TRANSFORMATION AND AMPLIFICATION OF GENES FOR HUMAN DIFFERENTIATION ANTIGENS IN MOUSE FIBROBLASTS[1]

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Following co-transfection of mouse L cells (TK-) with the herpes simplex thymidine kinase (TK) gene and total human DNA from JM, a human T lymphoma line, we selected Leu-2^+ and other human lymphocyte membrane antigen transferents by FACS sorting[1]. We found the frequency of transferents for Leu-2 to be about 10^{-3} of the TK+ cells selected in HAT medium. Though most cloned transferents had narrow ranges of antigen density per cell, one of the first four Leu-2^+ transferents found was strikingly more variable in the amount of Leu-2 antigen per cell. Further, this transferent, J10, had a mean Leu-2 staining per cell that was seven times greater than that of the other transferents (1).

In order to see if the J10 clone was amplified and whether it could be further amplified, we went through six cycles of selection of the brightest 0.1 - 0.3% of J10 cells stained with anti-Leu-2 and obtained a line which is 40 times brighter than the mean of J10. When we examined metaphase chromosome spreads of these cells, we found that they have numerous double minute chromosome fragments. Such structures are commonly found in mouse cells which have undergone gene amplification. We then looked to see if all the human DNA sequences present in the amplified J10 cells were similarly amplified. To do this, we cut the total DNA with different restriction endonucleases, electrophoresed the fragments in

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agarose gels, blotted the fragments onto cellulose nitrate (Southern blotting), and probed the blots with nick-translated ^{32}P human DNA or a cloned repetitive human ALU sequence. We found the same restriction fragments in the original J10 transfectant and in the highly amplified line, but these fragments were 10-50 fold more abundant from the amplified line. Thus, all the human DNA was amplified by selecting for brighter and brighter Leu-2 positive cells.

Since we do not yet have a DNA probe for Leu-2, we used a quantitative transfection assay to show that the number of copies of Leu-2 was increased in brightest J10 cells. Our data showed approximately 20 fold more transfectants could be obtained per microgram of DNA used.

We feel that these results present the first example of spontaneous gene amplification observed for membrane antigens. These amplified cells should facilitate cloning of the Leu-2 gene and characterization of the Leu-2 molecule. We have recently made a cDNA library from the amplified cell line and are screening this library with a "subtracted" cDNA probe made by exhaustive molecular hybridization with RNA from untransfected mouse L cells. At each step, the double stranded hybrids are removed by hydroxylapatite adsorption.

These results are presented in detail in the referenced publication and in an article recently accepted for publication in Nature (P. Kavathas and L.A. Herzenberg, "Amplification of a gene coding for a human T cell differentiation antigen").

REFERENCE

1. Kavathas, P. and Herzenberg, L.A., Proc. Natl. Acad. Sci. (USA) 80:524 (1983).