Role for the pre-BCR Signaling Complex in BCP-ALL Blast Cell Survival

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Abstract. **Strong evidence suggests that 'tonic signals' mediated through the pre-B Cell Receptor (pre-BCR) are essential for pre-B cell protection from apoptosis. We propose that B Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) blasts also require this pre-BCR survival mechanism, which may contribute to resistance to chemotherapeutic challenges. This work utilizes state-of-the-art single molecule imaging methods to look into the role of transient self-dimerization or galectin-mediated crosslinking of the pre-BCR and its role in 'tonic signaling' in a BCP-ALL cell model.**

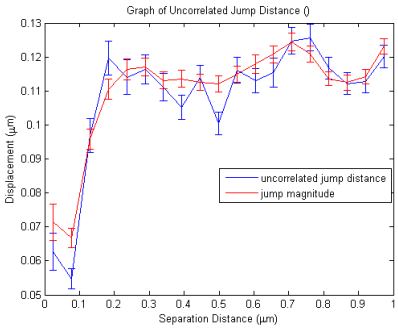
I. Significance

THE pre-B Cell Receptor (pre-BCR) is a highly specific target in Precursor B Acute Lymphoblastic Leukemia (BCP-ALL), the most prevalent neoplasm in children [1]. Though therapies have markedly increased the number of relapse-free patients in the past 40 years, there remain inherent concerns with existing treatments [2]. Because high-risk leukemia outcome appear to have generally plateaued with conventional therapy [3], a push for new therapeutic approaches and agents is of rising importance.

II. Experimental Approach

Introduction. Our study focuses on the pre-B Cell Receptor (pre-BCR) that plays a central role in the early stages of B cell development. Surface expression of pre-BCR complex components are required for survival of normal B cell progenitors. It has been postulated that tonic pro-survival signals derive from antigen-independent interactions between pre-BCRs through surrogate light chain (SLC) components [4-5]. Galectin binding appears to provide an alternative mechanism for pre-BCR oligomerization [6], acting as broadly reactive dimerizing "ligand". Whether the pre-BCR forms dimers and higher order oligomers through self-association or through bridging by galectin, it should trigger Lyn-mediated phosphorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) on the Igα and Igβ subunits [7]. ITAM phosphorylation forms binding sites for dual SH2-domains of spleen tyrosine kinase (Syk), a critical component of downstream signaling cascades that regulates cell fate decisions.

Rationale & Results. We have developed monovalent Quantum Dot labeled Fab fragments as probes for the cell surface pre-BCR, permitting us to use single molecule imaging and other innovative microscopy approaches to capture the dynamics of receptors interacting on the surfaces of live pre-B cells. Results show that diffusing pre-BCR engage in transient but frequent homotypic interactions. Motion is correlated only at short separations distances, consistent with formation of dimers and potential larger order oligomers (Figure below). These interactions likely drive between diffusing pre-BCRs may be implicated in pro-survival signals for the leukemia blasts. We are utilizing these same tracking methods to test the hypothesis that galectin produced as an autocrine factor [8] or by stromal cells [9] amplifies pre-BCR signaling capability by increasing the lifetime of pre-BCR complexes. Our long-term goal is to use this information as a motivation for the development of biologic agents that inhibit pre-BCR dimerization and offer potential for treatment of BCP-ALL.



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