Research on Antineoplastic Mechanism of Natural Product Arenobufagin

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Abstract. Arenobufagin is one of the major active components of toad venom, the molecular formula of which is C24H32O6 and its molecular mass is 416.511. Research has proved its antineoplastic activity. The major ways it achieves antineoplastic effects are inducing apoptosis, inhibiting cell proliferation, suppressing angiogenesis as well as adhesion, migration and invasion of tumor cells.

1. Introduction
Cancer is now one of the most severe threats to human health, and to find effective, safe and low-toxic antineoplastic drugs has become a major strategy to address this threat. Arenobufagin (ArBu), a major active component of toad venom, is a kind of steroid compound, the molecular formula of which is C24H3O6 and its molecular mass is 416.511; research has proved its broad-spectrum antineoplastic activity [1,2]. Research on the antineoplastic mechanism of ArBu is now a major field in research of antineoplastic drugs and it will direct a new way for development of antineoplastic drugs.

2. Induction of Apoptosis
Arenobufagin, when applied to PC-9 cells and A549 cells of non-small cell lung cancer (NSCLC), causes pyknosis of chromatin and karyorrhexis as well as formation of apoptotic bodies, which proves that ArBu can promote apoptosis [2,3]. Besides, ArBu can induce apoptosis of breast cancer cells, liver cancer cells (HepG2 and SMMC-7721), pancreatic cancer cells and esophageal squamous-cell carcinoma cells, but apoptosis of breast cancer cells shows dependence on time and dosage [4-9].

ArBu induces apoptosis mainly through two ways. The first is by interfering with genetic expression. ArBu can upregulate the expression of the pro-apoptotic protein Bax in PC-9 cells and liver cancer SMMC-7721 cells while downregulating expression of anti-apoptotic protein Bcl-2 [3,6]. In ArBu-processed lung cancer A549 cells, the Bax/Bcl-2 ratio increases and the expression of apoptosis factors, Caspace-3 and Caspase-9, are upregulated [2,10]. Yap (Yes-associated protein) can promote apoptosis of breast cancer cells, and when enabled to enter the cell nucleus by ArBu, it can combine with p73 to upregulate transcription of p21, Bax and p53AIP1 [4]. ArBu can lead to upregulation of expression of the HepG2 cell p-Erk and downregulation of expression of p-p38 and p-JNK [5] as well as downregulation of expression of p53 in non-small lung cancer cells [10]. In esophageal squamous-cell carcinoma cells, ArBu can induce in vitro and in vivo apoptosis by activating p35 [9]. ArBu can upregulate the expression of pro-apoptosis protein Noxa in lung cancer cells and inhibit expression of anti-apoptosis protein Mcl-1 [10]. When applied to HeLa cells, ArBu can inhibit upregulation of ataxin-1 protein and tumor cells [11].
Table 1. Induction of Apoptosis by ArBu through Genetic Regulation

| Regulating Target | Cell                                      | Regulation Effect by ArBu | Reference |
|-------------------|-------------------------------------------|---------------------------|-----------|
| Pro-apoptosis protein Bax | Lung cancer PC-9 cells; Liver cancer SMMC-7721 cells | Upregulation | 3, 6     |
| Anti-apoptosis protein Bcl-2 | Lung cancer PC-9 cells; Liver cancer SMMC-7721 cells | Downregulation | 3, 6     |
| Caspase-3; Caspase-9 | Lung cancer A529 cells | Upregulation | 2, 10    |
| p21, Bax, p53AIP1 | Breast cancer cells | Upregulation | 4        |
| p-Erk, p-p38; p-JNK | HepG2 cells | Downregulation | 5        |
| p53 | Non-small lung cancer cells; esophageal squamous-cell carcinoma cells | Upregulation | 9, 10    |
| Noxa | Lung cancer cells | Upregulation | 10       |
| Mcl-1 | Lung cancer cells | Downregulation | 10       |
| ataxin-1 | HeLa cells | Upregulation | 11       |

The second way through which ArBu induces apoptosis is by interfering the signaling pathway. ArBu can inhibit phosphorylation of proteins of the EGFR/RAF/MEK/ERK signaling pathway [3] and suppress the PI3K/Akt/mTOR signaling pathway of lung cancer A549 cells [2]. ArBu can also interfere signaling pathways related to Noxa and induce apoptosis of NSCLC cells [10]. AsBu can also induce apoptosis and autophagy of liver cancer cells by inhibiting the PI3K/Akt/mTOR signaling pathway [12].

3. Inhibition of Cell Proliferation

Research on ArBu’s inhibition of proliferation of PC-9 cells through the MTT research method shows that ArBu can suppress growth of PC-9 cells [3]. ArBu can inhibit growth of NSCLC A549 cells, reducing the cells’ proliferation speed and leading to dispersion of cells; meanwhile, the percentage of cells rises in G2/M phase increases and declines in G1 phase, and the ratio of cells in G2/M phase is dosage-dependent [2]. By suppressing the proliferative activity of breast cancer cells, ArBu presents in-vitro and in-vivo anti-proliferative activity [4]. When applied to the H22 solid liver tumor of mice, the S180 tumor of mice and the drug-resistant strain HepG2/ADM in human liver cancer cells, ArBu shows the inhibition effect [5, 13]. ArBu can inhibit the growth of human liver cancer SMMC-7721 cells, the IC50 of which is 0.415µg/mL, and application of ArBu can lead to increase of cells in G2/M phase while decrease of cells in G0/G1 phase [6]. ArBu can, by suppressing activation of the CDK1 cyclin-B1 compound, prevent the cell-cycle transition from the G2 phase to M phase, and in primary liver cancer cells, the anti-oncogene p53 enables continuous retardation of the cell cycle transition in the G2 phase [7].

ArBu suppresses survival and proliferation of pancreatic cancer cells, induces retardation of cell-cycle transition at the G2/M phase and downregulates the level of the epidermal growth factor receptor (EGFR) [8,14]. It also induces retardation of the cell cycle in G2/M phase of HeLa cells [11]. Also, by suppressing activation of the CDK1 cyclin-B1 compound, ArBu can prevent the transition of cell cycle from the G2 phase to M phase, and the anti-oncogene p55 enables continuous retardation of the cell-cycle transition in the G2 phase [15]. ArBu can inhibit proliferation of cells including CNE-2, Hep2, SH-SY5Y, LO VO, PC-3, DU 145 and HUVECs, the inhibition effect of which is dosage-dependent, and its effect of retarding the cell-cycle transition in the G/M phase of HUVECs has already been confirmed [16].
4. Inhibition of Angiogenesis

Folkman maintains that angiogenesis can provide nutrients for tumor growth, which facilitates migration and infiltration of tumor, so preventing formation of new blood vessels has become a new target for antineoplastic research [16, 17]. Research through the chick embryo chorioallantoic membrane model (CAM I Model) shows that ArBu does affect formation of new blood vessels: the number of newly formed blood vessels of the chorioallantoic membrane decreases substantially [16]. Also, through the VEGFR-2 signaling pathway, ArBu can inhibit VEGF-mediated angiogenesis, suppress VEGF-induced migration and invasion of cells in the blood vessel as well as formation of human umbilical vein endothelial cells (HUVECs) [18].

5. Extra

Research has found that ArBu can increase the content of ROS in HepG2 cells and substantially raise concentration of calcium ions [5]. It can also combine with Na and K-ATPase in HeLa cells to inhibit the activity of proteinase [10], but the exact inhibition mechanism remains unclear. ArBu can cause DNA damage of cancer cells: through the ATM/ATR-Chk1/chk2-cdc25c signaling pathway, it causes double-strand breaks (DSBs) and leads to DNA damage responses. In vitro experiments show that ArBu can react with the hydrogen bond in the GT base pair to bind directly to the DNA molecule [15].

Research shows that ArBu can inhibit adhesion, migration and invasion of liver cancer HepG2 cells by suppressing the expression of MMP2 and MMP9 [13]. Expression of β-catenin andβ-catenin/TCF4 target genes are related to invasion and migration of cancer cells, and by upregulating the expression of these genes, ArBu can inhibit invasion and migration of prostate cancer cells [19].

6. Conclusion and Prospect

Arenobufagin, a new potential antineoplastic compound, can also be used as a candidate for treatment of metastatic prostate cancer [19]. It shows good antineoplastic activity in inducing apoptosis, inhibiting proliferation of cancer cells and suppressing angiogenesis and migration of cancer cells.

Future research is expected to focus on the interaction mechanism of antineoplastic molecules of ArBu. Proteomic research has shown that the cytotoxicity of ArBu is related to 25 differently expressed proteins including proteasome-related proteins, calcium-binding proteins, oxidative stress-related proteins and metabolic enzymes [11], which provides more convincing evidence for its antineoplastic activity. Meanwhile, research on its toxicity shall be also highlighted, and safety should be considered before a new compound is used in clinical treatment, but existing research materials in this regard are rare.

Development and application of arenobufagin shall be combined with new pharmaceutical technology including drug targeting technology and nanotechnology. Previous research shows that application of nanotechnology can increase the drug concentration of arenobufagin in the liver and the lung, while decreasing the drug concentration in the heart and the brain [20]. These research results provide crucial guidance for targeted therapy of arenobufagin and are problems worth further research in development and application of arenobufagin.

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