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Screening for Natural Bioactive Compounds Targeting the Intracellular Signal Transduction Pathway : Natural Products Modulating the Expression of the Interleukin-2 gene

Takashi Hakamatsuka

Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan

Human Genome Project has recently been completed and the information on nucleotide sequences of our whole genome is now available at the public or commercial data banks. Next goals are to identify the functions of each gene and to elucidate the intracellular signal transduction pathways regulating gene expression.

We have established a PCR-based bioassay to search for biologically active compounds that can modulate the expression of genes encoding important proteins. Changes in the level of target mRNA in cell cultures were quantitatively determined by competitive PCR using internal standard DNA. With this bioassay system, we have been searching for lipophilic low-molecular-weight probes that can easily pass through the cell membrane and have an effect on some signal transduction steps. The compounds discovered will be useful tools to elucidate the mechanisms of intracellular signalings guiding the expression of the target genes.

As an application of the screening strategy, the expression of the interleukin-2 gene in Jurkat cells, a human T cell line, was examined. IL-2 is produced by activated T-lymphocytes and promotes proliferation of helper T cell by autocrine recognition. The activated helper T cells facilitate B cells to produce specific antibodies. Furthermore, IL-2 activates natural killer cells and cytotoxic T lymphocytes. Therefore IL-2 is a key regulator of immune and inflammatory responses, and compounds modulating IL-2 gene expression is expected to be effective with respect to immune-related diseases.

Screening of about a hundred crude drugs used in traditional Chinese medicines demonstrated that the methanol extracts of ten crude drugs enhanced the expression of the interleukin-2 gene by more than 4-fold as compared to the control. Chromatography by activity-guided fractionation yielded the active principles of six drugs out of ten (Figure 1). Active compounds enhancing the IL-2 gene expression have also been discovered from various sources, e.g. medicinal plants from South America, fungal metabolites and chemical libraries of our laboratory. The cellular target molecule(s) of the active compounds were

investigated by affinity chromatography. To detect the weak interaction between the compounds immobilized by the affinity resin and its target proteins, we developed a novel analytical technique, affinity chromatography cooperating with two-dimensional electrophoresis. Using this technique, one of target proteins of anthraquinones, active components of Rhei Rhizoma, was identified to be glyoxalase I which catalyzes the detoxification of methylglyoxal.

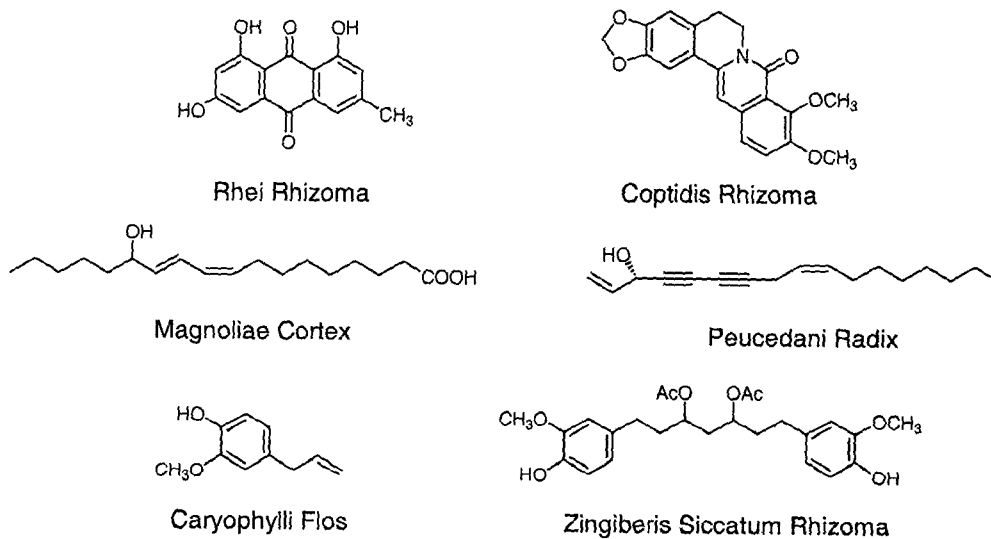


Figure 1 Activators of IL-2 gene expression isolated from Chinese crude drugs