AN INVESTIGATION OF THE (4;11)(q21;p15) TRANSLOCATION IN ACUTE LYMPHOCYTIC LEUKAEMIA

by

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Abstract

This thesis describes the results of an investigation to determine the molecular basis of an uncharacterised (4;11)(q21;p15) translocation in a patient with T-cell acute lymphocytic leukaemia (ALL). The chromosome 11 breakpoint had been narrowed to the region between the markers *D11S860* and *D11S470*.

A new zinc finger gene near the breakpoint region was cloned and characterised. This gene, subsequently named *ZNF195*, encodes an N terminal KRAB domain and 14 tandemly repeated Krüppel type zinc finger motifs. *ZNF195* was subsequently localised distal to *D11S470* and therefore distal to the chromosome 11 breakpoint in the (4;11)(q21;p15) translocation. This excluded disruption of *ZNF195* by the translocation.

Subsequently the nucleoporin 98 gene (*NUP98*) was identified at an acute myeloid leukaemia translocation. *NUP98* maps distal to *D11S470* and therefore within the breakpoint region. Analysis of somatic cell hybrids segregating the t(4;11) translocation chromosomes showed that the chromosome 11 breakpoint occurred within *NUP98*. The fusion partner of *NUP98* was identified as the *RAP1GDS1* gene using 3' RACE. In the *NUP98-RAP1GDS1* fusion transcript (abbreviated *NRG*), the 5' end of the *NUP98* gene is joined in frame to the coding region of the *RAP1GDS1* gene. This joins the phenylalanine-glycine (FG) repeat rich region of nup98 to smgGDS (the most common name for the protein encoded by *RAP1GDS1*) which largely consists of tandem armadillo repeats. *NRG* fusion transcripts were detected in the leukaemic cells of two other adult T-cell ALL patients with a t(4;11)(q21;p15) translocation. This is the first report of a *NUP98* translocation in lymphocytic leukaemia and the first time that *RAP1GDS1* has been implicated in any human malignancy.

The cellular localisation of nrg was determined and compared to that of its protein components nup98t (t for truncated) and smgGDS. cDNAs were cloned into the pEGFP-C2 mammalian expression vector to create green fluorescent protein (gfp) tagged proteins. The location of the gfp tagged proteins within transfected NIH-3T3 mouse fibroblast cells was visualised using confocal microscopy. Gfp-nup98t was located in a punctate pattern around the nuclear envelope. Gfp-smgGDS was found throughout the cytoplasm and was absent from the nucleus. The hybrid protein gfp-nrg was present throughout the cytoplasm but was also visible within specific subnuclear domains. Therefore the formation of nrg results in nuclear localisation of the normally cytoplasmic smgGDS protein. Nup98t has been shown by others to have strong transcriptional transactivation ability while smgGDS has been shown to be important in blocking apoptosis in thymocytes. It is therefore possible that nrg promotes leukemogenesis through two independent pathways. In the nucleus nrg may act to deregulate transcription pathways critical to normal T-cell development, while in the cytoplasm, nrg may act to increase T-cell survival by promoting an anti-apoptotic phenotype.