Natural triterpenoid avicins selectively induce tumor cell death

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Avicins, a family of plant-derived triterpenoids, have been shown to possess pro-apoptotic, anti-mutagenic and anti-inflammatory properties in mammalian cells. Through thiol binding, avicins can also mediate antioxidant defense. Accumulating evidence uncovered during the past several years suggests that avicins induce tumor cell death via multiple mechanisms. This review will focus on recent studies that provide insights into the cellular and molecular processes and pathways by which avicins induce tumor cell death, including the canonical intrinsic mitochondrial and the Fas-mediated apoptosis cascades as well as autophagy-associated non-apoptotic programmed cell death.

Introduction

Natural products are important sources of therapeutic compounds that can induce cell death in premalignant or malignant human cells.1 These compounds target various cellular components, such as tubulin,2 topoisomerase,3 mitochondria,4 etc. to induce cell apoptosis, necrosis or autophagic cell death. While a plenty of compounds have been studied in search of high efficient anti-tumor reagents, the development for clinical use of many has been terminated because of high toxicity to normal tissues. Thus, a major goal in clinical cancer research is to discover compounds that selectively inhibit the survival and growth of cancer cells with no or little adverse effects on normal cells.

We recently isolated and characterized a family of plant-derived triterpenoid saponins, avicins, from the Australian desert tree Acacia victoriae. Avicins have been shown to selectively inhibit growth in a wide variety of tumor cells.5,7 In addition to the cytotoxic properties, avicins also exhibit cytoprotective effects in non-transformed cells by activation of NF-E2-related factor-2 (Nrf-2) and its downstream targets,8,9 as well as by post-translational thioesterification of proteins.10 Avicins inactivate nuclear factor kappa-light-chain-enhancer of activated B cells (NF κB) pathway, an important mediator of cellular inflammation responses.11 In addition, avicins have been shown to reduce the frequencies of both H-ras mutations and aneuploidy in a mouse model for skin carcinogenesis, as well as decrease p53 mutations in a murine UVB skin model.12 These studies indicate that avicins can play a pleiotropic role in the maintenance of cellular homeostasis.

The molecular mechanisms by which avicins inhibit tumor cell growth have not been fully defined. Increasing evidence has shown that avicins may affect multiple cellular processes to suppress tumor cell growth or trigger cell death, including activation of apoptosis and cellular stress response pathways, induction of autophagic cell death by regulation of the AMPK-tuberous sclerosis complex 2 (TSC2)-mammalian target of rapamycin (mTOR) pathway, as well as inhibition of growth factor signaling, inflammation and oxidative stress response.

Avicin D Triggers Apoptosis by Mitochondrial Perturbation

It has been well established that mitochondria can serve as key regulators of apoptosis through cytochrome c-mediated signaling cascades.13 Our previous findings indicate that mitochondria are important targets of avicin D and G (two purified fractions identified in avicins’ component). After treatment with avicin D, permeabilization of outer mitochondrial membrane and release of cytochrome c can be detected in Jurkat cells within 30 min. Released cytochrome c binds to apoptotic protease activating factor-1 (APAF-1) and procaspase-9 to form the apoptosome, which triggers caspase-9 and downstream effector caspases, leading to the biochemical and morphological changes of apoptosis.5 Interestingly, avicin D-induced apoptosis has also been correlated with downregulation of pro-survival, anti-apoptotic proteins, such as heat shock protein 70 (HSP-70), X-linked Inhibitor of Apoptosis Protein (XIAP), phosphatidylinositol-3-kinase (PI3K), protein kinase B/AKT, and NF-κB.11,14,15 In addition, avicin D has also been shown to dephosphorylate and thereby inhibit the transcriptional activity of signal transducer and activator of transcription (Stat) 3. Downregulation of Stat3 activity and the resultant decrease in the expression of downstream Stat3-controlled pro-survival proteins also contribute to the induction of apoptosis in avicin-treated tumor cells.16,17

Avicin D Triggers Apoptosis by Fas Receptor-Mediated Cell Death

The extrinsic death receptor-initiated pathway of apoptosis is activated by an important family of proteins that locate on the
also induced by the treatment with avicin D. The observation was also confirmed in a human osteosarcoma cell line, U2OS, and a promyelocyte leukemia cell line, NB4.18 Thus, these data strongly suggested that avicin D-mediated cell death is associated with the activation of the death receptor-caspase-8 pathway (Fig. 1).

Theoretically, activation of caspase-8 may be mediated through Fas, TNFR1 or TRAIL-R2/DR5 receptor pathways.19 Avicin D-induced activation of caspase-8 may involve some or all of these receptors. However, since stimulation of TNFR1 is known to induce the activation of caspase-8 and NF κB simultaneously while avicins were shown to inhibit NF κB signaling,11 avicin D-induced activation of caspase-8 is unlikely through TNFR1 activation. In addition, functional assays showed that avicin D exerted a synergistic effect with TNFα and TRAIL, providing further evidence indicating that the action of avicin D is independent of TNFR1 and TRAIL-R2/DR5. Therefore, avicin D-induced caspase-8 activation and cell apoptosis are most probably associated with Fas receptors.

Interestingly, avicin D was able to induce caspase-8 activation in the presence of a neutralizing Fas antibody that blocks ligand binding. Thus, avicin D-induced activation of the Fas pathway is not dependent on the linkage of extracellular Fas ligands,18 which has led us to explore alternative mechanisms underlying the action of avicin D to regulate Fas activation and its downstream signaling events.

Lipid rafts are subdomains of the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. These dynamic structures exist as distinct liquid-ordered regions of the plasma membrane that are resistant to extraction with non-ionic detergents.23,24 It has been shown that various kinds of cell membrane proteins, especially those involved in cell signaling, can actively translocate into lipid rafts. Thus, these specialized membrane domains are thought to be involved in the regulation of signal transduction. Consistent with this notion, lipid rafts have been reported to play an important role in the activation of death receptors. Our recent data18 showed that avicin D was sufficient to induce the aggregation of the lipid rafts at cell surface and rapid recruitment of Fas and its downstream effectors into these rafts to form the DISC. This effect is dependent upon intact lipid rafts and the integrity of the Fas signaling pathway, since disruption of lipid raft organization by the cholesterol-depleting compound methyl-beta-cyclodextrin (MCD) not only abolished the clustering of Fas and the formation of DISC complex but reduced avicin D-induced cell apoptosis as well.18 These observations indicate that lipid rafts are novel cellular targets of avicin D that function as central structures involved in the amplification of Fas signaling by reorganizing membrane microdomains and bringing molecules together in a well-defined and reduced space, facilitating interactions among different signaling molecules and pathways.

Despite the ability of avicins to induce the activation of both mitochondrial and Fas-mediated cell death pathways, benzoyloxycarbonylvalyl-alanyl-aspartic acid (O-methyl)-fluoromethylketone (zVAD-fmk), a broad caspase inhibitor, could not fully protect avicin D-treated cells from cell death,19 suggesting...
that alternative cell death mechanisms are likely at work in response to avicin D administration.

**Avicin D Induces Autophagy by Activation of AMPK**

Autophagy is an evolutionarily conserved process by which intracellular long-lived proteins and cytoplasmic organelles are catabolized to provide cellular energy fuels and building blocks for biosynthesis. Autophagy has multiple physiological and pathophysiological functions.\(^{25-27}\) Autophagy dysfunction is associated with several pathological conditions, such as hereditary myopathies,\(^{28}\) retinal degeneration,\(^{29}\) tumorigenesis,\(^{30-32}\) and neurodegenerative disorders.\(^{33}\) It is thought that autophagy decreases the mutation rate and suppresses oncogenesis by eliminating damaged organelles that produce genotoxic stresses such as free radicals. In addition, if levels of autophagy are induced beyond a physiological range, the autophagy pathway can contribute to cell death, namely autophagic cell death. Consistent with this idea, many cancer cells might have lost the ability to undergo this form of non-apoptotic death as a growth advantage. Thus, induction of autophagic cell death has become an alternative therapeutic strategy being explored by investigators.

As discussed previously, avicins increase mitochondrial outer membrane permeability and decrease oxygen consumption, causing intracellular energy crisis. Since autophagy is a common response to nutrient starvation and mitochondrial dysfunction, thus, as a corollary, avicins may induce autophagy and/or autophagic cell death by interfering with cellular bioenergetics. Indeed, avicin D can decrease cellular ATP levels, stimulate the activation of AMPK, inhibit mTOR kinase activity, and induce autophagy associated with apoptosis-independent cell death (Fig. 1), which can be abrogated by specific knockdown of either autophagy-related gene-5 (Atg5) or Atg7. In addition, suppression of AMPK by compound C and dominant-negative AMPK can also decrease avicin D-induced autophagic cell death. Moreover, avicin D-induced autophagic cell death can be disrupted by knockdown of TSC2, a key mediator linking AMPK to mTOR inhibition.\(^{34}\) Taken together, these observations clearly demonstrate that avicins can trigger caspase-independent autophagic cell death by the regulation of AMPK-TSC2-mTOR pathway.

**Conclusions**

Resistance to cytotoxic drugs is the major factor limiting the success of cancer treatment. It has been documented that most, if not all, chemotherapy-resistant cancers have apoptotic defects,\(^{35}\) creating a pressing need to find reagents to induce non-apoptotic cell death. Avicins can induce not only apoptotic cell death via intrinsic and extrinsic pathways, but also autophagic programmed cell death by depletion of cell energy supply, implicating the therapeutic potentials of avicins for both apoptosis-sensitive and compromised individuals. Therefore, avicins could serve as an important new chemical template for the treatment and/or prevention of cancer.

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