Inhibitory Effect of Snake Venom on Colon Cancer Cell Growth Through Induction of Death Receptor Dependent Apoptosis

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국문초록

蛇毒이 세포자멸사와 관계있는 Death Receptor를 통한 인간 대장암 세포 성장억제에 미치는 영향

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목적 : 이 연구는 Vipera lebetina turanica 사독(蛇毒)이 인간 대장암 세포주인 HCT116 세포에서 세포주 기전행, death receptor 의존적 세포자멸사 경로 관련단백질 발현 및 NK-κB와 STAT3 활성에 미치는 영향을 규명함으로써 대장암 세포 성장에 대한 억제와 그 기전에 대하여 살펴보고자 하였다.

방법 : 사독을 처리한 후 HCT116의 세포주기를 분석하기 위해서 FACS analysis를 시행하였고, apoptosis 평가에는 TUNEL assay를 시행하였으며 death receptor 의존적 세포자멸사 경로 관련단백질 및 NF-κB와 STAT3 활성 변동 관찰에는 RT-PCR 및 western blot analysis를 시행하였다.

결과 :
1. 0.1, 0.5 및 1 ㎍/㎖ 등의 사독을 처리한 결과 농도 의존적으로 HCT116 대장암 세포활성의 억제가 나타났다.
2. 0.1, 0.5 및 1 ㎍/㎖ 등의 사독을 처리한 결과 농도 의존적으로 세포자멸사 활성세포의 증가가 나타났고, SVT 1 μg/ml에서는 60-70%의 대장암세포 억제 효과가 나타났다.
3. 0.1, 0.5 및 1 μg/ml 등의 사독을 처리한 결과 약한 G1 arrest와 강한 G2/M arrest가 나타났고, G0/G1 또는 G2/M 관련 cyclin D, E 및 B1의 증가가 나타났다.
4. 0.1, 0.5 및 1 μg/ml 등의 사독을 처리한 결과 death receptor에 따른 세포자멸사 5의 발현증가와 그에 따른 세포자멸사

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Ⅰ. Introduction

Colon cancer is a major malignancy with a worldwide cumulative incidence rate of 9.4% and the second leading cause of cancer\(^1\). It develops in the cecum, colon and rectum\(^2\), which confined within the intestinal wall is treatable by enucleation of the local lesions. However, in the advanced colon cancer metastasizing into the deeper regions or other organ such as liver, surgical treatment alone has the limitations\(^2\). Although chemotherapy with anticancer drugs, such as 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin is usually adopted for inhibiting the cancer growth and prolonging survival, it remains unsatisfactory due to current status of comparatively poor cancer drug development and cancer stem cell related highly chemoresistance in colon cancer\(^3\). Anti-cancer drug treatment generally results in apoptosis, programmed cell death through the activation of caspase cascade systems via triggering Death Receptor (DR) dependent apoptotic pathway at the cell surface, activating caspase-8 or cytochrome C dependent pathway at the mitochondria, characterized by loss of mitochondrial permeability transition, release of mitochondrial cytochrome C into the cytoplasm, consecutive activation of caspase-8 or -9, and caspase-3\(^7\). However, it is usual for the activated apoptosis related pathway to represent only some of the characteristics of the above classical apoptotic pathways in anti-cancer agents, depending on cancer cell type\(^1\). According to the previous reports\(^8\), acquired resistance to chemotherapeutics is closely related to the lower apoptosis rate. Therefore, a new remedy enhancing it for colon cancer is urgently needed.

A snake venom toxin (SVT) from \textit{Vipera lebetina turanica}, is a group of basic peptides, and important factor V activator made up of 236 amino acids with six disulfide bonds formed by twelve cysteins\(^1\). A few researchers revealed that SVT exerts its effect on cellular proteins such as Bax, Bcl-2, caspases in the classical apoptotic way, and that SVT inhibits cancer growth through the induction in cancers such as neuroblastoma and prostate cancer\(^1\). However, experiments demonstrating the molecular mechanisms of the anti-cancer effects of SVT in colon cancer cells have not been reported. Thus, in the present study, I investigated apoptosis related anti-cancer effects of SVT and confirmed whether increase of DR expression, cell cycle arrest, and inhibition of activity of NF-\(\kappa\)B signal molecules or STAT3 pathway in the human colon cancer, HCT116 cells.

Ⅱ. Materials and methods

A. Materials

SVT from \textit{Vipera lebetina turanica} was purchased from Sigma Chemical Co. (Saint Louis, USA). DR3, DR4, and DR6 siRNA were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). All of the secondary antibodies such as Bax, Bcl-2, caspase-3, cleaved caspase-3, caspase-8, caspase-9, cleaved caspase-9, p50, p65, PARP and STAT3,