MASTER'S THESIS

The role of MicroRNA in 20(R)-ginsenoside-Rg3-induced anti-angiogenesis

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The Role of MicroRNA in 20(R)-ginsenoside-Rg_3-induced Anti-Angiogenesis

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Abstract

The angio-suppressive effect of 20(R)-ginsenoside Rg3 (Rg_3-R) has been previously demonstrated. In this study, we are interested to investigate the underlying mechanisms of Rg_3-R at the RNA level. Over the last decade, study of microRNA (miRNA) has become an important issue in biomedical science. miRNA is a group of small single-stranded non-coding RNAs that function as post-transcriptional modulator of gene expression by completely or partially base pairing to the 3' untranslated region (3'-UTR) of target messenger RNA (mRNA). It takes part in many biological processes, including cell differentiation, development and pathogenesis. Recently, literature revealed that miRNAs act as regulators of both angiogenic processes and responses. Human umbilical vein endothelial cells (HUVECs) were employed as a model to evaluate the anti-angiogenic activities. Using microRNA microarray technique, miRNA expressions were profiled after Rg_3-R treatment. Among the screened 553 human miRNAs, 6 up-regulated (miR-520h, miR-487b, miR-197, miR-524*, miR-342 and miR-219) and 3 down-regulated (miR-23a, miR-489 and miR-377) miRNAs were detected in Rg_3-R treated vascular endothelial growth factor (VEGF)-induced HUVECs compared to VEGF alone. In addition, real time RT-PCR was performed to verify the miRNA microarray result. Among those miRNA candidates, miR-520h was found to be significantly modulated. Our result revealed that Rg_3-R induced the miR-520h expression about three-fold. Transfection of miR-520h precursor mimic (Pre-520h) into HUVECs showed that elevated level of miR-520h could reduce cell proliferation, tube formation of HUVECs on matrigel and subintestinal vessels (SIVs) formation in zebrafish embryo. Besides, computational approach was used to predict the gene targets of miR-520h. Experimental

target validation showed that protein expression of Eph receptor B2 (EphB2) and Eph receptor B4 (EphB4) were down-regulated in miR-520h over-expressed endothelial cells. In short, Rg_3-R induced over-expression of miR-520h will down-regulated the expression of EphB2 and EphB4 leading to anti-angiogenesis. By understanding the role of miRNAs in angiogenesis, especially the miR-520h, it may explain the mechanism of Rg_3-R in anti-angiogenesis and thus may help exploiting a new path for anti-angiogeneic therapy using RNAi approach.

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