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Application of N-terminal fusion protein partners for prokaryotic soluble expression of human VEGF165 and its simple purification with maltose binding protein tag

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Human vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis and plays a central role in the process of tumor growth and metastatic dissemination. *E. coli* is a convenient platform for protein expression. However, attempts to express human VEGF165 (hVEGF) in *E. coli* result in poorly soluble expression or protein misfolding and aggregation into inclusion bodies, hampering purification. To overcome these obstacles, seven N-terminal fusion partners, hexahistidine (His6), thioredoxin (Trx), glutathione S-transferase (GST), maltose-binding protein (MBP), N-utilization substance protein A (NusA), human protein disulfide isomerase (PDI) and the b'a' domain of PDI (PDIb'a') were constructed and tested for soluble overexpression of hVEGF in *E. coli*. We found that at 18°C, 92.8% of the MBP-tagged hVEGF to be soluble and this tag significantly increased the protein's solubility. 0.8 mg of pure hVEGF was successfully purified from 500 mL cell culture. The purified hVEGF is stable after tag cleavage, contains very low levels of endotoxin and is 97.6% pure. Using an $Flk1^+$ mesodermal precursor cell (MPC) differentiation assay, we show that the purified hVEGF is not only bioactive but has similar bioactivity to hVEGF produced in mammalian cells. Previous reports on producing hVEGF in *E. coli* have all been based on refolding of the protein from inclusion bodies. To our knowledge, this is the first study on successfully expressing and purifying soluble hVEGF in *E. coli*.

Biography

Minh Tan Nguyen completed his Master's degree in Medical Science from University of Ulsan College of Medicine, Seoul, South Korea, in February 2014. He is currently a PhD student at Physiology Laboratory, University of Ulsan College of Medicine. His research interest includes "Prokaryotic expression and purification of therapeutic proteins, synthetic antibody libraries for phage display".

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