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A Bach2 Link between Pre-B Cell Receptor Checkpoint and Pre-B Cell ALL

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Bach2 is a transcription factor required for affinity maturation of B cells. A recent study reveals, quite unexpectedly, that Bach2 also plays a key role in the pre-B cell receptor checkpoint and functions as a tumor suppressor in pre-B cell acute lymphocytic leukemia.

From lineage specification to the ultimate production of plasma cells, the B cell development program is not only marked by distinct phases of Ig rearrangement and diversification, but cell fate decisions are also often closely coordinated with the functional status of the surface B cell receptor (BCR). It is now generally accepted that B cell development is programmed with a series of checkpoints that control the initiation of key downstream events. The first major checkpoint, the pre-B cell receptor (pre-BCR) checkpoint, governs the transition from the pre-BI (completion of V_H-DJ_H rearrangement) to the pre-BII stage (onset of immunoglobulin [Ig] light chain rearrangement) (Herzog and Jumaa, 2012). Structurally resembling a mature BCR, the pre-BCR signaling complex is formed between a productively rearranged Ig heavy chain (IgH), the invariant, surrogate light chain, and two accessory signaling molecules, Igα and Igβ. Because V_H-DJ_H joining carries a great risk of disrupting the V segment open reading frame, the pre-BCR checkpoint is believed to function as a quality control step to monitor the structural integrity of the newly synthesized IgH chain on a pre-BI cell. Consequently, cells expressing nonfunctional pre-BCRs are either eliminated (negative selection) or allowed to rearrange the second IgH allele if still available. Cells equipped with a signaling competent pre-BCR are allowed to expand and proceed to the pre-BII stage, where Ig light chain rearrangement is initiated (positive selection).

Despite its importance in B cell development, regulation of the pre-BCR checkpoint remains incompletely understood. First, the transcription factor network operating at this checkpoint has yet to be defined. Second, it is not clear how signals from the pre-BCR are integrated into the cell fate decision in a manner that enables negative selection prior to positive selection. In a recent *Nature Medicine* article, Swaminathan et al. (2013) made exciting discoveries that shed light on both fronts. In searching for novel regulators of the pre-BCR checkpoint, the investigators honed in on Bach2 after analyzing relevant gene expression changes in both humans and mice.

Bach2 is a transcription factor previously implicated in Ig class switch recombination and efficient germinal center formation in mature B cells (Muto et al., 2004). Two attractive features about Bach2 were noted. Bach2 was significantly upregulated by Pax5 at the onset of V_H-DJ_H rearrangement. In addition, the dramatic cell death that results from Pax5-triggered V_H-DJ_H joining and subsequent negative section was greatly diminished in *Bach2*$^{-/-}$ pro-B/pre-BI cells. This was accompanied by reduced expression of p53 and Arf. Subsequent chromatin immuneprecipitation (ChIP) and gene expression analysis revealed that both *Cdkn2a* (which encodes Arf) and *Tp53* loci are under reciprocal regulation by Bach2 and Bcl6, a transcriptional repressor previously shown by the same group to facilitate positive selection by suppressing *Tp53* (Duy et al., 2011). The fact that additional genes involved in checkpoint function were also regulated by Bach2 and Bcl6 in opposite directions adds further support to the notion that the interplay between Bach2 and Bcl6 coordinates the orderly transition from negative to positive selection.

Two types of experiments provided the most definitive proof for a Bach2 requirement in negative selection. First, in a pre-B differentiation system based on tyrosine kinase inhibition (TKI) of BCR-ABL-transformed pre-B cells, Bach2 deficiency reduced the V(D)J rearrangement efficiency by ~20-fold, a defect concurrent with a similar reduction in the mRNA of *Rag1/2*. The notion that Bach2 can directly regulate *Rag1/2* transcription is supported by several assays including a Bach2 ChIP of the *Rag1* and *Rag2* promoters. The second set of experiments, which included an elegant test of V_H-DJ_H junction length distribution, showed that >50% of the *Bach2*$^{-/-}$ precursor B cells contain nonfunctional V_H-DJ_H joining, compared to only ~10% in wild-type controls. Most importantly, reexpression of Bach2 eliminated nonfunctional IgH rearrangements almost completely. Collectively, the results presented by Swaminathan et al. (2013) have clearly established Bach2 as a key regulator in the pre-BCR checkpoint. Mechanistically, these data are consistent with a model where Bach2, operating downstream of Pax5, promotes V_H-DJ_H rearrangement by sustaining Rag1/2 expression on the one hand, and, on the other hand, purges cells carrying nonfunctional IgH rearrangements through p53-dependent cell death (Figure 1A).

The second major and novel conclusion from this study carries significant clinical implications. Swaminathan et al. (2013) proposed that BACH2 is a novel tumor suppressor in pre-B cell acute lymphocytic leukemia (pre-B ALL), a notion that enforces the general concept that pre-B cell checkpoint regulators often also play roles in pre-B ALLs. PAX5, BCL6, and another pre-B cell checkpoint regulator, SLP-65, have all been previously implicated in pre-B ALLs (Duy et al., 2011;

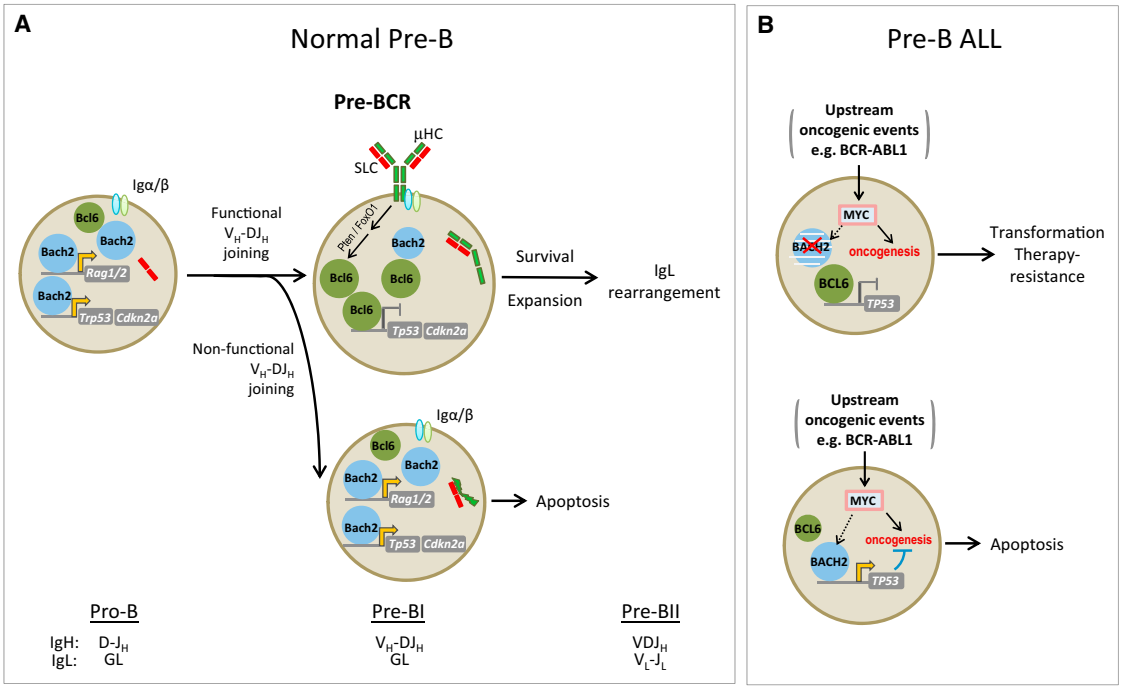

Figure 1. Model Illustrating the Cell Fate Outcome Influenced by the Interplay between Bach2 and Bcl6 at the Pre-Bcr Checkpoint and in Pre-B All

(A) In pre-BI cells, the absence of a functional pre-BCR leaves Bach2 expression at a relatively high level, which eventually triggers p53-dependent apoptosis when V_H-DJ_H joining has failed on both IgH alleles. This is the proposed mechanism for purging nonfunctional IgH rearrangement from the pre-B cell pool (bottom). During positive selection, signals transduced from a signaling competent pre-BCR lead to a shift in the Bach2-Bcl6 balance and the subsequent suppression of *Cdkn2a/Tp53* by Bcl6, which is a prerequisite condition for cell survival, expansion, and onset of the Ig light chain rearrangement at the pre-BII stage (top).

(B) In progenitor B cells, BACH2 expression favors p53 activation, which then imposes a barrier against transformation by aberrantly activated oncogenes (bottom). In cells where BACH2 expression or activity is reduced by either genetic or epigenetic changes, BCL6 overrides BACH2 influence and suppresses p53. This shift in the BACH2-BCL6 balance thus impairs the anti-cancer barrier, leading to de novo transformation of pre-B cells or acquisition of therapy resistance in established pre-B ALLs (top). Dashed arrows indicate it is currently unclear how aberrantly activated oncogenes might activate the BACH2-p53-apoptosis axis.

Herzog et al., 2006; Mullighan et al., 2007). The tumor suppressor function of BACH2 is supported by a large volume of results from cell culture-based experiments and genetic analysis of mouse and human pre-B ALLs, as well as clinical response data of pediatric B-ALL patients (Swaminathan et al., 2013). The most striking experiment among many is a Myc transformation assay performed in a bone marrow transplantation setting, a test well-known to evoke the ARF/p53-enforced tumor suppressive barrier (Lowe et al., 2004). Consistent with the ability of Bach2 to antagonize Myc-induced transformation, Myc-transduced *Bach2*$^{-/-}$ pre-B cells gave rise to lethal leukemia within 3 weeks, while recipients of Myc-transduced, Bach2-proficient cells remained leukemia-free for up to 10 weeks.

Clinical data from pediatric ALL patients demonstrated the ability of BACH2 expression to predict survival outcome (Swaminathan et al., 2013). Specifically, at the time of diagnosis, loss of BACH2 expression strongly correlated with predicted minimal residual disease and lower relapse-free survival. Comparing matched sample pairs collected at initial diagnosis and subsequent relapse, the authors found loss of BACH2 expression to be a common feature of disease relapse. How could BACH2 expression or function be lost during pre-B ALL development? The authors presented four possible scenarios, each supported by evidence from primary human ALL samples. These include a hot spot mutation in the BTB domain of BACH2 (found in five of ten Ph$^+$ ALL cases), promoter hypermethylation, PAX5 inactivation, and deletion of 6q15, where the human *BACH2* gene resides. Of note, in three out of four 6q15 deletion cases examined, the deletion was an acquired event at relapse. Combined with a general reduction of *BACH2* mRNA in all relapsed cases, this observation raises the distinct possibility that leukemia subclones with low BACH2 were more resistant than subclones with higher BACH2 expression to standard ALL treatment. This notion is in line with the differential toxicity of TKI in BCR-ABL-transformed wild-type and *Bach2*$^{-/-}$ pre-B cells. Because BACH2 and BCL6 play opposing roles in p53 regulation, checkpoint control, and patient outcome (Figure 1B), the authors propose to pharmacologically inhibit BCL6 using the BCL6 peptide inhibitor RI-BPI (Duy et al., 2011; Polo et al., 2004) in order to restore p53 expression and hence therapeutic response.

The study by Swaminathan et al. (2013) raises a number of tantalizing questions. Is the positive role of Bach2 on p53

expression exerted directly at the level of p53 transcription? Because Bach2 and Bcl6 recognize distinct DNA binding sequences, what is the mechanism underlying their competitive binding behavior in shared target promoters? In addition, at least under certain circumstances, Bach2 can shuttle between the cytoplasm and nucleus in a redox sensitive fashion (Chen et al., 2013; Muto et al., 2002). Therefore, is Bach2 subcellular localization modulated during the pre-BCR checkpoint? Since Bach2 has emerged as a key regulator of the pre-BCR checkpoint, these issues merit future studies.

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Mechanisms of Targeted Therapy Resistance Take a De-TOR

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The effectiveness of cancer therapeutics targeting signal transduction pathways is comprised of a diversity of mechanisms that drive de novo or acquired resistance. Two recent studies identify mTOR activation as a point of convergence of mechanisms that cause resistance to inhibitors of the Raf-MEK-ERK and PI3K signaling.

A critical turning point in the fight against advanced and metastatic melanomas occurred just over a decade ago with the discovery and characterization of the *BRAF* activating mutation V600E in about 60% of melanomas (Davies et al., 2002). This mutation causes constitutive activation of the B-Raf serine/threonine kinase, resulting in aberrant and persistent activation of the Raf-MEK-ERK mitogen-activated protein kinase cascade. Importantly, *BRAF* V600E correlated with poor prognosis in patients with metastatic melanoma. This prompted the development and clinical evaluation of Raf and MEK inhibitors for the treatment of *BRAF* mutant metastatic melanoma (Salama and Flaherty, 2013). The dramatic antitumor activities of these inhibitors led to Food and Drug Administration approval of two Raf (vemurafenib and dabrafenib) and one MEK (trametinib) inhibitor for the treatment of *BRAF* mutant melanoma (Chapman et al., 2011; Flaherty et al., 2012; Hauschild et al., 2012). Despite the clinical success of these inhibitors, resistance has limited their long-term clinical impact. Although patient selection based on *BRAF* mutation status defines the patient population that would benefit from Raf or MEK inhibition, 20%–50% of patients showed no initial response, suggesting de novo resistance in a significant subset of melanoma patients (Chapman et al., 2011; Hauschild et al., 2012). Furthermore, even for patients who do respond initially, within three months, essentially all suffer from relapsed tumors that have acquired drug resistance. This has led to numerous studies that have identified multiple mechanisms of de novo and/or acquired resistance to Raf, inhibition with mechanisms that cause ERK reactivation downstream of the inhibitor block, as well as ERK-independent mechanisms (Sullivan and Flaherty, 2013).

Corcoran et al. (2013) have recently identified a mechanism that may provide a more unifying model for the diverse mechanisms already identified. Although decreased phosphorylation of ERK (pERK) has thus far been the standard used to gauge tumor sensitivity in both clinical and preclinical studies, Corcoran et al. (2013) found that robust inhibition of pERK was still observed in melanoma