

Identification of BiP as target molecule for anti-angiogenesis therapy by subcellular proteome analysis

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Tumor angiogenesis is crucial for tumor growth and metastasis. Specifically expressed molecules in tumor endothelial cells would be effective targets of drug and drug delivery for cancer therapy. Aiming to discover the target molecules, we extracted and separated into cytosol and membrane/organelle fractions of vascular endothelial growth factor (VEGF)-activated human umbilical vein endothelial cells (HUVECs) and performed proteomic analysis using 2D-DIGE and MALDI-TOF/TOF-MS. Our subtractive proteomics revealed that the expression of various functional proteins such as molecular chaperones and cytoskeleton regulating proteins was altered in VEGF-activated HUVECs. Among them, BiP expression was most increased by the treatment with VEGF. Western blot analysis confirmed that its expression was increased in VEGF-activated HUVECs consistent with 2D-DIGE analyses.

Next, we evaluated the potential of BiP as a target molecule for drug delivery and anti-angiogenesis therapy. Firstly, we examined the expression of BiP on endothelial cell surface using NHS-SS-Biotin and NeutraAvidin column. The cell surface expression of BiP in HUVECs was increased by the treatment with VEGF. Moreover, BiP expression was elevated in tumor tissues compared with normal tissues of the cancer patients. Secondly, we investigated the functions of BiP in angiogenesis by RNA interference (RNAi). Small interfering RNA (siRNA) targeted to BiP successfully suppressed its protein expression. In addition, VEGF-induced endothelial cell proliferation and ERK1/2 activation were suppressed by the knockdown of BiP. These data suggest that BiP is highly expressed on the cell surface in VEGF-induced angiogenic endothelial cells and associated with VEGF-induced angiogenesis through the regulation of VEGF-MAPK signal transduction. In conclusion, BiP was identified as a candidate of molecular target for anti-angiogenesis therapy by our proteomics, suggesting that subtractive proteomic analysis may be the powerful tool for the discovery of target molecules.