Steroidal Saponins Isolated from the Rhizome of Dioscorea tokoro Inhibit Cell Growth and Autophagy in Hepatocellular Carcinoma Cells

Abstract

Our preliminary screening identified an extract from the rhizome of Dioscorea tokoro, which strongly suppressed the proliferation of HepG2 hepatocellular carcinoma cells and inhibited autophagy. This study aimed to isolate active compounds from the rhizome of D. tokoro that exert antiproliferative effects and inhibit autophagy. The bioassay-guided fractionation of the active fraction led to the isolation of two spirostan-type steroidal saponins, dioscin (1) and yamogenin 3-O-α-l-rhamnopyranosyl (1→4)-O-α-l-rhamnopyranosyl(1→2)-β-d-glucopyranoside (2), and the frostane-type steroidal saponin protodioscin (3) from the n-BuOH fraction. Furthermore, acid hydrolysis of 1 and 2 produced the aglycones diosgenin (4) and yamogenin (5), respectively. Compounds 1–5 suppressed proliferation of HepG2 cells. The analysis of structure-activity relationships indicated that the 25(R)-conformation, structures with a sugar moiety, and the spirostan-type aglycone moiety contributed to antiproliferative activity. Analysis of autophagy-related proteins demonstrated that 1–3 clearly increased the levels of both LC3-II and p62, implying that 1–3 deregulate the autophagic pathway by blocking autophagic flux, which results in p62 and LC3-II accumulation. In contrast, 1–3 did not significantly affect caspase-3 activation and PARP cleavage, suggesting that the antiproliferative activity of 1–3 occurred independently of caspase-3-mediated apoptosis. In summary, our study showed that 1–3, active compounds in the rhizome of D. tokoro, suppressed cell proliferation and autophagy, and might be potential agents for autophagy research and cancer chemoprevention.