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Dissertation Title : Discovery of Natural Products Suppressing Cancer Cell Proliferation and Autophagy

Dissertation Abstract

Autophagy is a catabolic process through which dysfunctional proteins and organelles are degraded, and that is associated with various human diseases. Although the relationship between autophagy and cancer development were evaluated, there have been few reports that have investigated the autophagic activity of natural products in cancer cells. The purpose of this thesis is to discover natural products that suppress cancer cell proliferation and autophagy modulation by screening our crude extract library based on traditional medicines and analyzing the molecular mechanisms of the identified extracts.

First, I screened for approximately 130 kinds of crude extracts to identify the extracts that would regulate the proliferation through autophagy of human hepatocellular carcinoma HepG2 cells. I found that 24 crude extracts increased LC3-II expression levels. Among these, Goboshi (burdock fruit), Soboku (sappan wood), Mokko (saussurea root), Rengyo (forsythia fruit), and Hikai (dioscorea) notably suppressed the cell proliferation and increased p62 expression levels, which suggested that these 5 extracts suppress the autophagic activity resulting in accumulation of p62.

Arctigenin (ARG) is a bioactive lignan contained in both Goboshi and Rengyo extracts. ARG inhibited the proliferation of HepG2 cells. Analysis of autophagy-related proteins demonstrated that ARG might block the autophagy that leads to p62 accumulation. The stage of inhibition in autophagy by ARG differed from those by the autophagy inhibitors 3-methyladenine (3-MA) or chloroquine (CQ). ARG could also inhibit starvation-induced autophagy. Further analysis of apoptosis-related proteins indicated that ARG did not affect caspase-3 activation and PARP

cleavage, suggesting that the antiproliferative activity of ARG can occur independently of caspase-3-mediated apoptosis. Taken together, these results indicate that ARG suppresses cell proliferation and inhibits autophagy.

Hikai extract showed the strongest antiproliferative activity among selected 5 extracts. A comparison of the antiproliferative activity by the fractions obtained from Hikai extract showed that the medium-polar (*n*-butanol) fraction exhibited most potent activity. Phytochemical investigations of the *n*-butanol fraction achieved the isolation of steroidal saponins, including dioscin (**1**), yamogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**2**), and protodioscin (**3**). Furthermore, acid hydrolysis of **1** and **2** produced the aglycones diosgenin (**4**) and yamogenin (**5**), respectively. Compounds **1**–**5** exerted antiproliferative activity and **1**–**3** inhibited autophagy leading to the accumulation of p62. On the other hand, **1**–**3** did not affect caspase-3 activation and PARP cleavage, suggesting that the antiproliferative activity of **1**–**3** occurred independently of caspase-3-mediated apoptosis.

Finally, I analyzed Mokko extract to identify the compounds modulating autophagy. Among the fractions prepared from Mokko extract, the nonpolar (hexane) fraction exerted strong antiproliferative activity. TLC and HPLC analysis demonstrated that costunolide (CL) and dehydrocostuslactone (DCL) were the major sesquiterpene lactones in the hexane fraction. CL and DCL suppressed the cell proliferation and inhibited autophagy leading to the accumulation of p62. Moreover, CL and DCL weakly induced caspase-3 activation and PARP cleavage, implying that the apoptotic cell death was associated with the antiproliferative activity of CL and DCL.

In conclusion, I identified 6 compounds which inhibit autophagy and cell proliferation from crude extracts used in traditional medicines. These compounds could serve as sources of lead compounds in the development of agents for cancer therapeutics and autophagy research.