

DOCTORAL THESIS

Mechanistic study of anti-tumor activity of sulforaphane on nasopharyngeal carcinoma

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Abstract

The incidence of nasopharyngeal carcinoma (NPC) is high in Southeast China, including Hong Kong. Current therapy on NPC relies largely on radiotherapy and chemotherapy, but treatment failures remain the major challenge for advanced stage and recurrent/metastatic NPC. Previous studies indicated that after completion of primary treatment, the tumor recurrent rate for NPC was between 15% and 58%. Recent researches suggest the existence of cancer stem cells (CSCs). CSC refers to a sub-population of cells within the bulk tumor. CSCs exhibit the stem cell property of self-renew and differentiation, and are responsible for sustaining tumorigenesis and establishing the heterogeneity in the tumor. CSCs are generally more resistant to conventional treatment methods and might be responsible for tumor recurrent after treatment. Therapies that can eliminate cells with CSCs-characteristics might provide a more durable response and better prognosis.

Sulforaphane (SFN) is a natural compound present in Cruciferous vegetables. SFN has been shown to inhibit the *in vitro* and *in vivo* growth of various types of tumor cells through the (i) induction of cell cycle arrest and apoptosis, (ii) inhibition of angiogenesis and metastasis, and (iii) suppression of cancer stem cells (CSCs). However, the effects of SFN on NPC have not been examined in detail. The present study aims to study the anti-tumor activities of SFN on NPC.

In the first part of this study, the effects of SFN on the *in vitro* growth of NPC cells were examined. The growth of both EBV-negative HONE-1 and EBV-positive C666-1 cells was found to be inhibited by SFN. The growth inhibition was associated with the induction of G2/M cell cycle arrest and apoptosis. The effects of SFN on the growth of NPC cells with CSCs characteristics were also examined by the tumor spheres formation assay. SFN was found to reduce the capacity of both HONE-1 and C666-1 cells to form CSCs-enriched tumor spheres. The population of cells with high expression of NPC CSCs-associated markers (Sox2 and ALDH) was found to be reduced after SFN treatment. Under the culture conditions for CSCs, ALDH inhibitor was found to reduce the capacity of NPC cells to form tumor spheres. Similarly, the capability of Sox2 siRNA-treated NPC cells to form tumor spheres was also reduced in the spheroids assay. Results from these studies indicated that the

growth of NPC cells with CSCs characteristics could be reduced by SFN.

After the *in vitro* study of SFN on the growth of NPC cells, mechanisms that are associated with the SFN-induced growth inhibition on NPC cells were examined. MIF is a NPC biomarker that is highly expressed in NPC patients. Previous study has shown that SFN could interact with MIF and affect the biological function of MIF. In the present study, SFN was found to down-regulate the expression of MIF in NPC cells. One of the receptors of MIF, CXCR2, was found to be down-regulated after the SFN treatment. The downstream Akt signaling was also inhibited. Results from the second part of this study indicated that SFN-mediated inhibition of MIF/CXCR2/Akt signaling was involved in the growth inhibitory effects of SFN on NPC cells.

In NPC, many genes were found to be down-regulated through hypermethylation and such down-regulation contributed to NPC development. In the present study, DNMT1 was found to be down-regulated after SFN treatment, and the effect was accompanied with the restored expression of WIF1 and Rassf1a. Further mechanistic study showed that siRNA-mediated DNMT1 knock-down could reduce the capacity of tumor spheres formation of NPC cells. Interestingly, the expression of the tumor suppressor genes WIF1 and Rassf1a was restored. In addition, exogenously added WIF1 could reduce the formation of tumor spheres. These findings suggested that SFN-mediated down-regulation of DNMT1 was associated with the growth inhibitory effects of SFN on NPC cells.

Finally, the *in vivo* efficacy of SFN alone or SFN in combination with cisplatin on the growth of NPC xenograft was examined. SFN was found to inhibit the growth of C666-1 xenograft and enhance the anti-tumor effects of cisplatin on the C666-1 xenograft.

Taken together, results from this study demonstrated the anti-tumor effects of SFN in NPC and suggested that SFN could be a potential therapeutic drug for NPC.

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