

Gene expression profiling in human hepatic and neuronal cells by Wasabi-derived isothiocyanates

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ABSTRACT

Wasabi (*Wasabia japonica* (Miq.) Matsumara) is a member of Brassicaceae vegetables, and a popular condiment in Japanese households. Wasabi contains high amount of isothiocyanates (ITCs) such as 6-(Methylsulfinyl)hexylisothiocyanate (6-MSITC), 6-(methylthio)hexyl isothiocyanate (6-MTITC) and sulforaphane (SFN). These ITCs have been reported to have antioxidant, anti-inflammatory, and anti-cancer activities, although the molecular mechanisms are not clear. Therefore, this study is designed to clarify the genome-wide gene expression profiles in hepatic cells, HepG2, and neuron cells, IMR-32 stimulated by the three ITCs, using microarray technology.

In HepG2 cells, comparative gene expression profiling was performed by treating the cells with ITCs, followed by DNA microarray analyses using HG-U133 plus 2.0 oligonucleotide array. Partial array data on selected gene products were confirmed by real-time polymerase chain reaction (PCR), and functional subsets of genes and biologically significant network were identified using Ingenuity Pathway Analysis (IPA). Array data showed that 6-MTITC had the highest number of differentially altered (≥ 2) gene expressions, consist of 114 upregulated genes and 75 downregulated genes. Furthermore, IPA revealed that Nrf2-mediated pathway was the common significantly modulated pathway across the ITCs treatment, along with glutamate metabolism pathway. Interestingly, 6-MSITC was the most potent activator of Nrf2-mediated pathway. Thus, our data suggest that 6-MSITC could exert cytoprotective function through the activation of Nrf2-mediated antioxidant pathway.

Next, DNA microarray profiling was designed in neuron cells, IMR-32, treated with the three ITCs to profile the global changes at the transcript level. Among the three ITCs, 6-MSITC caused expression changes of most genes, of which 100 genes were upregulated and 163 genes were downregulated. Biological gene categorization using Gene Ontology (GO) revealed that most of the differentially expressed genes are associated with oxidative stress response. Pathway analysis by IPA further demonstrated that Nrf2-mediated antioxidant pathway was the top of the most modulated pathways by ITCs. Lastly, real-time PCR and Western blot analyses confirmed the gene expressions and protein products which were mainly targeted by ITCs. Therefore, these results suggest that Wasabi-derived ITCs might target the Nrf2-mediated antioxidant pathway to exert its neuroprotective function.

In summary, comparative DNA microarray profiling analysis unveiled that 6-MTITC was the most potent inducer of gene expressions changes in HepG2 cells, whereas 6-MSITC was the most effective inducer in IMR-32 cells. Despite this cell-type response discrepancies, 6-MSITC prevailed as the strongest inducer of antioxidant-associated genes, which was through the activation of the Nrf2-mediated pathway. Altogether, this study provided comprehensive information on how structural differences of Wasabi-ITCs contribute to its efficacy and impact specific targets.