Rearrangement breakpoints and viral integration sites in the human genome

Deborah Burford

Darwin College

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Abstract

It is estimated that 1 in 2000 live births have an apparently balanced translocation and that the risk of congenital abnormalities in these carriers is approximately double that seen amongst individuals with normal karyotypes. To date, less than 50 de novo reciprocal balanced translocations have been sequenced across the breakpoint junctions and little is understood about the mechanisms that may cause these non-recurrent rearrangements. The aim of this thesis is to investigate methods of translocation breakpoint mapping. The results showed that array painting onto a Whole Genome Tile Path array followed by array painting onto a custom-made NimbleGen oligonucleotide array and subsequent amplification of the translocation junctions by long range PCR was the most efficient method tested for generating sequence across translocations. Analysis of the sequence generated showed that in the three patients studied, three of the six breakpoints directly disrupted a gene; CENTG2 in a patient with a t(2;7)(q37.3;p15.1) and PTPRZ1 and DACH1 in a patient with a t(7;13)(q31.3;q21.3). As an alternative approach, generation and screening of a custom-made fosmid library using flow sorted derivative chromosomes from an additional patient was used to generate sequence across the translocation breakpoints. This approach is useful for translocations in which additional complexity at the breakpoints confounds other methods for breakpoint mapping and sequencing. The fosmid library approach developed for the mapping of translocation breakpoints has also been applied to the mapping of viral integration into the human genome furthering our understanding of the integrated virus.

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