# MASTER'S THESIS

## The role of exosomes in nasopharyngeal carcinoma

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# The role of exosomes in nasopharyngeal carcinoma

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## Abstract

Exosomes are cell-secreted vesicles proposed to play an important role in cancer pathogenesis by facilitating tumor growth and metastasis. Nasopharyngeal carcinoma (NPC) has a high metastatic property among head and neck cancers of epithelial origin, and more than 60% of NPC patient diagnosed with cervical lymph node infiltration. Herein, the role of NPC-derived exosomes in cancer pathogenesis was investigated. Cultured media from NPC cell line C666-1 and immortalized normal nasopharyngeal epithelial cells NP69 and NP460 were utilized as the *in vitro* sources of exosomes. By means of differential centrifugation, exosomes were isolated, and the purity of the exosomal preparations was extensively confirmed by Western blotting with exosomal markers CD9 and CD63, sucrose density gradient centrifugation, transmission electron microscopy and qNano particle analysis system. The angiogenic properties of NPC exosomes were demonstrated using cellular models; in which, NPC exosomes have been found to increase HUVECs motility, invasiveness and tube formation ability significantly. Then, the protein content in exosomes was detected by iTRAQ-based comparative proteomic study, so as to reveal the possibility between proteins dysregulation in NPC exosomes and the observed

angiogenic properties. In a total of 642 proteins identified (≥ 1.3 fold compared to NP69 and NP460 exosomes), 78 proteins were classified as up-regulated and 106 proteins were classified as down-regulated in C666-1 exosomes. Among them, two pro-angiogenic proteins, intercellular adhesion molecule 1 (ICAM-1), and CD44, and one angiosuppressive protein thrombospondin-1 (THBS1) were found included, which implied the possibility of involvement of NPC exosomes in tumor angiogenesis switch. Whereas, the exosomal expressions of these 3 proteins candidates were validated by Western blot analysis. Additionally, internalization of exosomes by HUVECs was visualized using confocal microscopy, while Western blot analysis revealed the uptake of NPC exosomes in recipients increased the expressions of ICAM-1 and CD44, while reduced expression of THBS1 was observed compared with normal controls. These data suggested the involvement of NPC exosomes in tumor angiogenesis. In conclusion, this study provided the insights into the association between exosomal signaling pathway and tumor angiogenesis, and the findings would be useful for the future development of exosomes as potential therapeutic target in cancer.

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