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Expression, purification and *in vitro* study of VEGF121-PE38-KDEL fusion protein

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Background and Aims: Angiogenesis has a key role in solid tumors growth and progression. Hypoxic condition in solid tumors induces angiogenesis. Binding of Vascular Endothelial Growth Factor (VEGF121) to VEGFR1 and VEGFR2 triggers angiogenesis. As a consequence, blocking of angiogenesis can prevent tumor growth and progression. In this regard, Immunotoxins can be designed to target VEGFR2 on the surface of endothelial cells and cease angiogenesis. Immunotoxins are chimeric proteins which are composed of cell targeting moiety and cell killing moiety. In this study, a chimeric cassette contains truncated pseudomonas exotoxin A(PE38) and VEGF121 was expressed under T7 promoter, purified and studied in vitro.
Methods: The expression cassette was designed and ordered to synthesis in BMH cloning vector. BMH-VEGF121-PE38-KDEL vector was digested and subcloned into pET-28a expression vector. pET-28a-VEGF121-PE38-KDEL vector was transformed into Rosetta (DE3). The fusion protein expression was induced by IPTG (0.3 mM) at various incubation times. The fusion protein was purified by affinity chromatography. It was refolded by glutathione. The LPS contamination was removed by Triton x-114. Finally, cytotoxicity assay was studied on HUVEC and 293KDR cell line.
Results: The authenticity of pET-28a-VEGF121-PE38-KDEL was confirmed by digestion and sequencing. Maximum yield of the fusion protein expression occurred at 4 hours after induction. Running of purified protein showed an expected size on SDS-page and was confirmed by Western blot. LPS level was less than 0.025 unit/ml. IC50 of the fusion protein was 1.27 and 130 nM in 293 KDR and HUVECs respectively.
Conclusions: The VEGF121-PE38-KDEL fusion protein was highly cytotoxic in 293 KDR cells. 293 KDR cells express VEGFR2 more than 2×106 per cell. But the fusion protein was not highly cytotoxic in HUVECs. HUVECs express VEGFR2 approximately 0.017×105 per cell. This study showed cells over expressing VEGFR2 was 100 times more sensitive than cells not over expressing VEGFR2.

Keywords: Angiogenesis; Solid tumor; VEGFR2; VEGF121-PE38-KDEL