagraoui J, Krosl J, Gendron P, Wilhelm B, et al. (2012) A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. Genes Dev 26: 651-656.

110. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, et al. (2012) The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481: 157-163.

111. Ntziachristos P, Tsirigos A, Van Vlierberghe P, Nedjic J, Trimarchi T, et al. (2012) Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat Med 18: 298-301.

112. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, et al. (2007) Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med 13: 1203-1210.

113. Chan SM, Weng AP, Tibshirani R, Aster JC, Utz PJ (2007) Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood 110: 278-286.

114. Oswald F, Liptay S, Adler G, Schmid RM (1998) NF-kappaB2 is a putative target gene of activated Notch-1 via RBP-Jkappa. Mol Cell Biol 18: 2077-2088.

115. Oakley F, Mann J, Ruddell RG, Pickford J, Weinmaster G, et al. (2003) Basal expression of IkappaBalpha is controlled by the mammalian transcriptional repressor RBP-J (CBF1) and its activator Notch1. J Biol Chem 278: 24359-24370.

116. Bash J, Zong WX, Banga S, Rivera A, Ballard DW, et al. (1999) Rel/NF-kappaB can trigger the Notch signaling pathway by inducing the expression of Jagged1, a ligand for Notch receptors. EMBO J 18: 2803-2811.

117. Shin HM, Minter LM, Cho OH, Gottipati S, Fauq AH, et al. (2006) Notch1 augments NF-kappaB activity by facilitating its nuclear retention. EMBO J 25: 129-138.

118. Espinosa L, Cathelin S, D'Altri T, Trimarchi T, Statnikov A, et al. (2010) The Notch/Hes1 pathway sustains NF-kappaB activation through CYLD repression in T cell leukemia. Cancer Cell 18: 268-281.

119. Guan E, Wang J, Laborda J, Norcross M, Baeuerle PA, et al. (1996) T cell leukemia-associated human Notch/translocation-associated Notch homologue has I kappa B-like activity and physically interacts with nuclear factor-kappa B proteins in T cells. J Exp Med 183: 2025-2032.

120. Wang J, Shelly L, Miele L, Boykins R, Norcross MA, et al. (2001) Human Notch-1 inhibits NF-kappa B activity in the nucleus through a direct interaction involving a novel domain. J Immunol 167: 289-295.

121. Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, et al. (2005) Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol 6: 314-322.

122. Espinosa L, Inglés-Esteve J, Aguilera C, Bigas A (2003) Phosphorylation by glycogen synthase kinase-3 beta down-regulates Notch activity, a link for Notch and Wnt pathways. J Biol Chem 278: 32227-32235.

123. Rodilla V, Villanueva A, Obrador-Hevia A, Robert-Moreno A, Fernández-Majada V, et al. (2009) Jagged1 is the pathological link between Wnt and Notch pathways in colorectal cancer. Proc Natl Acad Sci U S A 106: 6315-6320.

124. Balint K, Xiao M, Pinnix CC, Soma A, Veres I, et al. (2005) Activation of Notch1 signaling is required for beta-catenin-mediated human primary melanoma progression. J Clin Invest 115: 3166-3176.

125. Leong KG, Niessen K, Kulic I, Raouf A, Eaves C, et al. (2007) Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. J Exp Med 204: 2935-2948.

126. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, et al. (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 126: 955-968.

127. Stecca B, Ruiz I Altaba A (2010) Context-dependent regulation of the GLI code in cancer by HEDGEHOG and non-HEDGEHOG signals. J Mol Cell Biol 2: 84-95.

128. Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, et al. (2003) Notch1 functions as a tumor suppressor in mouse skin. Nat Genet 33: 416-421.

129. Nakashima H, Nakamura M, Yamaguchi H, Yamanaka N, Akiyoshi T, et al. (2006) Nuclear factor-kappaB contributes to hedgehog signaling pathway activation through sonic hedgehog induction in pancreatic cancer. Cancer Res 66: 7041-7049.

130. Simon JA, Kingston RE (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 10: 697-708.

131. Min J, Zaslavsky A, Fedele G, McLaughlin SK, Reczek EE, et al. (2010) An oncogene-tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kappaB. Nat Med 16: 286-294.

132. Lee ST, Li Z, Wu Z, Aau M, Guan P, et al. (2011) Context-specific regulation of NF-kappaB target gene expression by EZH2 in breast cancers. Mol Cell 43: 798-810.

133. Inui M, Martello G, Piccolo S (2010) MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 11: 252-263.

134. Curtale G, Citarella F, Carissimi C, Goldoni M, Carucci N, et al. (2010) An emerging player in the adaptive immune response: microRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. Blood 115: 265-273.

135. Boldin MP, Baltimore D (2012) MicroRNAs, new effectors and regulators of NF-kappaB. Immunol Rev 246: 205-220.

136. Kuchen S, Resch W, Yamane A, Kuo N, Li Z, et al. (2010) Regulation of microRNA expression and abundance during lymphopoiesis. Immunity 32: 828-839.

This article was originally published in a special issue, entitled: "Signal Transduction Mechanisms in T lymphocytes", Edited by Dr. Noah Isakov, Ben Gurion University of the Negev, Beer Sheva, Israel.