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

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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 VVC (rVVC) 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 colourimetry
- 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 cytoplasma 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

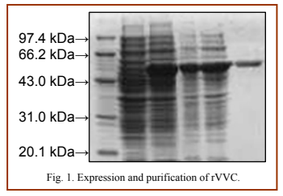

Fig. 1. Expression and purification of rVVC.

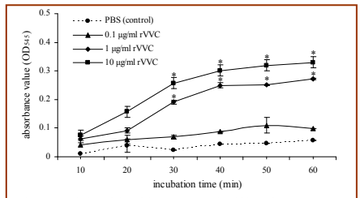

Fig. 2. Hemolytic activity of the rVVC towards rabbit erythrocytes (Mean ± SD, n=5). Rabbit erythrocytes were incubated with 0.1, 1, 10 μg/ml rVVC for various time intervals, and the OD_{541} in supernatants was fetched to reflect the hemolytic activity of rVVC. *: $P<0.01$ vs the control.

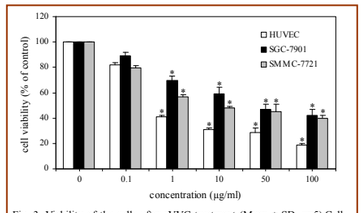

Fig. 3. Viability of the cells after rVVC treatment (Mean ± SD, n=5).Cells were incubated with 0.1~100 μg/ml rVVC for 24 h, control cells were left untreated. Cell viability was determined by MTT test from quintuple culture wells, and its percentage was calculated as a ratio of A490 of control cells. *: P<0.01 vs the control.

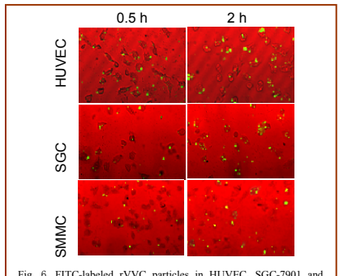

Fig. 6. FITC-labeled rVVC particles in HUVEC, SGC-7901 and SMMC-7721 cells. Cells were incubated with 10 μg/ml FITC-labeled rVVC for different time periods, and the 30 min and 2 h incubation effects in the cells were presented.

Table 1. Changes of LDH and free K^+ contents in supernatants of rVVC-treated cells (Means ± SD, n=3)

Incubation time (h)	LDH (U/L)			[K^+] (mmol/L) treated by rVVC			[K^+] (mmol/L) treated by rVVC+TEA		
	HUVEC	SGC	SMMC	HUVEC	SGC	SMMC	HUVEC	SGC	SMMC
0	108.22±2.14	128.81±1.32	106.99±3.00	5.15±0.64	4.79±0.84	5.17±0.09	5.15±0.20	4.76±0.91	4.91±0.03
0.5	112.34±2.29	130.07±4.79	102.01±2.14	9.29±0.85*	8.19±0.82*	12.35±1.33*	9.34±1.25*	8.43±0.12*	12.01±2.04*
2	110.47±4.12	116.92±2.97	98.82±7.94	9.68±2.03*	8.91±1.48*	13.20±1.42*	10.18±0.47*	8.65±1.38*	13.00±3.02*
6	103.92±3.91	123.08±2.38	102.28±5.59	9.36±1.02*	8.90±1.08*	13.03±2.56*	9.25±1.78*	8.29±1.93*	12.39±2.91*

TEA: potassium channel blocker; *: $P<0.01$ vs the control.

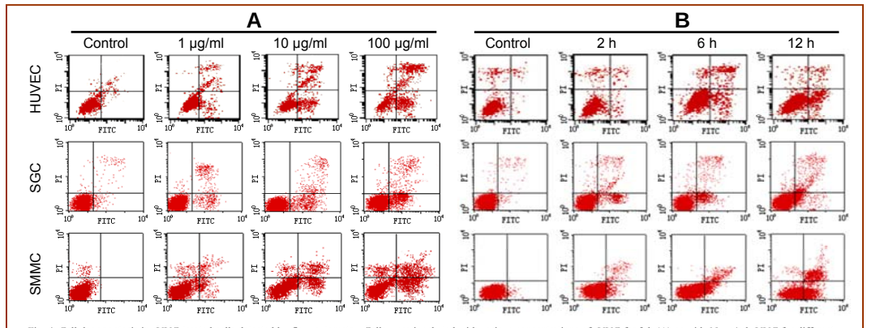

Fig. 4. Cellular apoptosis in rVVC-treated cells detected by flow cytometry. Cells were incubated with various concentrations of rVVC for 2 h (A) or with 10 μg/ml rVVC for different time periods (B), and apoptosis was measured by flow cytometric analysis after staining with Annexin V-FITC and propidium iodide (PI).

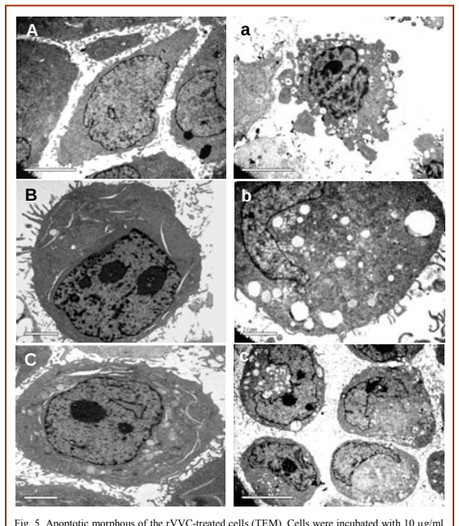

Fig. 5. Apoptotic morphous of the rVVC-treated cells (TEM). Cells were incubated with 10 μg/ml rVVC for 4 h, and photographed under transmission electron microscope at ×4000 or ×6000 magnification. Typical apoptotic characteristics were observed in HUVEC (a), SGC-7901 (b) and SMMC-7721 (c), compared with untreated HUVEC (A), SGC-7901 (B) and SMMC-7721 (C).

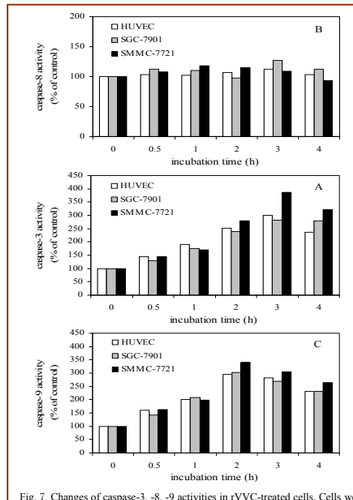

Fig. 7. Changes of caspase-3, -8, -9 activities in rVVC-treated cells. Cells were incubated with 10 μg/ml rVVC for various time periods. Cytosolic extracts were assayed for caspase-3,-8, -9 activities based on the detection of the fluorescence of AFC from cleavage of their specific substrate. Comparison of the fluorescence from apoptotic samples with un-induced controls allows determination of the fold increase in caspase activities.