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Research Highlight

A New Avenue to Cure Cancer by Turning Adaptive Immune T Cells to Innate Immune NK Cells via Reprogramming

Dong-Ming Su [1,*] and Ramakrishna Vankayalapati [2]

1 Department of Biomedical Research, University of Texas Health Science Center at Tyler, Tyler, TX 75708, USA
2 Center for Pulmonary and Infectious Disease Control, University of Texas Health Science Center at Tyler, Tyler, TX 75708, USA
* Correspondence to: Dong-Ming Su, Tel: +1-903-877-7587; Fax: +1-903-877-7968; E-mail: dong-ming.su@uthct.edu

Thymocytes after T-lineage commitment develop in the T-cell pathway. However, in a recent study, Li et al. (2010) demonstrated that inducing to delete *Bcl11b* gene in these thymocytes, even in mature T cells turns these cells into natural killer (NK) cells during the culture. They called this conversion 'reprogramming', and the reprogrammed killer cells 'ITNK cells'. The ITNK cells possessed tumor-killer ability and did not indiscriminately kill normal cells. This exciting finding represents a major breakthrough towards curing cancer and identifies an important, novel transcription factor in the thymus development.

The immune response consists of the innate and adaptive immune response, which cooperate to eliminate cancer cells, pathogens and infected cells from the host. The adaptive immune reaction is largely dependent on specific T lymphocytes ($CD4^+$ and $CD8^+$ T cells), which are primed by foreign antigens (immunogenic proteins or components from pathogens and cancer cells) prior to the reaction. On the other hand, the innate response, in which the natural killer (NK) cells play a prominent role, is a form of natural immunity in which the immune cells have never previously encountered the pathogens or cancer cells, but can nevertheless eliminate them. Besides lysing cancer cells and infected cells, NK cells also secrete cytokines that shape the adaptive immune response. Normal proteins in the body are not antigenic to the self-immune system because of the self-tolerance (Kyewski and Klein, 2006) generated in the thymus—a central T-cell system organ. Since tumors develop from normal tissues due to mutations, the tumor antigens in most situations are very similar to the proteins in normal tissue, called tumor-associated antigens, and are present not only on some tumor cells but also on some normal cells. The absence of 'foreign' antigen makes it difficult for the immune system to be primed, and it is also not easy for us to develop tumor vaccines to prime the body's immune system in an anti-tumor strategy because the development of vaccines needs tumor-specific antigens, present only on tumor cells but not on any other normal cells. Therefore, innate immunity, particularly NK cells, in the anti-tumor response becomes very important. Although infectious agents, such as bacterial or viral pathogens have sufficient specific 'foreign' antigens, the NK cells are still critical for the first line of defense against infections. In addition, some cancer cells and virus-infected cells often down-regulate their MHC-I expression to avoid being recognized by T cells. However, this results in their becoming NK cell targets, because NK cells function by targeting and killing cells that lack the self-MHC-I molecule, and are tolerant to self-cells carrying self-MHC-I (Borghaei et al., 2009). Thus, cooperation between the innate and adaptive immune response is essential to eliminate cancers and infections. In recently published studies Li et al. (2010) made a breakthrough by converting adaptive immune T cells to innate immune NK cells via reprogramming of the *Bcl11b* gene, which promises a new avenue to cure cancer.

Development of T cells

The thymus is a cradle that fosters T-lymphocyte, mainly αβ T-cell receptor (TCR)-lineage T-cell development. T-cell progenitors in the thymus are recruited from bone marrow via the blood in postnatal life. Inside the thymus, the developing T-precursors, namely thymocytes, undergo complicated developmental processes, including migration, proliferation, lineage-specification, differentiation, apoptosis and β- then α-TCR rearrangements, as well as several checkpoints based on CD3, CD4, CD8, CD44 and CD25 cell surface molecules (Figure 1). Early thymocytes are $CD3^-$, $CD4^-8^-$ double negative (DN) cells (Ceredig and Rolink, 2002) that can be divided into four developmental stages from immature to relatively mature: $CD44^+25^-$ (DN1), $CD44^+25^+$ (DN2), $CD44^-25^+$ (DN3) and $CD44^-25^-$ (DN4) (Godfrey et al., 1993, 1994). The DN1 subset is a heterogeneous population that includes multi-potent cells that can produce T cells, B cells, dendritic cells (DC) and NK cells (Shortman and Wu, 1996). In the DN1 subset, early T-cell progenitors (Allman et al., 2003) are a canonical T-cell lineage progenitor subset with $CD117^+$, which develops into the DN2 subset. The DN2 subset still has the

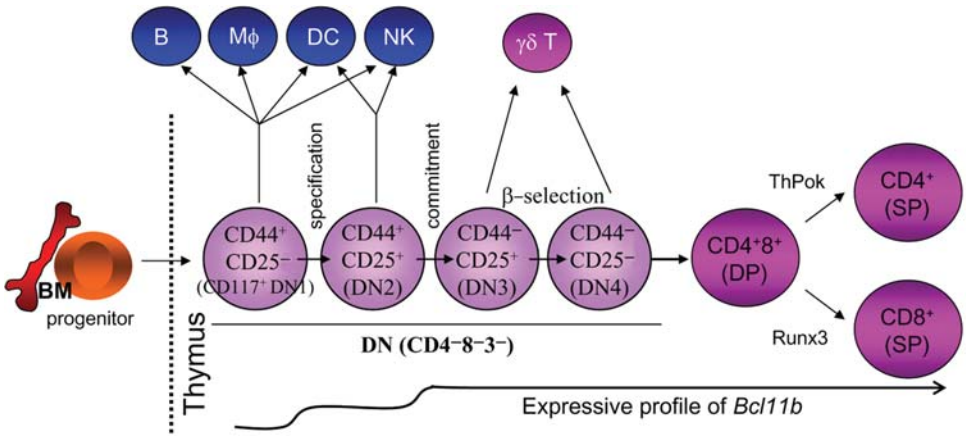

Figure 1 Outline of T-cell development pathway from bone marrow to the thymus. Lympho-hematopoietic progenitor cells leave the bone marrow for the thymus. Intrathymic T-cell development includes starting with none of CD4 and CD8 markers (double negative, DN), then gaining both CD4 and CD8 (double positive, DP), to losing one of them (single positive, SP). In the DN stage, there are four subsets: DN1, DN2, DN3, and DN4, based on the gain and loss of CD44 and/or CD25. DN1 contains $CD117^+$ and $CD117^-$ subsets. The figure shows $CD117^+$ DN1, which is the canonical subset giving rise to DN2. Expression of *Bcl11b* gene was shown to be increased from DN2 to DN3 stage and subsequently maintained at a high level based on the Li et al.'s paper (2010).

potential to give rise to DC and NK cells. However, after the DN2 checkpoint, the thymocytes generally develop in the T-cell pathway, because thymocytes undergo T-lineage commitment between DN2 and DN3, and undergo β-selection at DN3 (Figure 1). The whole developmental process is tightly controlled by stage-specific transcription regulators, such as notch and its mediators, GATA-3 and E2A/HEB, and so on (Rothenberg and Taghon, 2005; Rothenberg et al., 2008). Bcl11b is a zinc-finger transcription factor, and its function in the T-cell development in the thymus was only recently recognized (Wakabayashi et al., 2003), so the information is very limited.

Turning T cells into NK cells

The OP9-DL1 monolayer co-culture system is an *in vitro* model of T-cell lymphopoiesis, generated by Dr. Zuniga-Pflucker's group in 2002 (Schmitt and Zuniga-Pflucker, 2002). It is now widely used for the culture and observation of T-cell development *in vitro* (Zuniga-Pflucker, 2004). In this system, the OP9 bone marrow-derived stromal cells are transformed to express notch ligand delta-like 1 (DL1). Hematopoietic progenitor cells from BM or thymus are loaded on top of the OP9-DL1 cells and undergo expansion and differentiation to develop into mature T cells. Dr. Liu's group used the OP9-DL1 culture system to culture immature thymocytes, in which the floxed-*Bcl11b* gene was inducibly deleted (conditional knockout) during the culture, and made some exciting discoveries that were published in a recent issue of Science (Li et al., 2010). They found that the immature thymocytes in this culture system did not undergo expansion and differentiation, but instead killed the OP9-DL1 stromal cells. On further investigation, they found that these conditional *Bcl11b* gene knockout thymocytes were altered into NK cells gaining an NK marker—NKp46, while losing a T-cell marker—CD3, and the TCR-β. They called this change 'reprogramming' and the reprogrammed stromal cell-killing $NKp46^+CD3^-$ cells 'ITNK (T-to-natural killer) cells'. ITNK cells represent a new type of killer cells, because they are different from natural thymic NK cells and conventional mature NK cells in some of their molecular markers, but possess their killing ability. Almost all T-cell populations, including immature (DN1, DN2, DN3, and DP) and mature ($CD8^+$, except for $CD4^+$) T-lineage cells, have the potential to undergo reprogramming. However, B and γδ T cells did not show this potential, which coincides with observations in the *Bcl11b* conventional gene knockout mouse (Wakabayashi et al., 2003). Furthermore, the reprogramming can occur both *in vitro* (OP9-DL1 culture) and *in vivo* (conditional *Bcl11b* gene knockout lymphocyte transplantation into lymphocyte-deficient $Rag2^{-/-}$ $IL2rg^{-/-}$ mouse) models.

Transcription factor *Bcl11b* is involved in T-cell lineage determination

The Liu's group, using their newly generated *Bcl11b-tdTomato* knock-in reporter mouse (Li et al., 2010), found that expression of the *Bcl11b* gene is up-regulated from the DN2 stage and maintained at a high level from the DN3 stage through all late developmental stages of T cells (Figure 1). This expression profile suggests the importance of *Bcl11b* as a crucial regulator in all T-cell developmental stages, including mature T cells. However, in the past, shortage of *Bcl11b* had been reported to only arrest immature T-cell development at earlier stages, such as DN3 and DP (Inoue et al., 2006; Albu et al., 2007). It is apparent that much remains unknown about the role of *Bcl11b* in T-cell development. Recent reports have enhanced its importance since it corrects the expression of approximately 1000 genes in thymocytes, including transcription factors that are required for the late stage of T-cell development, such as

ThPok—required for $CD4^+$ T-cell differentiation and *Runx3* implied for $CD8^+$ T-cell differentiation (Kastner et al., 2010).

During normal T-cell development in the thymus, DN1 and DN2 thymocytes have the potential to give rise to thymic NK cells (Rothenberg and Taghon, 2005), although details of the regulatory pathway are not clear. Li et al. (2010) may provide a clue by suggesting that changes in the expression of *Bcl11b* at the physiological level in the natural thymus lead to this pathway. After T-lineage commitment, which occurs during the DN2 to DN3 checkpoint, the thymocytes have never previously been known to give rise to thymic NK cells. Therefore, Dr. Liu's group has made a great ground breaking discovery by demonstrating that the potential to give rise to NK cells after the DN2 checkpoint still exists. The cells after T-commitment still can be reprogrammed into NK cells.

An immunotherapeutic regiment and several unknown questions

The report by Li et al. (2010) showed that the *ex vivo* expanded ITNK cell did not indiscriminately kill normal cells nor were they malignantly transformed, but they exclusively killed cancer cells with powerful ability (10-fold reduction of lung metastatic foci of melanoma cells). The results without doubt provide a new avenue of promising interventional medicine for the treatment of cancers. Scientists have previously attempted to expand and activate a patient's own (autologous) tumor-reactive T cells by isolating, culturing and manipulating them, then re-injecting these cells back into the patient to treat the cancer. However, successful examples of this *ex vivo* T-cell treatment are variable and limited (Borghaei et al., 2009). This technique was also used in an attempt to expand NK cells *ex vivo* (Klingemann and Martinson, 2004). However, the purification of scarce NK cells from patients' blood and the cell-expansion variability are hard to control. Henceforth, scientists can isolate and alter a patient's own T cells, which are much more than NK cells in the blood, into ITNK cells, by knockout or knockdown of the *Bcl11b* gene using RNA interference.

Although ITNK may present a promising immunotherapeutic regiment to treat cancer (Li et al., 2010), a couple of issues still need to be addressed before it is used to clinic. First, since the T cells used in the reprogramming were largely from the thymus, whether the T cells from the blood, which is easily obtained from patients, can be converted into ITNK cells with the same efficacy still needs to be tested, because there is a finalized process (Weinreich and Hogquist, 2008) for T cells just before leaving the thymus. Second, while ITNK cells carry out the same function as physiological NK cells, whether they use the same mechanism to kill tumor cells and whether they are as safe to normal tissues, for example, tolerant to self-tissues by expression of inhibitory receptor killer cell Ig-like receptor, still need to be investigated. Third, when testing the *in vivo* ITNK tumor-cell killing capability the experimental design was the injection of ITNK cells into healthy mice prior to inoculation of melanoma cells into these mice. This is generally considered to test the prevention of tumor formation/metastasis. The effectiveness of the curative experiment, i.e. applying ITNK cells to the animals already suffering from cancer, remains to be tested. Finally, in the authors' own words, 'If we can see the same effect in humans—could be of enormous value in cancer treatment'. In any event, this exciting story represents a major breakthrough towards curing cancer and identifies an important, novel transcription factor in thymus development.

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