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Guggulsterone Targets Smokeless Tobacco Induced PI3K/Akt Pathway in Head and Neck Cancer Cells

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Abstract

Background: Epidemiological association of head and neck cancer with smokeless tobacco (ST) emphasizes the need to unravel the molecular mechanisms implicated in cancer development, and identify pharmacologically safe agents for early intervention and prevention of disease recurrence. Guggulsterone (GS), a biosafe nutraceutical, inhibits the PI3K/Akt pathway that plays a critical role in HNSCC development. However, the potential of GS to suppress ST and nicotine (major component of ST) induced HNSCC remains unexplored. We hypothesized GS can abrogate the effects of ST and nicotine on apoptosis in HNSCC cells, in part by activation of PI3K/Akt pathway and its downstream targets, Bax and Bad.

Methods and Results: Our results showed ST and nicotine treatment resulted in activation of PI3K, PDK1, Akt, and its downstream proteins - Raf, GSK3β and pS6 while GS induced a time dependent decrease in activation of PI3K/Akt pathway. ST and nicotine treatment also resulted in induction of Bad and Bax phosphorylation, increased the association of Bad with 14-3-3ζresulting in its sequestration in the cytoplasm of head and neck cancer cells, thus blocking its pro-apoptotic function. Notably, GS pre-treatment inhibited ST/nicotine induced activation of PI3K/Akt pathway, and inhibited the Akt mediated phosphorylation of Bax and Bad.

Conclusions: In conclusion, GS treatment not only inhibited proliferation, but also induced apoptosis by abrogating the effects of ST / nicotine on PI3K/Akt pathway in head and neck cancer cells. These findings provide a rationale for designing future studies to evaluate the chemopreventive potential of GS in ST / nicotine associated head and neck cancer.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) remains a significant cause of morbidity and mortality worldwide with five year survival rates of about 50%, marred by frequent recurrence or formation of second primary tumors (10–25% of cases)[1,2]. On a global scale, the use of tobacco products is the major risk factor, with smokeless tobacco (ST) consumption being linked to the high incidence of HNSCC [3-5]. ST is used in multiple forms namely naswar, gutkha, khaini (a mixture of ST with lime) and has or with betel quid, been classified as a human carcinogen by International Agency of Research in Cancer (IARC) [6,7]. The association of smoking with HNSCC is well known, but the link between ST use and head and neck cancer is emerging. In a recent report, Stepanov *et al.* [8] identified 23 polycyclic aromatic hydrocarbons (PAH) in ST in addition to nitrosamines and nicotine as reported in earlier studies [7]. Nicotine enhances proliferation, accelerates tumor growth and inhibits apoptosis in certain types of human cancer cell lines and induces angiogenesis in vivo by sustained activation of the mitogenic pathways [9-11]. Nicotine has been reported to inhibit apoptosis induced by opioids and genotoxic stress induced by etoposide, cisplatin, or UV irradiation in lung cancer cells [12,13]. In addition, nicotine activates PI3K/Akt pathway and inhibits the pro-apoptotic functions of Bax and Bad through phosphorylation [14,15]. These findings prompted us to investigate whether ST (khaini) induces PI3K/Akt pathway activation in head and neck cancer cells.

Further, identification of pharmacologically safe chemopreventive agents that can suppress ST/nicotine induced PI3K/Akt pathway in HNSCC is likely to have the potential to prevent ST induced head and neck carcinogenesis. Guggulsterone (GS), (4,17(20)-pregnadien- 3,16-dione), a constituent of Indian Ayurvedic medicinal plant *Commiphora mukul* is a biosafe nutraceutical with anti-neoplastic properties [16-18]. GS has been reported to