氏名  朴 俊彦

学位の種類  博士（薬学）

学位記番号  富医薬博甲第142号

学位授与年月日  平成26年3月21日

学位授与の要件  富山大学学位規則第3条第3項該当

教育部名  富山大学大学院医学薬学教育部  薬学領域  博士課程

生命薬科学専攻

学位論文題目  Effects of shikonin and deoxyshikonin on lymphangiogenesis
（インビトロにおけるシコニンおよびデオキシシコニンのリンバ管新生に対する効果）

論文審査委員
（主査） 教授  柴原 直利（指導教員）
（副査） 教授  櫻井 宏明
（副査） 教授  門脇 真
Increasing evidence indicates that lymphangiogenesis plays an important role in promoting tumor progression and metastasis. The discovery of immunohistochemical markers for the identification of lymphatic vessels, as well as the characterization of lymphatic-specific growth factors and receptors, has provided insight into the mechanisms involved in new lymphatic vessel formation and the process of metastasis via the lymphatic system. Quantitative assessment of lymphangiogenesis in malignant tumors has emerged as a promising prognostic indicator. However, the results are conflicting regarding the usefulness of lymphatic vessel density for predicting lymph nodal metastases and overall survival. Solid tumors were recently found to induce new lymphatic vessel growth in draining lymph nodes before the onset of metastasis; this has generated immense interest in studying lymphangiogenesis in the lymph nodes.

The vascular system plays important roles in supplying fresh oxygen and nutrients to tissues while the lymphatic system plays pivotal roles in mediating tissue fluid homeostasis and immunity. Excessive formation of blood and lymphatic vessels is also implicated in many pathological conditions, including inflammation and tumor metastasis, as tumor cells metastasize to other distant organs via newly formed blood and lymphatic vessels. Therefore, angiogenesis, lymphangiogenesis, and lymph node metastasis are very important diagnostic indicators for the prediction of tumor progression.

In this study, we demonstrated the effects of deoxyshikonin on lymphangiogenesis. Deoxyshikonin enhanced the ability of human dermal lymphatic microvascular endothelial cells (HMVEC-dLy) to undergo time-dependent in vitro cord formation. The increased ability for cord formation following deoxyshikonin treatment correlated with increased expression of
VEGF-C mRNA, compared to VEGF-A and VEGF-D mRNA expression. Deoxyshikonin also regulated cord formation of HMVEC-dLy cells by increasing the HIF-1α mRNA level, HIF-1α protein level, and the accumulation of HIF-1α in the nucleus. Knockdown of the HIF-1α gene by transfection with siHIF-1α decreased VEGF-C mRNA expression and cord formation ability in HMVEC-dLy. Deoxyshikonin treatment could not restore VEGF-C mRNA expression and cord formation ability in HIF-1α knockdown cells. This indicated that the deoxyshikonin induction of VEGF-C mRNA expression and cord formation in HMVEC dLy on Matrigel occurred mainly via HIF-1α regulation.

Deoxyshikonin also promoted *in vitro* wound healing by the induction of HMVEC-dLy migration into the wound gap. This study describes a new effect of deoxyshikonin, namely, the promotion of cord formation by human endothelial cells via the regulation of HIF-1α. The findings suggest that deoxyshikonin may be a new drug candidate for wound healing and treatment of lymphatic diseases.

The effects of shikonin on *in vitro* lymphangiogenesis were also examined. First, its effects were evaluated in temperature-sensitive rat lymphatic endothelial (TR-LE) cells. A WST-8 assay indicated that shikonin induced viability of TR-LE cells. Adhesion increased in shikonin-treated TR-LE cells compared to control cells. Capillary formation also decreased in the shikonin-treated TR-LE cells, with the strongest suppression observed at 4 h. However, no effect was observed on cell migration. The effects of shikonin were also tested on *in vitro* lymphangiogenesis in human endothelial cells (human dermal lymphatic microvascular endothelial cells, HMVEC-dLy) and reduced capillary formation in the shikonin-treated cells was observed.
Our study demonstrated *in vitro* anti-lymphangiogenic effects of shikonin for the first time. These results suggest that shikonin may be a new drug candidate for treatment of cancer metastasis to the lymph nodes.