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Commentary

A Brief Discussion about Polymerase Chain Reaction (PCR)

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DESCRIPTION

PCR of chromosomal breakpoint junctions is also a popular method for identifying translocations because of its rapidity and simplicity. Two types of translocations occur in lymphoid neoplasms, those that cause normal cellular genes to be deregulated without affecting the coding sequence (type 1) and those that result in the fusing of two normal cellular genes to form a new chimeric gene (type 2).

One of the antigen receptor gene loci is involved in the majority of type 1 translocations. These include translocations such as t (8;14) involving the IGH region on chromosome 14 and the MYC gene on chromosome 8, t (11;14) involving the IGH locus and the CCND1 locus on chromosome 11, and t (14;18) involving the IGH locus and the BCL2 gene.

Translocations damage the target gene's regulatory elements or place it under the control of elements from the relevant antigen receptor gene in all of these circumstances.

A minority of the type 1 translocations do not involve an antigen receptor gene, such as some of the variant translocations involving the BCL6 gene on chromosome 3q27. Nonetheless, the result is similar in that the target gene's coding sequence is left intact, but its regulatory elements are altered, through the substitution of control elements from the various genes on the partnering chromosomes. Whereas the type 1 translocations leave the coding sequences of the potential oncogenes intact, Type 2 translocations occur between two unrelated genes and result in the creation of a novel mRNA and protein encoded by portions of the two fused genes. Typically both genes are transcription factors, and the resulting chimeric proteins display highly aberrant transcriptional activities. Representative of the type 2 translocation is the pre-B-cell Acute Lymphocytic Leukemia (ALL) t (12; 21) translocation, which fuses the TEL gene on chromosome 12 to the AML1 gene on chromosome 21.

The molecular detection strategies for the two types of translocations differ in type 1 translocations. The targeted template is generally the junctional sequence within the DNA while in, the type 2 translocations the primers usually target the junctional sequence within the message (cDNA) encoding the novel protein. For both types of translocations, a PCR product will be generated only from the tumor cells bearing the junctional DNA or cDNA sequences created by the translocation, which therefore serves as a tumor-specific marker. Detection is generally carried out either by gel or capillary electrophoresis. Alternatively, PCR and detection may be combined in real-time PCR instruments. These instruments monitor the accumulation of product during the PCR reaction using fluorescent detection technologies.

The use of PCR to detect type 1 translocations can be highly effective when the breakpoints on the partnering chromosomes are clustered, as occurs with the t (14;18) translocations in follicle centre lymphomas. While DNA-based PCR assays have a very high analytic sensitivity, capable of identifying 1 in 106 abnormal cells, these tests have a much lower diagnostic sensitivity than fish, since each PCR primer set can target only a few hundred base-pair segments of DNA, while an appropriate FISH probe can identify translocations involving all potential breakpoint regions. Type 2 translocations occur most frequently in pre-B-cell and B-cell lymphoblastic lymphomas/leukemia, but they also occur in some of the mature B and T-cell lymphomas, including ALCL and MALT lymphoma.

These are interesting events from the biological point of view, as they create chimeric proteins that have novel oncogenic activities. For diagnostic purposes, the chimeric RNA serves as the tumor-specific PCR product. Reverse Transcription (RT)-PCR assays targeting the chimeric RNA sequences generally have a much higher diagnostic sensitivity than those that target the corresponding chromosomal breakpoint regions because in most cases, gene splicing events create junctions precisely between the exons of the fused genes, even when the breakpoint sequences themselves may be spread diffusely throughout intronic sequences. For example, 75% of ALCLs possess a t (2; 5) translocation joining the Nucleophosmin Gene (NPM) on chromosome 5 to the ALCL tyrosine kinase gene (ALK) on chromosome 2. The breakpoints are spread throughout intron 4 of the NPM gene and within a single intron of the ALK gene, which presents difficulties in the design of a simple DNA-based PCR test. However, splicing events in the novel transcript brings exon 4 of NPM into contiguity with a 5′ coding exon of ALK in all cases. This allows the design of a simple RTPCR assay, using

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only a single primer pair that can reliably identify all of the t (2; 5) translocations.

CONCLUSION

PCR is a highly accurate and rapid method for duplicating genetic material. The discovery of thermostable polymerase enzymes has permitted the automation of PCR, thus reducing the manpower required to conduct these experiments.