BioTechnologia vol. 96(3) • 2015
Journal of Biotechnology, Computational Biology and Bionanotechnology SUPPLEMENTARY MATERIALS

The shRNA-mediated silencing of *VEGF-C* illustrates its role in proliferation, chemosensitization, tumor colonization, and anchorage independence

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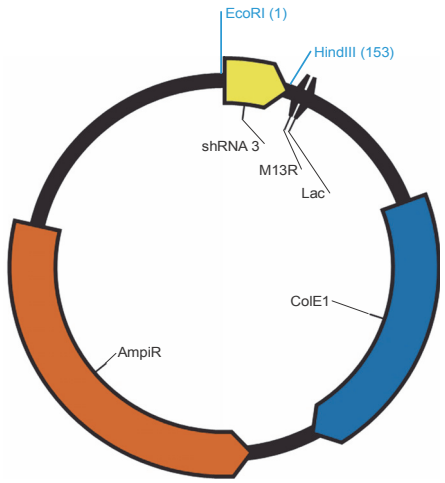
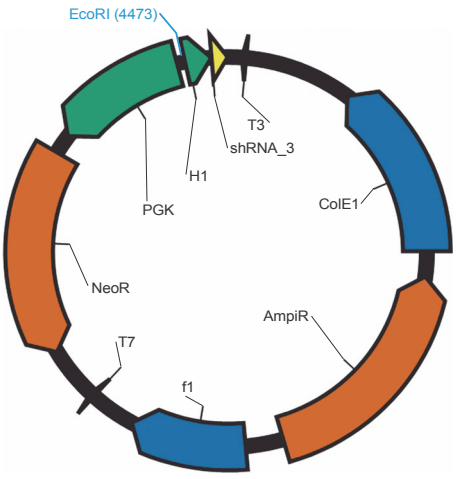
Abstract

Vascular endothelial growth factor C (VEGF-C) is known to stimulate growth of endothelial cells to form tumor-associated lymphatics, which leads to the migration of tumor cells to distant places. However, the function of VEGF-C is not limited to lymph angiogenesis only. VEGF-C is produced by breast cancer cells and binds to the receptors present on them and to lymphatic endothelial cells. The binding of VEGF-C to its receptors on a cancer cell is referred to as autocrine signaling. This ligand-receptor binding, activates a cascade of events that are different from angiogenesis. Moreover, autocrine transmission of signals may facilitate the growth, migration, and drug sensitivity of cancer cells, including the malignancy of the breast. In this study, we investigated the effect of inhibition of *VEGF-C* gene expression via RNAi. A vector-based siRNA was constructed and transfected into breast cancer cell line MDA-MB-231. *VEGF-C* gene expression was analyzed via RT-PCR. *In vitro* proliferation, soft agar colony formation, and scratch healing assays were performed. A relative decrease in *VEGF-C* gene expression observed in transfected cells indicated the silencing effect of shRNA. Due to unavailability of VEGF-C protein, the replicative potential of cancer cells decreased. No colonies on soft agar formed by these tumor cells were observed, which explains their incapability of being anchorage independent. Similarly, scratch healing and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay showed a non-migratory and drug-sensitive nature of the transfected cells. All our results indicate that abundance of VEGF-C in tumor microenvironment will affect various non-angiogenic cell functions such as proliferation, migration, colonization, and chemoresistance.

Key words: VEGF-C, breast cancer, lymphangiogenesis, metastasis, shRNA, RNAi



Supplementary Fig. 1. Vector map of insert vector as procured from Geneart, Germany



Supplementary Fig. 2. Vector map of final vector psNIPERDH1C1