Denbinobin induces apoptosis in human lung adenocarcinoma cells via Akt inactivation, Bad activation, and mitochondrial dysfunction

許銘仁

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摘要

Abstract

Increasing evidence demonstrated that denbinobin, isolated from Ephemerantha lonchophylla, exert cytotoxic effects in cancer cells. The purpose of this study was to investigate whether denbinobin induces apoptosis and the apoptotic mechanism of denbinobin in human lung adenocarcinoma cells (A549). Denbinobin (1−20 μM) caused cell death in a concentration-dependent manner. Flow cytometric analysis and annexin V labeling demonstrated that denbinobin increased the percentage of apoptotic cells. A549 cells treated with denbinobin showed typical characteristics of apoptosis including morphological changes and DNA fragmentation. Denbinobin induced caspase 3 activation, and N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk), a broad-spectrum caspase inhibitor, prevented denbinobin-induced cell death. Denbinobin induced the loss of the mitochondrial membrane potential and the release of mitochondrial apoptotic proteins including cytochrome c, second mitochondria derived activator of caspase (Smac), and apoptosis-inducing factor (AIF). In addition, denbinobin-induced Bad activation was accompanied by the dissociation of Bad with 14-3-3 and the association of Bad with Bcl-xL. Furthermore, denbinobin induced Akt inactivation in a time-dependent manner. Transfection of A549 cells with both wild-type and constitutively active Akt significantly suppressed denbinobin-induced Bad activation and cell apoptosis. These results suggest that Akt inactivation, followed by Bad activation, mitochondrial dysfunction, caspase 3 activation, and AIF release, contributes to denbinobin-induced cell apoptosis.