Alterations in Bruton’s tyrosine kinase affect the transcriptional profile and phenotype of chronic lymphocytic leukemia cells

March 25, 2016
James B050
1:00
VITA

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ABSTRACT

Chronic Lymphocytic Leukemia (CLL) is currently an incurable disease. The current front-line therapy is chemoimmunotherapy, which is associated with significant toxicity and immunosuppression. Targeted therapeutics have the potential to provide well-tolerated therapies, however the lack of a unifying genetic alteration in CLL has made target identification difficult. Activation of B cell receptor signaling (BCR) is aberrant leading to enhanced pro-survival signaling. An integral kinase in the BCR pathway, Bruton’s Tyrosine Kinase (BTK) was identified as a viable target in CLL. Targeting BTK with ibrutinib, an irreversible inhibitor, can decrease pro-survival signaling, has significant clinical activity and is now FDA approved. Some patients on long-term ibrutinib therapy acquire a point mutation in ibrutinib binding site (BTK\textsuperscript{C481S}). This mutation renders ibrutinib a reversible inhibitor reducing the efficacy of the drug. Determining the transcriptional and phenotypic differences after alteration of BTK is integral for understanding the role of BTK in CLL progression and development of targeted agents to subvert resistance.

It is known that ibrutinib targets CLL survival signaling, but it is not known how targeting BTK alters the microRNA (miR) profile. Lymphocytes from ibrutinib treated patients were collected pre- and post-therapy and were analyzed by miR Nanostring. A unique profile of differentially expressed miRs was identified. OncomiR, miR-155 was confirmed to be down-modulated with ibrutinib therapy. Analysis of miR-155 expression was done on responding versus relapse patients on long-term ibrutinib therapy. miR-155 expression remained down-modulated in responding patients, but was elevated in relapsed patients. We conclude that miR-155 can be favorably down-modulated by ibrutinib treatment and may play a role in resistance. Another novel miR identified was tumor suppressive, miR-126. In our studies, we show that miR-126 is reduced with CLL disease progression. miR-126 expression is negatively correlated with levels of p85β, a
regulatory subunit of PI3K complex, in CLL patients and in miR-126 overexpression cells lines. This study identified a novel miR and its role in targeting PI3K complex in CLL.

The development of the $BTK^{C481S}$ ibrutinib resistant mutation is associated with shorter patient survival after relapse. To study the growth advantage the resistant clone provides, B lymphocyte cell lines that are lacking BTK were obtained and $BTK^{WT}$ and $BTK^{C481S}$ were expressed. Infection with $BTK^{C481S}$ rendered the cells ibrutinib resistant. The $BTK^{C481S}$ shows enhanced kinase activity, migration, pro-survival signaling and increased levels of immune modulatory molecules. After engraftment of $BTK^{WT}$ and $BTK^{C481S}$ cell lines into NSG mice, the mice who received mutant cells had a shorter median survival. Overall we see $BTK^{C481S}$ significantly alters the phenotype of the cells leading to survival of the resistant clone.

In conclusion, this work demonstrates that targeting BTK with ibrutinib can alter the miR profile of the CLL cells, identifying miR-155 and miR-126 as having a role in CLL disease biology. We have also shown that cells that acquire the $BTK^{C481S}$ mutation have enhanced activity and can accelerate disease progression. Overall we can conclude that alterations to BTK through pharmacological inhibition or mutation can affect the CLL disease phenotype.
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FUTURE PLANS

Following graduation, Daphne will be transitioning into the field of clinical research and regulatory affairs. She is currently seeking a fellowship or position in the clinical regulatory field in the pharmaceutical industry or government.