**Identification of Candidate Oncogenes and Chromosomal Breakpoint Sequencing by Targeted Locus Amplification in T-cell Acute Lymphoblastic Leukemia**

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**INTRODUCTION**

T-cell acute lymphoblastic leukemia is characterized by clonal and mutual exclusive chromosomal rearrangements that recurrently activate TAL1, LM22, TLKX, NXK2, TLX2, HOXA or MB22 oncogenes. Most of these translocations or chromosomal rearrangements occur as erroneous D or V-J rearrangement attempts of T cell receptor beta (TCRB) or TCR alpha/delta (TCRd) genes, mostly positioning oncogenes under the transcriptional control of TCR enhancer elements. Alternatively, oncogenes can also be activated as consequence of BCL11B chromosomal rearrangements. Although many oncogenes are known in T-ALL, the driving oncogenic lesion in particular T-ALL cases remains unknown.

**OBJECTIVE(S)**

In this study, we aimed to clone reciprocal breakpoint sequences to elucidate cellular mechanisms that activate oncogenes in T-LX2-translocated patients. Moreover, we want to identify oncogene candidates in various T-ALL patient samples with BCL11B, TCRB- or TCRd-locus translocations for which the candidate oncogene so far was not identified.

**METHOD(S); TARGETED LOCUS AMPLIFICATION (TLA) TECHNOLOGY**

We used Targeted Locus Amplification (TLA) procedure that allows us to identify neighboring DNA sequences that surround a site of interest [I]. The TLA method relies on the crosslinking of DNA in live cells (II), enzymatic digestion of DNA (III) followed by re-ligation (IV) and reverse crosslinking (V) to allow formation of circular DNA ligations (VI). Inverted polymerase chain reaction from a locus specific sequence (i.e. a viewpoint); (VII) amplifies the locus specific sequences. Structural variants can be detected by comparing the sequence to the normal genome (V). Multiple locus specific sequence inverted PCRs have been developed for various oncogenes (XI) (BCL11B, TCRD(TRA61) and TCRD(TCB62) genes) to identify breakpoints in primary T-ALL patient samples.

**RESULT(S)**

**A**

**TAL1-TCRAD translocation**

Using a primer set for TAL1 (chr11), a signal was detected on chr14 (A).

**B**

The signal on chr14 is in the vicinity of the TCRD locus (B).

**C**

Target locus amplification was applied to a TAL1 positive T-ALL sample. The TAL1 locus (chr1) was used as a viewpoint and a signal of chromosome 14 was detected (A). The signal was located at the TCRD locus (B). A sequence read of the breakpoint and a transition sequence was identified (C).

**CONCLUSION(S)**

1. Targeted Locus Amplification is a powerful technique to discover translocation partners in chromosomal translocations, e.g. oncogenic partners that become activated due to chromosomal rearrangements to TCRB, TCRD or BCL11B translocations in T-ALL, and may improve classification of malignancies into risk-categories.

2. The targeted locus amplification procedure allows for rapid cloning of translocation breakpoints in diagnostic patient samples. This way, it may provide patient-specific minimal residual disease markers for disease monitoring during the course of treatment.

3. Pinpointing exact locations of chromosomal breakpoints may specify regulatory elements (e.g. enhancers, promoters) that drive oncogene expression levels, improving our understanding of pathogenic drivers of malignancies.

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