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AF4 encodes a ubiquitous protein that in both native and MLL-AF4 fusion types localizes to subnuclear compartments

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Abstract

Acute leukemia with t(4;11)(q21,q23) translocation results from the in-frame fusion of the MLL to the AF4/FEL gene. In previous studies, we and others demonstrated that AF4 transcripts are present in a variety of hematopoietic and nonhematopoietic human cells. To further study the wild-type and leukemia fusion AF4, we used glutathione S-transferase (GST)-fusion proteins as immunogens to produce rabbit polyclonal antibodies that were specific for normal and chimeric AF4 proteins. Using Western blotting analysis, we demonstrated that the AF4 gene encodes proteins with apparent molecular weight of 125 and 145 kD. A 45-kD protein coprecipitated with AF4 protein in immunoprecipitation. Also, the anticipated MLL-AF4-encoded 240-kD protein was detected in all cell lines with t(4;11) translocations; fusion proteins were present in lesser quantity than the wild-type AF4. The proteins recognized by the antibodies are of the predicted sizes of the AF4 and MLL-AF4-encoded proteins based on previous DNA sequencing analysis. The MLL-AF4 fusion protein had a similar subcellular distribution as AF4. Both t(4;11) and non-t(4;11) leukemic cells showed a similar pattern of punctate nuclear staining in all cell lines tested using confocal immunofluorescence microscopy. AF4 antibodies should be useful for further elucidation of the function of AF4 in normal cellular physiology, as well as the function of MLL-AF4 in leukemogenesis. The antibodies should also be helpful for the diagnosis of the MLL-AF4 fusion proteins in t(4;11) leukemias.

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