**Vibrio vulnificus** cytolysin induces apoptosis in HUVEC, SGC-7901 and SMMC-7721 cells via caspase-9/3-dependent pathway

Jin-fang Zhao, Ai-hua Sun, Ping Ruan, Xu-hong Zhao, Miao-quan Lu, Jie Yan*

Department of Medical Microbiology and Parasiology, Medical College of Zhejiang University, Hangzhou, Zhejiang, P. R. China, * Corresponding author

---

**Introduction**

*Vibrio vulnificus* is a gram-negative halophilic bacterium which is known to be a life-threatening pathogen for its high lethality rate of 70%. *V. vulnificus* cytolysin (VVC) has been considered to be a critical agent in the pathogenesis of *V. vulnificus* infection among various virulence factors. Ordinarily, VVC is believed to be a pore-forming toxin which shows cytotoxicity for mammalian cells in culture and induces apoptosis in endothelial cells. In order to determine whether VVC induces apoptosis in vascular endothelial cells and tumor cells, the cytotoxicity induced by recombinant *V. vulnificus* r(VVC) and its potential mechanism in HUVEC, SGC-7901 and SMMC-7721 cells were investigated, which reveals that the apoptosis-induction of rVVC via caspase-9/3 cascade is closely related with its cytotoxic mechanism.

**Materials and Methods**

- *V. vulnificus* strain GTC333; HUVEC, SGC-7901 and SMMC-7721 cells
- Prokaryotic expression and purification of rVVC
- Hemolysis assay & Cell viability assay
- Detection of cellular LDH and [K+] lever by DPNH and TPhBNa colorimetry
- Morphologic observation of rVVC-treated cells by TEM
- Cellular apoptosis detected by flow cytometry
- rVVC location monitored by confocal microscopy
- Detection of caspase activity with Fluorometric Assay Kits in spectrofluorometer

**Conclusion**

*V. vulnificus* cytolysin (VVC) exerts apoptotic action on HUVEC, SGC-7901 and SMMC-7721 cells, which is triggered by caspase-9/3 dependent apoptotic signaling pathway. The cytolysin is able to quickly enter the cytoplasm of target cells after a brief superficial attachment, rather than act locally at the cell membrane. VVC not only acts as a hemolysin but also has an ability to induce apoptosis in human vascular endothelial cells and tumor cells.

---

**Results**

**Introduction**

Ordinarily, VVC is believed to be a pore-forming toxin which shows cytotoxicity for mammalian cells in culture and induces apoptosis in endothelial cells. In order to determine whether VVC induces apoptosis in vascular endothelial cells and tumor cells, the cytotoxicity induced by recombinant *V. vulnificus* r(VVC) and its potential mechanism in HUVEC, SGC-7901 and SMMC-7721 cells were investigated, which reveals that the apoptosis-induction of rVVC via caspase-9/3 cascade is closely related with its cytotoxic mechanism.

**Materials and Methods**

- *V. vulnificus* strain GTC333; HUVEC, SGC-7901 and SMMC-7721 cells
- Prokaryotic expression and purification of rVVC
- Hemolysis assay & Cell viability assay
- Detection of cellular LDH and [K+] lever by DPNH and TPhBNa colorimetry
- Morphologic observation of rVVC-treated cells by TEM
- Cellular apoptosis detected by flow cytometry
- rVVC location monitored by confocal microscopy
- Detection of caspase activity with Fluorometric Assay Kits in spectrofluorometer

**Conclusion**

*V. vulnificus* cytolysin (VVC) exerts apoptotic action on HUVEC, SGC-7901 and SMMC-7721 cells, which is triggered by caspase-9/3 dependent apoptotic signaling pathway. The cytolysin is able to quickly enter the cytoplasm of target cells after a brief superficial attachment, rather than act locally at the cell membrane. VVC not only acts as a hemolysin but also has an ability to induce apoptosis in human vascular endothelial cells and tumor cells.