Ribozyme and Macroarray: Identification of AP-2-Regulated Genes by Macroarray Profiling of Gene Expression

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1 Introduction

A ribozyme is an RNA enzyme that catalyzes specific cleavage of RNA molecules and cleaves complementary substrate RNAs [4, 8, 10]. Because ribozymes can cleave RNAs that contain an NUX triplet (where N corresponds to any ribonucleotide and X corresponds to A, U, or C), they are considered to have a great potential as therapeutic agents. Thus, ribozymes can cleave mRNAs specifically in eukaryotic cells and they have been successfully used in the functional analysis of specific genes in cells [7, 9].

Metastasis, the biggest threat to survival for patients with tumour, is the spread of tumour cells from original growth to the other site in the body. Extensive studies in recent past have shown that progression of tumour cells from nonmetastatic to metastatic stage involves a complex multistep process [12] that remains largely undefined [3]. Therefore, it is important to identify functional genes and clarify their functions in metastasis. It has been shown that there is a correlation on the expression level of transcription factor AP-2 and the malignancy of melanoma [5]. Transcription factor AP-2 is a retinoic acid inducible 52kDa protein that binds to a consensus palindromic core recognition element with the sequence 5'-GCCN(3,4)GGC-3' [11] and regulates several genes involved in the progression of human melanoma [1]. In recent studies, the decrease of AP-2 was shown to be a cause of the malignant transformation of melanoma [2] and AP-2 expression was inversely correlated with melanoma progression [6]. Based on these investigations, it was suggested that decrease in the expression of AP-2 contributes to the progression of melanoma by regulation of expression of some of its downstream genes. However, the whole network of gene cluster transcriptionally controlled by AP-2 is not clarified. If we can control expression level of AP-2, it is expected that the genes, regulated by AP-2, can be identified by the profiling of the gene expressions.

In this report, we propose a new technique of gene profilings that combined ribozymes with the macroarray and try to elucidate the function of metastasis related factor AP-2 in the signal transduction network. We constructed ribozyme expression vectors to knock down AP-2 and performed profiling of gene expression in melanoma cells that stably expressed ribozyme by macroarray analysis. As a result of the gene profiles of A375P human melanoma cells that expressed ribozyme against AP-2 or not, we observed alteration of expression levels of several genes and confirmed alteration of expression levels of the genes by RT-PCR.

2 Method and Results

To identify genes regulated by transcription factor AP-2, we prepared human A375 melanoma cell lines that stably expressed ribozymes targeted to AP-2. Following transfection of A375P cells with an expression vector pPUR-KE [7] or its derivative encoding ribozymes against human AP-2 (AP-2-Rz), we treated cells with antibiotic (puromycin) for four weeks and then we established antibiotic-resistant cells as stable transfectants. Expression of ribozyme and reduction of the expression level of AP-2 in these cell lines was confirmed by RT-PCR.

For macroarray analysis, total RNA was isolated from cells treated with empty vector as a control and treated with AP-2-Rz respectively. Isolated RNAs were treated with DNase I and were used for purification of mRNA and preparation of ^{32}P-labeled first strand cDNAs that were subjected to macroarray analysis. For profiling of gene-expression, we used nylon macroarray membranes spotted with cDNA fragments of 1176 cancer related genes (Atlas Human Cancer 1.2 Array, Clontech, CA, USA). Phosphor signals on membrane were detected by phosphor imager Storm (Molecular Dynamics Inc, CA, USA). We performed removal of a background and normalization of a signal intensity in the raw data using Array Vision 6.0 (Amersham, NJ, USA). In addition, to limit the candidate genes, the rate of signal change and the threshold of the signal intensity change were determined by expression profiling data analysis with comparison between control cells and cells that expressed AP-2 Rz. Then we confirmed the expression levels of approximately twenty candidate genes by an RT-PCR analysis. The correlation between the experimental data by the RT-PCR analysis and that by the macroarray was determined. The alterations of expression level of candidate genes that were identified by macroarray analysis were confirmed by RT-PCR.Comparison of the level of 1176 gene expression in cells treated with AP-2-Rz and empty vector indicated that AP-2 had effect on the expression of multiple genes as follows.

3 Discussion

Macroarray analysis indicated that AP-2 affects the expression of multiple genes including c-kit and p21/WAF (the genes known to be regulated by AP-2). Besides, we observed the alternation in the expression level of mda-7, fibronectin 1, KIT ligand, cyclin-dependent kinase 6 and cyclin H. Since AP-2 plays a major role in metastasis of human melanoma by regulating expression of multiple gene expressions, genes identify in this analysis might contribute metastasis more directly. This study and further analysis might let us understand cellular function of AP-2 and molecular mechanism of metastasis more clearly. In the near future, we will try the construction of database of transcriptional network that based on the analysis using technologies of the cDNA macroarray and ribozymes.

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