STUDY OF MN1 AND DLK1 GENES IN THE POLYOMAVIRUS MIDDLE-T ONCOPROTEIN INDUCED TRANSFORMATION

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Polyomavirus is associated with the development of animal tumors, and has been used as a model for studies of malignant transformation and cell proliferation control. Polyomavirus Middle T antigen (MT) is essential for cell transformation. Using a DNA microarray approach, the MN1 and Dlk1 genes were identified by our group as being completely repressed by MT activity. The objective of this study is to analyze the function of MN1 and Dlk1 genes by overexpressing MN1 in the MT expressing cell line (MTWT) and inhibiting Dlk1 in the control cell line (PLJ). The MN1 gene full-length cDNA was amplified using the "Rapid Amplification of cDNA Ends" (RACE) technique. Through 3' RACE we were able to clone the 3' portion of the gene. However, using 5' RACE only non-specific amplifications were obtained. Using primers designed throughout the predicted sequence of the gene we were able to amplify a larger portion of the gene. To obtain the 5' portion, we undertook another 5' RACE experiment using these primers, the result has been submitted to sequencing. For Dlk1 inactivation, we used the small interference RNA technique. We have been able to transfect the 293T packaging cell line and use the resulting viral particles to successfully infect the PLJ cell line, the infected cells are being analyzed.

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