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Extra Views

Interference of BCR-ABL1 Kinase Activity with Antigen Receptor Signaling in B Cell Precursor Leukemia Cells

Florian Klein
Niklas Feldhahn
Markus Müschen*

Laboratory for Molecular Stem Cell Biology; Institute for Transplantation Diagnostics and Cell Therapeutics; Heinrich-Heine-Universität Düsseldorf; Düsseldorf, Germany

*Correspondence to: Markus Müschen; Laboratory for Molecular Stem Cell Biology; ITZ, Building 14.80; Heinrich-Heine-Universität Düsseldorf; 40225 Düsseldorf, Germany; Tel.: +49.211.811.9964; Fax: +49.221.811.9961; Email: markus.mueschen@uni-duesseldorf.de

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KEY WORDS

Antigen receptor, B cell precursor leukemia, BCR-ABL1, STI571

ABBREVIATIONS

CML	chronic myeloid leukemia
IGH	immunoglobulin heavy chain
SAGE	serial analysis of gene expression

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ABSTRACT

The chromosomal translocation t,(9;22) resulting in the fusion of the *BCR* and *ABL1* genes, represents a recurrent aberration in B cell precursor leukemia cells. Their normal counterparts, B cell precursor cells, are positively selected for survival signals through the antigen receptor, whose expression requires a functional immunoglobulin heavy chain (*IGH*) gene rearrangement. Unexpectedly, B cell precursor leukemia cells harboring a *BCR-ABL1* gene rearrangement do not depend on antigen receptor mediated survival signals. Genes involved in the signaling cascade of the antigen receptor are silenced and in most cases, the dominant tumor clone does not carry a functional *IGH* gene rearrangement. However, upon inhibition of the BCR-ABL1 kinase activity by STI571, only leukemia cells expressing an antigen receptor are able to survive. Since resistance to STI571 is frequent in the therapy of *BCR-ABL1*$^+$ B cell precursor leukemia, antigen receptor signaling may represent a mechanism through which these cells can temporarily evade STI571-induced apoptosis. This may open a time frame, during which leukemia cells acquire secondary transforming events that confer definitive resistance to STI571.

INTRODUCTION

Early B cell development takes place in the bone marrow, where the expression of different transcription factors, e.g., *PAX5*, *EBF*, *E2A*, commit hematopoietic stem cells to the B cell lineage.[1] During differentiation, B cell precursors undergo a defined sequence of immunoglobulin heavy chain (*IGH*) gene rearrangements.[2] A productively rearranged *IGH* variable (V) region gene functions as a component for the assembly of the antigen receptor,[3] whose expression is required for survival and further differentiation along the B cell lineage. B cell precursors are destined to die by apoptosis unless they are rescued by survival signals through their antigen receptor.[4]

Defects of molecules that confer antigen receptor signaling can also lead to a developmental block at an early B cell stage or critically decrease the number of mature B cells.[5] For example, deleterious mutations of Bruton's tyrosine kinase (*BTK*) result in a B cell immunodeficiency, called X-linked agammaglobulinemia (XLA) in humans.[6]

However, the expression of an antigen receptor exposes B cells also to a negative selection process, where cells carrying an auto-reactive receptor are eliminated.[7] These cells can only avoid programmed cell death by undergoing secondary rearrangements of a functional *IGH* V region gene. Thus, antigen receptor signaling is essential for survival and further differentiation but can also transduce apoptosis signals.

During development, B cells may acquire genetic aberrations, which can give rise to malignant transformation. The most frequent recurrent translocation leading to B cell precursor leukemia in adults involves chromosomes 9 and 22 and results in the expression of BCR-ABL1.[8] This translocation is also found in nearly all cases of chronic myeloid leukemia (CML). The protein encoded by *BCR-ABL1* functions as a constitutively active tyrosine kinase and is responsible for the oncogenic transformation.[9]

A specifically designed inhibitor of the BCR-ABL1 kinase, called STI571 (Imatinib), is now successfully used for CML therapy[10] but failed to increase long-term survival in the treatment of *BCR-ABL1*$^+$ B cell precursor leukemias.[11] Treatment failures of STI571 in CML and B cell precursor leukemia are in general attributed to the acquisition of STI571-resistance.

BCR-ABL1$^+$ B CELL PRECURSOR LEUKEMIA CELLS ARE INDEPENDENT OF SURVIVAL SIGNALS THROUGH THE ANTIGEN RECEPTOR

While the essential role of the antigen receptor has been described for B cells and their precursors, we recently started to investigate the function of antigen receptor signaling in *BCR-ABL1*$^+$ B cell precursor leukemia cells:[12] Analyses of the *IGH* loci identified that in 9 of 12 leukemia cases, the dominant tumor clone does not carry a potentially functional *IGH* gene rearrangement, which would be required for the expression of an antigen receptor. In these nine cases, we identified traces of secondary V region gene rearrangements, which may have rendered an initially productive rearrangement nonfunctional, indicating ongoing recombination of *IGH* V region genes.

Comparing serial analysis of gene expression (SAGE) profiles of *BCR-ABL1*$^+$ B cell precursor leukemias and their normal counterpart, we observed decreased expression of antigen receptor-associated genes in the leukemia cells, namely genes encoding membrane-located receptor molecules as *CD19, IGHC*μ*, VpreB, Ig*α*, Ig*β and signaling molecules including *LYN, BLK, BTK, SLP65, SYK, BAP37, PLC*γ*2, VAV1-3, LCK, FYN, SHC1, NIK,* and *IKK*. Of note, genes related to the antigen receptor cascade were transcriptionally silenced even in leukemia cells carrying a potentially functional *IGH* V region gene rearrangement. Measuring Ca^{2+} flux in B cell precursor leukemia cells revealed autonomous oscillatory signaling activity, which was independent of antigen receptor engagement. We conclude that the majority of B cell precursor leukemia cells harboring a *BCR-ABL1* fusion gene can survive and even clonally expand in the absence of antigen receptor-mediated survival signals. Unlike normal B cell precursors, *BCR-ABL1*$^+$ leukemia cells bypass selection for the expression of an antigen receptor.[12]

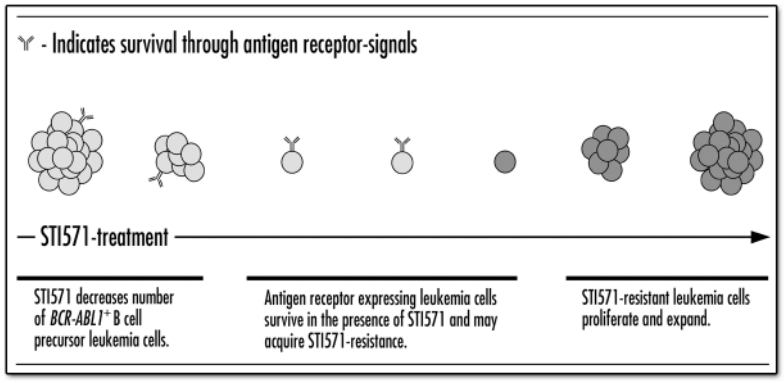

Figure 1. Hypothesis for the acquisition of STI571- resistance in *BCR-ABL1*$^+$ B cell precursor leukemia cells. STI571-treatment induces apoptosis in the majority of *BCR-ABL1*$^+$ B cell precursor leukemia cells (lightly shaded cells). Rescued by survival signals through the antigen receptor, a subset of leukemia cells can avoid STI571-induced apoptosis. However, the time frame to acquire additional mutations that eventually cause STI571-resistance is extended. Once such an event takes place (e.g., mutation within the ATP binding site), the cell (darkly shaded) is again driven by BCR-ABL1 kinase activity and can clonally expand.

THE FUNCTION OF THE ANTIGEN RECEPTOR IN OTHER B CELL MALIGNANCIES

Conversely, the majority of B-cell-non-Hodgkin's lymphomas express an antigen receptor and are most likely selected for its expression.[13] For instance, engagement of the antigen receptor in chronic lymphocytic leukemia (CLL) cells inhibits apoptosis signals including decreased caspase activity and activation of NF-κB.[14] Follicular lymphoma cells usually carry the chromosomal translocation t(14;18)[15] resulting in the overexpression of *BCL2*, but still require survival signals through their antigen receptor. Furthermore, analyses of *IGH* V region genes indicated a positive selection process for follicular lymphoma cells expressing an antigen receptor of high antigen affinity.[16,17]

However, other B cell tumors are independent of the antigen receptor. For example, Hodgkin and Reed-Sternberg (H/RS) cells in classical Hodgkin's lymphoma carry to a large extent "crippling" mutations within the variable region genes of the *IGH* locus.[18] Independence from the antigen receptor, which was also observed for B cells in angioimmunoblastic lymphadenopathy with dysproteinemia (AILD),[19] does not only reflect absence of selection but might also confer a survival advantage. In order to avoid apoptosis signals through the antigen receptor, these tumor cells may render a previously functional receptor nonfunctional.

While in most B cell malignancies, the antigen receptor is indispensable, *BCR-ABL1*$^+$ B cell precursor leukemia cells can survive in the absence of antigen receptor signaling. This raises the question of how these survival signals can be replaced in *BCR-ABL1*$^+$ B cell precursor leukemia cells.

THE BCR-ABL1 KINASE INTERFERES WITH THE ANTIGEN RECEPTOR SIGNALING CASCADE

Interaction with several antigen receptor signaling molecules was recently found to play a crucial role for the transforming activity of *BCR-ABL1*. For example, a truncated isoform of the linker molecule SLP65, which is no longer accessible to tyrosine phosphorylation from the antigen receptor, is expressed upon BCR-ABL1 kinase activity and was frequently detected in leukemia cells.[12,20] Src-kinases, including LYN, are activated in *BCR-ABL1* expressing B lymphoid cells and required for the leukemogenesis of B cell precursor leukemia.[21] One could envision, that *BCR-ABL1* integrates molecules from the antigen receptor signaling cascade into its own signaling pathway.

Of note, Epstein-Barr Virus (EBV) is associated with several B cell proliferative disorders and encodes the protein LMP2A, which can promote survival and differentiation even in antigen receptor negative B cells.[22,23] Moreover, LMP2A can prevent the function of the antigen receptor by excluding it from glycolipid-rich membrane domains.[24] Like the antigen receptor, LMP2A contains immunoreceptor tyrosine based activation motifs (ITAMs) and interacts with the tyrosine kinase LYN.[25] Therefore, LMP2A exploits the antigen receptor signaling cascade by mimicking upstream receptor signals.

Both, LMP2A and *BCR-ABL1* confer independence of the antigen receptor and target its associated molecules. In order to drive malignant transformation, they require components of the antigen receptor signaling cascade, but can replace the function of the antigen receptor.

INHIBITION OF THE BCR-ABL1 KINASE ACTIVITY RECONSTITUTES SELECTION FOR THE EXPRESSION OF AN ANTIGEN RECEPTOR

Upon inhibition of the BCR-ABL1 kinase using STI571, only antigen receptor expressing cells were able to survive.[12] STI571-treated B cell precursor leukemia cells exhibit reconstitution of antigen receptor responsiveness, indicating signaling activity through the antigen receptor complex, as measured by Ca^{2+} flux. In the absence of BCR-ABL1 kinase activity, the malignant cells would undergo apoptosis unless they successfully reactivate other survival signals by default, most likely through the antigen receptor. Therefore, inhibition of the BCR-ABL1 kinase reconstitutes selection for the expression of an antigen receptor.

Even *BCR-ABL1*$^+$ B cell precursor leukemias which do not carry a functional *IGH* gene rearrangement in the dominant tumor clone, showed preferential outgrowth of antigen receptor expressing cells upon STI571-treatment.[12] In these cases, B cell precursor leukemia populations may include subclones that carry a functional heavy chain gene rearrangement prior to STI571-treatment. Alternatively, STI571-mediated inhibition of BCR-ABL1 might induce secondary *IGH* V region gene rearrangements that can replace a non-functional with a productive *IGH* gene rearrangement.

In STI571-treated patients, expression of the antigen receptor on a few leukemia subclones may confer a survival advantage and, hence, represent an escape mechanism, through which some leukemic cells can temporarily evade STI571-induced apoptosis. With respect to the prolonged survival, these cells can acquire additional mutations (e.g., mutations within the ATP binding site of *BCR-ABL1*) and develop an STI571-resistant clone (Fig. 1).

Indeed, resistance to STI571 therapy is a severe and yet not resolved problem.[11,26] In the treatment of *BCR-ABL1*$^+$ B cell precursor leukemias, STI571 has initially a pronounced antileukemic activity but resistance develops rapidly. Hence, treatment failure and relapse occurred mostly after a short period of time (median 58 days; ref. 27). Conversely, STI571 is effectively used in the therapy of CML. In contrast to B cell precursor leukemias, STI571-resistance in CML is observed at a much lower frequency and after longer latency. The high frequency of STI571-resistance in B cell precursor leukemia is possibly owing to the presence of an antigen receptor, which can be expressed by B cell precursor leukemias but not by CMLs.

Besides other mechanisms leading to STI571-resistance, the expression of an antigen receptor could enable *BCR-ABL1*$^+$ B cell precursor leukemia cells to extend the time frame during which STI571-resistance can be acquired. In order to prove this hypothesis, further experiments are currently in progress.

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