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Transcription factor network regulating CD4⁺CD8⁺ thymocyte survival

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Abstract

More than 80% of thymocytes are CD4⁺CD8⁺ double positive (DP) cells which subject to positive/negative selection. The lifespan of DP thymocytes is critical in shaping the peripheral T cell repertoire essential for mounting immune responses against foreign, but not self, antigens. During T cell maturation, if the first round of T cell receptor (TCR) α chain rearrangement fails to generate a productive T cell receptor, DP cells start another round of α chain rearrangement until positive selection or cell death intervenes. Thus, the lifespan of DP cells determines how many rounds of α chain rearrangement can be carried out, and influences the likelihood of completing positive selection. The anti-apoptotic protein Bcl-x_L is the ultimate effector regulating DP cell survival, and several transcription factors critical for T cell development, such as TCF-1, E proteins, c-Myb, and ROR γ t, regulate DP survival via a Bcl-x_L-dependent pathway. However, the relationship between these transcription factors in this process is largely unclear. Recent results are revealing an interactive network among these critical factors during regulation of DP thymocyte survival. This review will discuss how these transcription factors potentially work together to control DP thymocyte survival that is critical for successful completion of T cell development.

T cell development

T cells are important players in adaptive immunity against infection and parasites. Similar to other developmental processes, T cell development in the thymus, which is believed to be the only organ where T cell development occurs, is highly-organized and involves a network of multiple players. The goal of T cell development is to arm T cells with all necessary machineries to, upon activation of T cells in the periphery, launch responses to the insult by either direct killing (CD8⁺ T cells) or helping cells in the battle against the insult (CD4⁺ T cells). However, during T cell development, T cells must be educated to target only “non-self” antigens, and this is accomplished by purging self-responsive T cells. Both acquisition of the armature to fight insults and the ability to recognize “non-self” antigens as targets are obtained during T cell development in the thymus, and underscore how critical T cell development is in T cell-mediated cellular immunity.

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T cell development is usually divided into three stages:¹ double negative (DN), double positive (DP), and single positive (SP). At the DN stage, thymocytes express neither CD4 nor CD8, and based on their expression of CD25 and CD44, they are further divided into the DN1 (CD25⁻CD44⁺), DN2 (CD25⁺CD44⁺), DN3 (CD25⁺CD44⁻) and DN4 (CD25⁻CD44⁻) subsets. Strictly, only DN cells at stage DN3 or later should be called thymocytes, since starting from early T cell precursors, which are newly immigrated into the thymus from the bone marrow, to the DN2 stage, cells gradually lose the potential to differentiate into other non-T cell lineages, such as NK cells, monocytes and dendritic cells. Once cells reach the DN3 stage, they are committed to the T cell lineage and can no longer develop into alternative cell types.² At the DN3 stage, thymocytes start rearranging T cell receptor (TCR) β chain locus to produce TCR β chain, and only those thymocytes that have a successfully rearranged TCR β chain are able to survive and move on in the process of T cell development. A successfully rearranged TCR β chain, combined with the invariant pre-TCR α chain, form a pre-TCR, which is critical for proliferation of post- β thymocytes and the transition from the DN to DP stage. DN4 thymocytes proliferate extensively to prepare for the stringent DP selection process. Although more than 80% of total thymocytes are DP, only 5% are positively selected and mature into either CD4⁺ or CD8⁺ SP cells if a DP thymocyte bears a TCR that interacts with an MHC-self peptide complex with sufficiently low affinity. The other cells are either negatively selected and eliminated through apoptosis if the TCR is recognized by an MHC-self peptide with high affinity, or die by neglect if the TCR cannot be recognized at all.³ Once thymocytes mature into SP cells, they migrate out of the thymus and into peripheral lymphoid organs, such as lymph nodes and spleens, to take effect in adaptive immunity.

A network of transcription factors regulates DP thymocyte survival

DP thymocytes are able to initiate multiple rounds of TCR α chain locus rearrangement until they are positively selected or die due to limited lifespan. Thus, the duration of the window for positive/negative selection determines the productivity of the selection process.⁴ A longer lifespan for DP cells leads to greater opportunity for the thymocytes to eventually generate a TCR that responds to foreign, but not self, antigens. Given the importance of DP thymocyte survival, it is of great interest to exploit the mechanisms that prevent DP thymocytes from undergoing pre-mature apoptosis. Several factors that regulate DP thymocyte survival have been implicated, including the Wnt/ β -catenin/TCF-1 pathway,^{5, 6} ROR γ t (Retinoid-related orphan nuclear factor γ t),⁷ c-myb (myeloblastosis oncogene),⁸ and HEB (also called TCF-12, transcription factor 12).⁹ In this review, we summarize recent findings on these factors that play important roles in extending DP thymocyte survival and attempt to weave these “dots” into “strings” of signaling pathways, aiming to eventually reveal the molecular network that supports DP thymocyte survival.

Role of ROR γ t in DP thymocyte survival

ROR γ t is a transcription factor that belongs to the steroid nuclear receptor superfamily. Similar to other members of this family, ROR γ t contains a conserved DNA binding domain and a ligand binding domain. Upon binding to its ligand, which has yet to be identified, ROR γ t is thought to be activated, at which point it stimulates target gene expression. ROR γ t was initially identified by expression cloning to screen for molecules that regulate activation-induced cell death.¹⁰ We created ROR γ t knockout mice and demonstrated that ROR γ t is required for DP thymocyte survival and lymph node genesis,¹¹ which was also confirmed with an independently generated ROR γ t knockout mouse strain.¹² ROR γ t^{-/-} DP thymocytes undergo accelerated apoptosis accompanied by greatly reduced Bcl-x_L levels, and overexpression of Bcl-x_L rescues ROR γ t^{-/-} thymocyte apoptosis, demonstrating that ROR γ t enhances DP cell survival by up-regulating Bcl-x_L expression.¹¹ We further demonstrated that the recruitment of the steroid receptor coactivator (SRC) through the

motif of activation function 2 (AF2) of ROR γ t is essential for ROR γ t to support thymocyte survival. With both the ROR γ t downstream effector, Bcl-x_L, and interacting partner, SRC, being identified, the upstream signaling pathways that regulate ROR γ t during thymocyte survival, however, remained unknown.

Role of the Wnt/ β -catenin/TCF-1 signaling pathway in thymocyte development

The Wnt pathway includes the canonical Wnt pathway mediated by β -catenin/TCF-1 complexes, the non-canonical Wnt pathway, which involves intracellular calcium mobilization, and the planar cell polarity pathway mediated by activation of the small GTPase RHOA (RAS homologue gene-family member A). Although non-canonical Wnt signaling has been implicated in immunological process such as regulation of $\alpha\beta$ -lineage thymocyte survival,¹³ we will mainly focus on canonical Wnt signaling because it is the most significant and extensively studied among all Wnt pathways.

Activation of the canonical Wnt signaling pathway is initiated by binding of ligands, one of the 19 different Wnts, to the cell membrane-localized receptor complex composed of Frizzled and LDL receptor-related protein 5 (LRP5) or LRP6.¹ Ligand binding initiates a signaling cascade that dissociates the anchor protein AXIN1 of the destruction complex, which contains GSK3 β . GSK3 β phosphorylates and degrades β -catenin in the absence of Wnt signals. Dissociation of AXIN1 prevents phosphorylation and degradation of β -catenin, leading to its accumulation in the cytoplasm. The ultimate effector of the canonical Wnt pathway is TCF-1/LEF-1. In the absence of Wnt signals, TCF-1 is bound by co-repressors such as Groucho/Transducin-like enhancer (GRG/TLE) and turns off target gene expression. Once Wnt initiates the signaling pathway, the accumulated β -catenin translocates into the nucleus, replaces the GRG/TLE repressor, and binds TCF-1 as a co-activator, leading to transcriptional activation of the target genes.

The Wnt pathway regulates multiple basic developmental processes, such as cell-fate specification, progenitor cell proliferation, establishment of the dorsal axis, and control of asymmetric cell division.¹⁴ In addition, Wnt signaling has been linked to pathogenesis of diseases such as colon carcinoma¹⁵ and leukemia.¹⁶ Wnt signaling is also required for hematopoietic stem cell self-renewal and the proliferation of progenitor cells. In addition, it appears that Wnt signals can confer a more undifferentiated phenotype on lineage-committed progenitors, inhibiting them from differentiating into various lineages.¹⁷

Wnt signaling is also important at multiple stages of thymocyte development (Fig. 1). The role of Wnt signaling pathway in T cell development was first evaluated through fetal thymic organ cultures (FTOC) grown in the presence of soluble Frizzled receptors as decoys for WNT proteins.¹⁸ *in vitro* thymocyte differentiation was inhibited by the soluble Frizzled receptors, suggesting an essential role for the Wnt pathway in thymocyte development. In addition, the inhibition primarily negatively affected thymocyte proliferation, which was confirmed by several other studies. For instance, Schilham *et al.*¹⁹ used *TCF-1*^{-/-} mice, which lack exon 7 of TCF-1, to study the potential role of the effector of the Wnt signaling pathway, TCF-1. They found very few actively cycling DN2 and DN4 thymocytes, which should have been extensively proliferating to prepare for β -selection and positive/negative selection, respectively. DN4 thymocyte proliferation was also negatively affected in a β -catenin-deficient mouse line.²⁰ In accordance with the role of TCF-1 in regulating thymocyte proliferation, the thymi of mice deficient in both Wnt1 and Wnt4 had low cellularity.²¹

In addition to promoting proliferation of DN thymocytes, Wnt signaling has been proposed to be important for the DN to DP transition. Work by Verbeek *et al.*, showed that differentiation of thymocytes from *TCF-1*^{-/-} mice was blocked at the ISP (CD8⁺ Immature

Single positive) to DP transition.²² This work was further supported by later studies. For instance, when ICAT, the inhibitor of β -catenin and TCF, which blocks the binding of β -catenin to TCF-1 and LEF-1, was over-expressed through retroviral transduction, thymocyte development was blocked at the DN to DP transition in FTOC assays,²³ indicating an essential role for β -catenin/TCF-1 in the DN to DP transition during thymocyte development.

Wnt signaling has also been implicated in thymocyte development at the DP stage. DP thymocytes from *TCF-1*^{-/-} mice undergo rapid apoptosis during *in vitro* culture, and thymocyte survival can be restored by a transgene that expresses full length TCF-1 but not truncated TCF-1 that lacks the domain mediating the interaction with β -catenin, suggesting that the Wnt signaling pathway mediated by β -catenin is required to support DP thymocyte survival.⁶ To further establish the importance of Wnt signaling pathway in DP thymocyte survival, we have established a β -catenin transgenic mouse strain (β -cat^{Tg}) that overexpresses constitutively active β -catenin under the control of a CD4 promoter.⁵ The β -catenin transgene is not expressed until the DP stage, which ensures that thymocyte development at DN or earlier stages is not affected. As expected, the four DN subsets have normal distribution and cell numbers in these mice. In contrast, both the frequency and numbers of thymocytes at the DP stage are significantly greater in β -cat^{Tg} mice compared to wild-type (WT). In addition, when DP thymocytes from β -cat^{Tg} mice are grown *in vitro*, they undergo much slower apoptosis than WT counterparts in both spontaneous and glucocorticoid-induced apoptosis. Furthermore, the promotion of DP thymocyte survival by the transgene of β -catenin is mediated by up-regulation of Bcl-x_L, a key anti-apoptosis factor that supports DP thymocyte survival. These data demonstrated that β -catenin/TCF-1 extends DP thymocyte survival by upregulating Bcl-x_L. However, whether Wnt signaling mediated by β -catenin directly targets Bcl-x_L or acts through factors downstream of the β -catenin/TCF-1 complex remained unknown. Our recent work has shed light on this question by showing that enhancement of DP thymocyte survival by β -catenin/TCF-1 is mediated by ROR γ t. More than 20 genes have been reported to be direct targets of the canonical Wnt/ β -catenin/TCF signaling pathway,²⁴ including *Drosophila Ultrabithorax (Ubx)*, the *Xenopus* homeobox genes *siamois*, *twinned*, and *engrailed-2*, PPAR δ (a member of the nuclear receptor superfamily), c-Jun and Fra-1 (two components of the AP-1 transcription complex), and E-cadherin. However, in spite of the apparent significance of the canonical Wnt signaling pathway in the hematopoietic and immunological system, the target genes of the pathway in these systems remain largely unknown. ROR γ t seems to be one of the target genes of β -catenin/TCF-1 pathway during thymocyte development because TCF-1-deficient thymocytes express strikingly lower level of ROR γ t whereas β -catenin transgenic thymocytes have significantly higher expression of ROR γ t compared to WT counterparts (unpublished data). In addition, forced expression of ROR γ t rescued survival of TCF-1-deficient DP thymocytes in an *in vitro* thymocyte differentiation system (unpublished data). To explore on the molecular mechanism, we tested the capability of β -catenin to stimulate a reporter construct harboring ROR γ t promoter region, and found that β -catenin/TCF-1 complex is able to activate ROR γ t promote. And furthermore, an antibody specific for β -catenin was able to pull down a fragment of ROR γ t promoter region contained by the reporter construct in chromatin immuno-precipitation assays (ChIPs) (unpublished data). Therefore, it appears that β -catenin/TCF-1 is able to activate ROR γ t transcription by directly targeting its regulatory region in thymocytes. Although more investigations on identifying TCF-1-responsive elements within ROR γ t regulatory region is needed to ultimately confirm the assignment of ROR γ t as a target of β -catenin/TCF-1 pathway, our studies demonstrated for the first time that during DP stage of thymocyte development, β -catenin/TCF-1 supports thymocyte survival by activating ROR γ t expression.

The window for DP thymocytes to complete positive and negative selection must be sufficiently long that thymocytes can have a reasonable lifespan, but not so long that detrimental effects such as promoting the accumulation of “unwanted” thymocytes occur. Guo *et al.* reported that in transgenic mice that overexpressed constitutively active β -catenin due to deletion of β -catenin exon 3, thymocyte development was blocked at the DP to SP transition. In addition, these mice developed T cell lymphomas that contained mainly DP thymocytes,²⁵ suggesting that Wnt signaling activity at the DP stage must be controlled tightly to enable thymocytes to finish positive and negative selection properly. Furthermore, these findings indicated that inappropriate activation of Wnt signals may lead to tumorigenesis.

In addition to enhancing DP thymocyte survival, the Wnt signaling pathway has also been implicated in regulating positive and negative selection of DP thymocytes prior to their eventual maturation into either CD4 or CD8 SP thymocytes. Through use of a β -catenin transgenic mouse strain that expressed an Lck promoter-driven stabilized β -catenin (due to deletion of the N terminal 87 amino acids), Yu *et al.* showed that positive selection was promoted, and that the transgene expression accelerated the generation of CD8 SP thymocytes, which usually occurs later than the generation of CD4 SP thymocytes, such that the generation of both types of thymocytes took place with the same kinetics.²⁶ These data suggest that β -catenin/TCF-1 also plays important roles in positive selection, and maybe in CD4/CD8 lineage differentiation as well.

Given the versatile roles of β -catenin/TCF-1 in other developmental processes, it is not surprising that this pathway is required for thymocyte development at multiple stages. Starting from the commitment of earliest stage of ETPs (early T cell progenitors) to T cell lineage commitment, positive selection and CD4 versus CD8 lineage decision, β -catenin/TCF-1 could be regulated/influenced by different upstream events such as preTCR signals and the binding of Wnt ligands to their receptors; and it might also regulate distinct downstream factors to meet the developmental requirement at different stages. So far, the roles of β -catenin/TCF-1 at each individual stage has been emerging; however, the more important questions to answer are that how thymocytes dynamically regulate their β -catenin/TCF-1 signaling, and also how β -catenin/TCF-1 differentially regulates its downstream factors at different stage of thymocyte development. To answer these questions, approaches that allow *in vivo* tracing of preTCR or β -catenin/TCF-1 activity at different thymocyte development stages could be of great help. In addition, a systematic analysis on β -catenin/TCF-1 target genes at different thymocyte developmental stages would also reveal important information as to how β -catenin/TCF-1 shifts the targets from a specific stage to the next during thymocyte development.

c-Myb in DP thymocyte survival

A recent paper by Yuan *et al.* identified another transcription factor, c-Myb, encoded by the proto-oncogene *Myb*, as an important factor for regulating DP thymocyte survival.⁸ Previously, using conditional mutagenesis at the *Myb* locus,²⁷ the same group showed that c-Myb is crucial at multiple stages of thymocyte development, including transition from the DN to DP stage, survival of preselection DP thymocytes and differentiation of CD4 SP thymocytes. In their recent work, more efficient deletion of c-Myb starting from DP stage was achieved by crossing the *Myb*^{f/f} and *cd4/cre* mouse strains. Deficiency of c-Myb led to premature DP thymocyte apoptosis due to decreased expression of Bcl-x_L. More specifically, due to enhanced dependence on Bcl-x_L for survival, small pre-selection DP thymocytes underwent faster premature apoptosis than large pre-selection and post-selection DP thymocytes. Forced expression of Bcl-x_L rescued thymocyte survival, and re-introduction of c-Myb restored both Bcl-x_L expression and the small preselection DP compartment. The defective DP thymocyte survival due to reduced expression of Bcl-x_L

exhibited by c-Myb-deficient mice is reminiscent of what has been observed in *TCF-1*^{-/-} and *RORγt*^{-/-} mice. However, the authors proposed that the transcriptional regulation of Bcl-x_L by c-Myb is independent of both TCF-1 and RORγt, since c-Myb expression in both TCF-1- and RORγt-deficient thymocytes was comparable to that in WT thymocytes, indicating that multiple pro-survival pathways could synergize to ensure proper survival of DP thymocytes.

HEB is required for DP thymocyte survival

The E protein family of transcription factors regulates lymphocyte differentiation in multiple processes, including controlling the expression of genes essential to lineage commitment, targeting antigen-receptor gene rearrangement and enforcing key developmental checkpoints.^{28, 29} Mammals have four E proteins (E2A, HEB, E12 and E2-2), of which E2A and HEB promote TCR rearrangement and control the developmental progression, survival and proliferation of developing T cells, thereby accounting for the majority of E protein function in the thymus.²⁹ E2A is involved in regulating the pre-TCR and Notch signaling pathway at the earliest stages of thymocyte development, restraining DN to DP progression until rearrangement of a productive TCRβ chain.^{28, 30, 31} E2A and HEB have been thought to compensate for each other during lymphocyte development. For instance, HEB-deficient thymocyte development are blocked at the DN to DP transition, but this can be partially overcome by the compensatory activity of E2A.^{32, 33} Recently, HEB was found to have a distinctive role in promoting DP thymocyte survival.⁹ Thymocytes from T lineage-specific HEB-deleted mice undergo rapid apoptosis and have reduced Bcl-x_L expression. As observed in c-Myb or RORγt-deficient thymocytes, forced expression of Bcl-x_L rescued DP thymocyte survival, indicating that HEB is another transcription factor that functions upstream of Bcl-x_L to promote DP thymocyte survival. In contrast to the independence of RORγt and TCF-1 in c-Myb-mediated DP thymocyte survival regulation, HEB regulates RORγt expression by binding to the two E-box sites present in the RORγt promoter and stimulates its transcription, which suggests that HEB could act upstream of RORγt in the same pathway to promote DP thymocyte survival.

Regulation of Bcl-x_L expression in DP thymocytes

All four factors discussed to this point promote DP thymocyte survival by upregulating Bcl-x_L, and transgenic expression of Bcl-x_L rescued thymocyte survival when each of the factors was deficient, indicating that Bcl-x_L is the main anti-apoptotic factor protecting thymocytes from apoptosis. Although another pro-survival Bcl-2 family member, Mcl-1, has also been implicated in the regulation of DP thymocyte survival, it is believed to only compensate for the loss of Bcl-x_L when later is deficient.³⁴ Both RORγt and c-Myb can regulate the transcription of Bcl-x_L, indicative of possible synergy between these two transcription factors in stimulating Bcl-x_L transcription. Whether RORγt and/or c-Myb can bind to the Bcl-x_L promoter and thereby promote its transcription needs further investigation. We have shown that β-catenin/TCF-1 is able to target the RORγt promoter to activate its activity (unpublished data). Interestingly, HEB is also able to bind to the two E-box sites located approximately 200 bp upstream of RORγt transcription starting site, activating RORγt transcription. Therefore, it appears that TCF-1 and HEB could bind close to each other on the RORγt promoter, suggesting that these two factors might interact with each other and cooperate to stimulate the RORγt promoter (Figure 2).

preTCR signals and the transcription factor network regulating DP thymocyte survival

preTCR signals are major components of the signaling network in immature T cell development, and are key to survival and proliferation of DN cells.^{35, 36} Whether there is any relationship between preTCR/TCR and the β -catenin/TCF-1 signaling pathway remains controversial. β -catenin has been suggested as a target of both preTCR and TCR signaling.²⁰ Stimulation of isolated thymocytes or splenic CD4⁺ T cells with either phorbol 12-myristate 13-acetate or CD3 monoclonal antibody caused accumulation of more β -catenin in the nucleus than in nuclei of untreated samples, suggesting that both preTCR and TCR signaling could activate β -catenin. This idea was corroborated by a recent observation that, in primary human T cells, TCR signaling led to stabilization of β -catenin.³⁷ However, in another study, the non-phosphorylated, active form of β -catenin was readily detected in Rag-1-deficient thymocytes which lack preTCR due to impaired TCR β chain locus rearrangement, and, furthermore, when Rag-1-deficient mice were injected with anti-CD3 antibodies to induce preTCR signaling, neither total nor active β -catenin increased. Interestingly the authors found that TCF-1 levels were significantly increased after preTCR induction through anti-CD3 antibody injection, indicating that preTCR signals might induce TCF-1 independently of Wnt-induced β -catenin.³⁸ However, because TCF-1 is usually repressed by Groucho, unless Groucho is replaced by the activated β -catenin, it is hard to explain how an increase in the absolute quantity of TCF-1 alone would lead to activation of target genes. Therefore, we believe that if TCF-1 is regulated by preTCR signals, it could be mediated by both activation of β -catenin, which “turns on” TCF-1, and induction of TCF-1 transcription.

Interestingly, preTCR signals have also been shown to induce a delayed elevation in ROR γ t expression, which is thought to allow for a period of proliferation to occur after preTCR signaling.³⁹ Based on these findings, Xi *et al.* proposed a molecular model to explain the mechanisms that regulate the delayed induction of ROR γ t by preTCR signals.⁴⁰ preTCR signals initially upregulate both ROR γ t and early response genes-3 (Egr3); however, the upregulated ROR γ t is kept at moderate level due to the absence of E proteins (E2A/HEB), which are sequestered by Id3, the E protein inhibitor, induced by Egr3. Expression of Egr3 is transient, and once Egr3 subsides, E proteins are released from Id3-mediated inhibition, bind to the E box sites located in the ROR γ t promoter region and stimulate its transcription. The binding of E proteins to the ROR γ t promoter eventually leads to upregulation of ROR γ t to its maximal levels. However, two questions remain to be answered: 1) how preTCR signals initially induce ROR γ t, and 2) why overexpression of Egr3 in Egr3 transgenic mice could not completely abolish expression of ROR γ t. To answer these two questions, we propose that the initial moderate induction of ROR γ t by preTCR signals could be mediated by TCF-1. When E proteins are absent during the earliest period of preTCR signals, due to the transient up-regulation of Egr3, TCF-1 is only able to stimulate ROR γ t transcription to a moderate level. When E proteins are relieved of Id3-mediated inhibition due to the reduction in Egr3 levels, synergy between TCF-1 and E proteins would induce a maximal level of ROR γ t transcription. Lack of E proteins at the ROR γ t promoter due to overexpressed Egr3 would lead to a substantial reduction in ROR γ t; however, TCF-1 alone might still be able to induce some expression of ROR γ t, which could explain the incomplete abolishment of ROR γ t expression in Egr3 transgenic mice. Taking into account the model proposed by Xi *et al.* and our own findings that TCF-1 regulates DP thymocyte survival through a ROR γ t pathway, we propose a new model for the molecular mechanism regulating DP thymocyte survival (Figure 2). At the DN4 to DP transition, preTCR signals might initially lead to up-regulation of Egr3 and activation of TCF-1; subsidence of Egr3 relieves E proteins from inhibition by Id3, which, together with already activated TCF-1, induces the maximal level

of ROR γ t expression. Then ROR γ t, joined by c-Myb, upregulate Bcl-x_L, which eventually promotes DP thymocyte survival.

Some transcription factors discussed above somehow have a similar function in mature T cells in addition as in immature thymocytes. Stabilized β -catenin is able to extend regulatory T cell (Treg) survival through up-regulating Bcl-x_L,⁴¹ suggesting that T cells at different development stages may utilize the same pathway to regulate their survival. On the other hand, we also showed that in conventional T cells β -catenin is able to promote FasL-mediated activation-induced cell death,⁴² suggesting that β -catenin pathway can also be used to promote cell death. TCF-1 is shown to be essential for differentiation and persistency of memory CD8⁺ T cells. TCF-1-deficient memory CD8⁺ T cells express diminished survival molecule Bcl-2, part of the mechanism whereby TCF-1 regulates memory CD8⁺ T cell differentiation thus could be through promoting the survival.⁴³ In this case, TCF-1 regulates memory T cell survival via Bcl-2 instead of Bcl-x_L as in DP thymocyte. The same transcription factor are also involved in promoting survival and proliferation in other cell types. For example, c-myb has been shown to regulate B cell development likely via affecting IL-7-dependent survival pathway.⁴⁴ Both c-myb and TCF-1 promote tumorigenesis and self-renewal of hematopoietic stem cells by stimulating proliferation.^{14, 45–49} Similarly, Heb has been associated with lymphoma and expansion of neuron tissues.^{47, 50} Taken together, the same transcription factors regulating DP thymocyte survival may also affect survival pathways in other types of cells. However, ROR γ t is unique for T cells, since it is only expressed in developing thymocytes and Th17 cells. Therefore, the same transcription factors although regulate survival in different cell types but likely via stimulating different target genes.

Conclusion

Life and death are the central theme of T cell development, which maintains survival of T cells that recognize foreign antigens presented by self-MHC, but induces death of self-reactive T cells. A critical event in DP thymocytes, α chain rearrangement, allows for the generation of a repertoire of T cells subject to positive and negative selection. The lifespan of DP cells determines the rate of successful selection, and thereby limits the size of the peripheral T cell repertoire. Previous single gene or multiple gene studies with traditional molecular biology techniques have revealed that many transcription factors critical for T cell development regulate the survival of DP cells. Instead of working alone, these transcription factors regulate or cooperate with each other to form a network to ensure the survival of DP thymocytes critical for completion of T cell development. Our proposed model (Figure 2) is surely oversimplified since it is based on results generated mostly from one gene-one function analysis. However, with the development of microarray and deep-sequencing technology, it is now possible to perform genome-wide analysis to reveal a global network responsible for regulating thymocyte survival. Genome-wide analysis is also expected to reveal novel critical genes that otherwise would be difficult to identify. Detailed mechanisms responsible for these candidate genes that regulate thymocyte survival in turns require study by traditional molecular biology. Future work therefore requires a combination of genome-wide and traditional single/multi-gene analyses to define the complete picture of regulation of DP thymocyte survival.

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Figure 1.

Wnt/β-catenin/TCF-1 regulates thymocyte development at multiple stages. At the DN stage, particularly for DN2 and DN4, β-catenin/TCF-1 promotes thymocyte proliferation to expand the thymocyte pool extensively in order to prepare for β-selection and positive/negative selection. DP thymocyte survival requires β-catenin/TCF-1, which acts upstream of RORγt in the upregulation of Bcl-x_L. β-catenin/TCF-1 also promotes positive selection.

Figure 2.

Model for possible mechanisms of regulation of DP thymocytes by multiple factors. Activation of ROR γ t transcription requires cooperation of the transcription factors TCF-1 and HEB. There are three possible pathways responsible for activation of TCF-1. (1) preTCR signals might activate β -catenin, which translocates into the nucleus and switches TCF-1 from a repressor to an activator, eventually leading to induction of ROR γ t transcription. (2) Activation of β -catenin could also result from the binding of Wnt ligands to the Frizzled receptor in the canonical Wnt signaling pathway. (3) preTCR signals might induce TCF-1 transcription independently of β -catenin. These three possible pathways may be non-exclusive, and could have synergy that leads to maximal activation of TCF-1 at the DN4 and DP stages. Compared to TCF-1, which upon activation functions to stimulate ROR γ t transcription, HEB seems to mediate fine-tuning of ROR γ t transcription. At the DN to DP transition, preTCR signals upregulate Egr3 transiently. Egr3-induced Id3 sequesters E proteins such as HEB from binding to the ROR γ t promoter. Because both HEB and TCF-1 are required for substantial induction of ROR γ t, the absence of HEB leads to reduction of ROR γ t expression. When thymocytes differentiate to the DP stage, Egr3 subsides and Id3 is removed, which leads to recovery of HEB activity. HEB then binds to the ROR γ t promoter, together with already activated TCF-1, and leads to stimulation of maximal ROR γ t transcription. HEB binds to two E-box sites 200 bp upstream of the ROR γ t transcription starting site, whereas TCF-1 targets the 200 bp fragment between the ROR γ t transcription starting site and translation starting site, as determined based on our ChIP assay results. Whether HEB and TCF-1 interact with and influence each other's activity remains unknown. ROR γ t and c-Myb cooperate in upregulating Bcl-x_L, which eventually leads to protection of DP thymocytes from apoptosis. Mcl-1 could also enhance DP thymocyte survival; however, it is not essential and may only partially compensate for lack of Bcl-x_L. Dashed lines indicate pathways and interactions that have not yet been proven.