Abstract

Cytokinins are important plant hormones which regulate various physiological events in plant growth and development according to the intrinsic developmental program and in response to environmental stimuli. As one of the functions of cytokinins, it have been well-known that they have activity to induce the redifferentiation of callus into a whole plants. Callus are the cluster of dedifferentiated cells which proliferate indefinitly in a disorganized manner. On the other hand, induction of differentiation from dedifferentiated cells has also been known in many cancer cells. Leukemia cells are resulted from immature blood cells which can not undergo the cell differentiation that normally leads to mature functional blood cells. The arrest of maturation in myeloid leukemia cells can sometimes be reversed by differentiation inducers. Since there is a similarity between human leukemia cells and callus, I examined effect of cytokinins on the growth and differentiation of human myeloid leukemia cells.

Cytokinins such as isopentenyladenine (IPA), kinetin and benzyladenine (BA) which are most effective in plant were also very effective at inducing the granulocytic differentiation of human myeloid leukemia HL-60 cells. They inhibited growth and induced nitroblue tetrazolium (NBT)-reducing activity and morphological changes of HL-60 cells into mature granulocytes. On the other hand, cytokinin ribosides such as kinetin riboside, isopentenyladenosine (IPAR) and benzyladenine riboside (BAR) were the most potent for the growth inhibition and apoptosis of HL-60 cells. Cytokinin ribosides greatly reduced the intracellular adenosine-5'-triphosphate (ATP) content, disturbed the mitochondrial membrane potential and induced the accumulation of reactive oxygen species (ROS), whereas

cytokinins did not. When the cells were incubated with cytokinin ribosides in the presence of O_2^- scavenger, antioxidant or caspase inhibitor, apoptosis was significantly reduced and differentiation was greatly enhanced. These results suggest that both cytokinins and cytokinin ribosides can induce granulocytic differentiation of HL-60 cells, but cytokinin ribosides also induce apoptosis prior to the differentiation process.

Cytokinins were rapidly converted to nucleotides in human leukemia cells. When the cells were incubated with [^{14}C]-BA, most of the radioactive metabolites in acid-soluble fractions were BAR-monophosphate, and the radioactivity was significantly incorporated into RNA and DNA. However, the radioactive nucleotides in RNA or DNA were adenine nucleotides, not BA nucleotides, suggesting that cytokinins were not incorporated into RNA and DNA in human leukemia cells. Next, to determine an association between metabolisms of cytokinins and the cytokinin-induced differentiation, HL-60 cells were treated with various concentrations of BA in the presence of Ado or 5'-amino-2'-deoxyadenosine (dAdo), the differentiation-enhancing or -inhibitory agent, respectively. Growth inhibition and NBT reduction were synergistically induced in HL-60 cells by BA and Ado, while 5'-amino-dAdo counteracted the effects of BA on the growth and differentiation of HL-60 cells. The incorporation of labeled BA into nucleotides was increased and decreased by Ado and 5'-amino-dAdo, respectively. They are consistent with the effects of Ado or 5'-amino-dAdo on growth inhibition and differentiation. These results suggest that incorporation of cytokinin into nucleotides is an important step in the cytokinin-induced differentiation and cytokinin nucleotides themselves play an important role in inducing the differentiation of HL-60 cells.

Changes in signal transduction pathways during the cytokinin-induced differentiation were investigated. Cytokinins effectively induced a phosphorylated (active) form of mitogen-activated protein kinase (MAPK). MAPK activation was necessary for cytokinin-induced differentiation, because PD98059, an inhibitor of MAPK kinase, suppressed the differentiation induced by cytokinins. Next, I examined gene expression profiles by cDNA microarray analysis and compared the differentiation-associated expression patterns induced by cytokinins and other differentiation inducers. The results indicate that the gene expression pattern induced by cytokinins is different from that by typical inducers of granulocytic differentiation such as all-*trans* retinoic acid (ATRA) and dimethylsulfoxide, and rather similar to that induced by cotylenin A and methyl jasmonate, which are regulators of plant growth and development. I have finally found that CCAAT enhancer binding protein (C/EBP) δ and S100P are involved in the differentiation of leukemia cells. Mechanism of differentiation of leukemia cells induced by cytokinins is quite different from that by ATRA or other inducers of granulocytic differentiation. These results suggest that cytokinins might be useful tools for further molecular analysis of differentiation of leukemia cells.