Society for Endocrinology

Endocrine Oncology (2025) **5** e240076
https://doi.org/10.1530/EO-24-0076

Received 10 November 2024
Accepted 25 February 2025
Available online 25 February 2025
Version of Record published 12 March 2025

RESEARCH

Oral cancer cells secrete stress neurotransmitter and proliferate in response to tobacco carcinogen NNK

Flávia Alves Verza[1], Ana Lívia Santos-Sousa[1], Sandra Helena Penha Oliveira[1,2] and Daniel Galera Bernabé[1,3]

[1]Psychoneuroimmunology Laboratory, Psychosomatic Research Center and Oral Oncology Center, School of Dentistry, São Paulo State University (Unesp), Araçatuba, Brazil

[2]Department of Basic Sciences, School of Dentistry, São Paulo State University (Unesp), Araçatuba, São Paulo, Brazil

[3]Department of Diagnosis and Surgery, School of Dentistry, São Paulo State University (Unesp), Araçatuba, São Paulo, Brazil

Correspondence should be addressed to D G Bernabé: daniel.bernabe@unesp.br

Abstract

Purpose: Although there is a growing body of evidence showing the effects of stress-related catecholamines on oral cancer progression, to date there are no studies that have investigated whether oral squamous cell carcinoma (OSCC) cells can produce these hormones and whether this phenomenon is modulated by tobacco-related nitrosamines.

Methods: In this study, we investigated whether keratinocytes (HaCaT) and OSCC-derived cell lines (SSC9 and SCC25) can secrete the neurotransmitter norepinephrine, as well as the effects of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on norepinephrine secretion and OSCC proliferation.

Results: Supernatant from the HaCaT, SCC9 and SCC25 cells showed higher norepinephrine levels (6-, 14.9- and 15.1-fold higher, respectively) compared to the culture medium without cells. When the cells were stimulated with NNK, a tobacco-specific carcinogen, there was an increase in the levels of norepinephrine secretion by HaCaT and SCC25 cells but not by SCC9 cells. NNK (10 μM) induced cell proliferation in the HaCaT, SCC9 and SCC25 cell lines, and these effects were totally inhibited by blocking β-adrenergic receptors with propranolol. The NNK-induced OSCC cell proliferation was furthermore dependent on the activation of nicotinic acetylcholine receptors α4 (nAChR-α4) (completely in SCC9 cells and partially in SCC25 cells) but not on the activation of nAChR-α7. Inhibition of the β-adrenergic receptors, nAChR-α4 and nAChR-α7 did not block NNK-induced HaCaT proliferation.

Conclusion: Our findings suggest that oral cancer cells secrete the neurotransmitter norepinephrine and that the tobacco nitrosamine NNK promotes increased cell proliferation through a stress-related cellular adrenergic pathway.

Keywords: oral squamous cell carcinoma; norepinephrine; beta-adrenergic receptor; nicotinic receptors; stress

Introduction

Tobacco consumption is a worldwide epidemic, with 155 million tobacco smokers worldwide aged 15–24 years (Reitsma *et al.* 2021; https://www.who.int/en/news-room/fact-sheets/detail/tobacco). Smoking is one of the main cancer-inducing factors, being related to 29% of all cancer deaths (Danaei *et al.* 2005). Cigarette smoking increases the risk of developing tumors in almost all organs, being a strong factor in the development of

Published by Bioscientifica Ltd.
https://eo.bioscientifica.com
© 2025 the author(s)

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

https://doi.org/10.1530/EO-24-0076

oral squamous cell carcinoma (OSCC) (Jethwa & Khariwala 2017). According to the International Agency for Research on Cancer, an estimated 389,485 new cases of oral cancer were reported worldwide in 2022, with 188,230 deaths resulting from this malignancy (Bray *et al.* 2024). Tobacco contains multiple types of chemicals and carcinogens associated with smoking-induced cancer. Tobacco-specific nitrosamines (TSNAs), including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been associated with carcinogenic activity as well as tumorigenic and mutagenic abilities (Hecht 2003). Nicotine is one of the main components of tobacco and is considered a genotoxic and tumor-promoting substance (Grando 2014). Nicotinic acetylcholine receptors (nAChR) are found in neuronal synaptic transmission, keratinocytes and bronchial epithelium, suggesting that these receptors have features that go beyond neurotransmission (Zoli *et al.* 2018).

Nicotine and NNK, present in tobacco, are nAChR agonists and play a relevant role in mediating the malignant transformation of oral epithelial cells (Kalantari-Dehaghi *et al.* 2012). The activation of nAChRs modulates the expression of genes encoding proteins involved in signal transduction, cell cycle regulation, apoptosis and cell adhesion (Lin *et al.* 2023, Schuller 2013). NNK is a pro-carcinogen that requires metabolic activation to exert its carcinogenic functions. Multiple cytochrome P450 enzymes catalyze the metabolic activation of NNK and other nitrosamine derivatives (Yamazaki *et al.* 1992). This activation induces metabolites that can promote a series of damage to nucleobases in DNA and form DNA adducts (Phillips 2002). In addition to DNA adduction, NNK can also promote increased cell proliferation, survival, and migration of mutated cells, as well as cancer cell invasion (Xue *et al.* 2014).

Neurotransmitters derived from chronic stress affect many biological functions, including cancer progression (Antoni *et al.* 2006). The stress response is mediated by the hypothalamic–pituitary–adrenal axis in addition to the sympathetic nervous system. This activation results in the increased release of the neurotransmitters norepinephrine and epinephrine in different tissues and organs (Itoi & Sugimoto 2010, Armaiz-Pena *et al.* 2013). The biological effects of stress-related neurotransmitters are mediated by beta-adrenergic receptors (β-ARs), detected directly on tumor cells in numerous malignancies (Armaiz-Pena *et al.* 2013, Santos-Sousa *et al.* 2024). Stress neurotransmitters incite tumor production of pro-inflammatory cytokines and growth factors, affecting cancer progression (Lutgendorf & Andersen 2015, Bernabé 2021). Norepinephrine upregulates the expression of vascular endothelial growth factor (Thaker *et al.* 2006), interleukin-6 (IL-6) (Yang *et al.* 2009, Bernabé *et al.* 2011) and is responsible for the modulation of the matrix metalloproteinases (MMPs) in the tumor microenvironment (Moreno-Smith *et al.* 2010).

Patients with oral cancer display sympathetic nervous system hyperactivation, with high systemic levels of norepinephrine and epinephrine (Bastos *et al.* 2018). Increased catecholamine levels can enhance the proliferation, migration and invasion of OSCC cells in preclinical models (Bernabé *et al.* 2011, Xie *et al.* 2015). Moreover, recent findings from our group identified that norepinephrine was able to induce chemoresistance in OSCC cells through β-ARs (Tjioe *et al.* 2022). The structure of the tobacco nitrosamine NNK is similar to norepinephrine and epinephrine, and it can act as an agonist for β-ARs and signaling pathways that activate transcription, cell proliferation, and survival (Schuller *et al.* 1999, Al-Wadei *et al.* 2012*a*).

Previous studies have shown that different cell lines (i.e., colon cancer (Wong *et al.* 2007), pancreatic cancer (Al-Wadei *et al.* 2012*b*), small airway epithelial cells (Al-Wadei *et al.* 2010) and breast cancer (Shi *et al.* 2011, Amaro *et al.* 2020)) are able to produce and release stress-related neurotransmitters. Moreover, this event can be potentiated by tobacco nitrosamines such as NNK. Although there is a growing body of evidence showing the effects of stress-related catecholamines on OSCC progression, to date, there are no studies that have investigated whether OSCC cells can produce these hormones and whether this phenomenon is modulated by tobacco-related nitrosamines. In this sense, the present study investigated the autocrine production of norepinephrine in human cell lines derived from OSCC and normal keratinocytes, as well as the effects of NNK on neurotransmitter secretion and cell proliferation mediated by β-ARs and nAChRs.

Materials and methods

Cell culture

The cell lines derived from keratinocytes (HaCaT) and oral squamous cell carcinoma (OSCC)-derived cell lines (SSC9 and SCC25) were obtained from the American Type Culture Collection (ATCC). The culture medium used in this experiment was Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Life Technologies, USA) supplemented with 0.5 or 10% fetal bovine serum (FBS) (GIBCO, Life Technologies, USA), 100 U/mL penicillin, 100 μg/mL streptomycin and 0.1% gentamicin for HaCaT cells, and equal parts DMEM and F12 culture medium (Invitrogen, USA) (DMEM/F12) supplemented with 0.5% or 10% FBS, 100/mL penicillin, 100 μg/mL streptomycin, 0.1% gentamicin and 400 pg/mL hydrocortisone for SCC9 and SCC25 cell lines. To assess whether HaCaT, SCC9 and SCC25 cells can secrete the neurotransmitter norepinephrine, the cell lines were cultured with 10% FBS, seeded in 6-well plates, and incubated at 37°C in a moist atmosphere with 5% CO_2.

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

Endocrine Oncology (2025) **5** e240076
https://doi.org/10.1530/EO-24-0076

After reaching approximately 80% confluency, the respective culture medium was changed, and the cells were cultured for 48 h at normal (10%) or reduced FBS concentrations (0.5%). As a control, 6-well plates containing the same medium used for cell lines but without cells were maintained in the same conditions and periods used for cell cultivation. The supernatants from the cultures and control plates were collected and stored at −80°C for norepinephrine measurements. To evaluate if the concentrations of norepinephrine in the medium are dependent on the amount of FBS, norepinephrine levels were measured in the culture medium without cells supplemented with normal FBS concentration (10%) or reduced concentration (0.5%). This analysis was performed in three technical replicates for each experimental group.

Measurement of norepinephrine concentration

To evaluate the norepinephrine levels in the culture medium (supernatants) with or without cells at two different concentrations of FBS, hormone concentrations were quantified using a specific kit (EIAb Science Co. Ltd, China) through enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's recommendations. The minimum detectable concentration of the assay (assay sensitivity) was less than 7.5 pg/mL, and intra-assay and inter-assay coefficients of variation were <4.3 and 7.5%, respectively. The optical density (OD) was read using a spectrophotometer set to a wavelength of 450 nm.

Treatment with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

To evaluate the effects of the carcinogen NNK on norepinephrine secretion and cell proliferation rates in HaCaT, SCC9 and SCC25 cells, two different experimental sets were carried out. First, the cells were expanded in 24-well plates at a density of 2×10^5 in specific culture medium (10% FBS) in accordance with the respective cell line. After the cells reached 80% confluency, the FBS concentration was reduced to 0.5%, and the cells were incubated for another 24 h. Then, the cells were stimulated with NNK (Sigma, USA) at concentrations of 100 nM, 1 μM or 10 μM (Laag *et al.* 2006, Chen *et al.* 2021) for 6 h. After this period, cell supernatants from each line were collected, and norepinephrine levels were measured by enzyme immunoassay as described above. This analysis was performed in four technical replicates for each experimental group.

Cell proliferation assays

To evaluate the effects of NNK on the proliferation rates of HaCaT, SCC9 and SCC25 cells, they were plated in 96-well plates (1×10^4 cells/well) in specific culture medium (10% FBS) in accordance with the respective cell lines. After the cells reached 60% confluency, the FBS concentration was reduced to 0.5%. After 24 h, the cells were incubated in the absence or presence of NNK (Sigma, USA) at concentrations of 1 nM, 10 nM, 100 nM, 1 μM and 10 μM for 48 h. After 36 h of this period, BrdU was added for 12 h until the end of the experiment. The BrdU incorporation levels were measured by ELISA using a specific kit (Millipore, USA) according to the manufacturer's recommendations. The cell proliferation rates were represented by the BrdU incorporation levels, which were detected according to a resulting color. The OD values were analyzed by spectrophotometry. This analysis was performed in four technical replicates for each experimental group.

Effects of β-adrenergic and nicotinic antagonists on NNK-induced cell proliferation

To investigate whether the effects of NNK on HaCaT and oral cancer cell proliferation were dependent on β-adrenergic receptors and nicotinic nAChR-α7 and nAChR-α4, the cells were expanded in 96-well plates until they reached 60% confluency. Then, the FBS concentration was reduced to 0.5%. Thirty minutes before NNK treatment, the cells were either treated or not with the antagonists for β-adrenergic receptors (propranolol at 10 μM; Calbiochem, Merck, Germany), for nAChR-α4 (dihydro-beta-erythroidine at 200 nM; Tocris Bioscience, UK) and for nAChR-α7 receptors (α-bungarotoxin at 100 nM; Calbiochem, Merck, Germany). The antagonists were diluted in the respective culture media. Then, the cells were stimulated with 10 μM NNK. This NNK concentration was chosen because it had been determined to have the best stimulatory effect on cell proliferation in previous tests. Cell proliferation after treatment with NNK, in association or not with antagonist drugs, was assessed by BrdU incorporation rates as previously described. This analysis was performed in five technical replicates for each experimental group.

Statistical analysis

Data were checked for normality, and Student's t-test or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple-comparison test was used to determine the statistical significance of differences between groups. P values < 0.05 were considered significant. All wells from all experimental groups were assessed for cell viability before stimuli. Wells with cells that did not show satisfactory viability were previously excluded. This explains why some groups have a different number of replicates than others.

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

Endocrine Oncology (2025) **5** e240076
https://doi.org/10.1530/EO-24-0076

Results

Culture medium for normal keratinocytes and oral cancer cell lines contains measurable norepinephrine levels that are dependent on FBS concentrations

To evaluate whether the culture medium used for the cultivation of HaCaT (DMEM), SCC9 and SCC25 (DMEM/F12 for both) cells have basal levels (culture medium without cells) of norepinephrine, samples of the two types of media in normal (10%) and reduced (0.5%) FBS concentrations were tested for hormone measurement. Our results indicated that both DMEM and DMEM/F12 medium supplemented with FBS had measurable norepinephrine levels even before being placed in contact with the cell lines (Fig. 1). Furthermore, norepinephrine levels were correlated with FBS concentrations in the medium, since for both studied culture media, those with the highest concentration of FBS (10%) had higher hormone levels than those with reduced FBS concentrations (0.5%) ($P < 0.05$) (Fig. 1). DMEM culture medium showed higher norepinephrine levels than DMEM/F12 when compared with the same FBS concentrations, but these differences did not reach statistical significance ($P > 0.05$) (Fig. 1).

Oral cancer cells and normal keratinocytes secrete norepinephrine

To assess whether the HaCaT, SCC9 and SCC25 cell lines (Fig. 1C, D, E) secrete norepinephrine, the cells were cultured in normal culture medium (10% FBS) or in reduced FBS concentration (0.5%). The norepinephrine levels in the cell supernatant for both FBS conditions were measured by ELISA and were compared with the hormonal levels found in the culture medium without cells. In general, the results indicated that the supernatants from all cell lines showed higher norepinephrine levels compared to the culture medium without cells (Fig. 1C, D, E). The supernatant from the HaCaT cells showed a higher norepinephrine concentration compared to the culture medium without cells, both for the medium with lower FBS concentration (0.5%) (31.11 ± 5.2 pg/mL vs 4.70 ± 2.0 pg/mL, respectively; $P < 0.01$) and for the medium with 10% FBS (51.72 ± 08.03 vs 16.73 ± 3.6 pg/mL, respectively; $P < 0.01$) (Fig. 1C). Following the same profile, which was observed with the cell-free culture media, the supernatant from the HaCaT cells cultured with 10% FBS showed higher norepinephrine levels when compared to the supernatant from cells cultured with 0.5% FBS ($P < 0.05$) (Fig. 1C). Similar results were found for SCC9 (Fig. 1D) and SCC25 (Fig. 1E) cells. Regarding SCC9 cells, the cell supernatant at 0.5% FBS exhibited a norepinephrine level about 15 times higher (17.3 ± 2.6 pg/mL) than the medium without SCC9 cells (1.41 ± 0.3 pg/mL) ($P < 0.01$) (Fig. 1D). When tested in culture medium DMEM/F12 with 10% FBS, the norepinephrine levels in the SCC9 cell supernatant were 4.3 times higher (45.23 ± 3.4 pg/mL) than the basal culture medium without cells (10.34 pg/mL ± 0.6) ($P < 0.001$) (Fig. 1D). The supernatant from the SCC9 cells cultured with 10% FBS showed increased norepinephrine levels after 48 h compared to the supernatant from the same cells cultured with 0.5% FBS ($P < 0.001$) (Fig. 1D). The results from the SCC25 cells were very similar to those found with SCC9 cells. The supernatant from the SCC25 cells showed a norepinephrine level 15 times higher than the medium without cells ($P < 0.01$) when SCC25 cells were cultured in 0.5% FBS (Fig. 1E); and about 6 times higher than the culture medium in the absence of cells using the 10% FBS condition ($P < 0.001$) (Fig. 1E). Similar to what was observed for SCC9 cells, norepinephrine levels in the supernatant from the SCC25 cells cultured with 10% FBS were higher than the hormone levels found when 0.5% FBS was used ($P < 0.001$) (Fig. 1E). In order to assess which cell line showed a higher potential to secrete norepinephrine, we have estimated the percentage increase of norepinephrine levels for each line when the cell supernatant was compared to its respective culture medium in basal conditions (without cells). The data showed that depending on the FBS concentration, there were changes in cellular response and consequent norepinephrine levels in the supernatant. For example, when HaCaT cells were cultured with 0.5% FBS, they increased the norepinephrine levels by about 6 times after 48 h of cultivation. The OSCC cells secreted higher norepinephrine levels in the supernatant compared to HaCaT cells (14.9 times for SCC9 cells and 15.1 times for SCC25 cells) (Fig. 1F, G, H, I). As for the cells cultured with 10% FBS, the percentage of increasing of norepinephrine levels in the supernatant was lower than when the cells were cultured in 0.5% FBS, although maintaining the greatest potential for norepinephrine secretion by the cancer cells (4.3 times for SCC9 cells and 6.1 times for SCC25 cells) compared to keratinocytes (3.0 times) (Fig. 1F, G, H, I). These data suggest that cancer cells may secrete a greater amount of norepinephrine than non-neoplastic HaCaT cells.

NNK induces norepinephrine secretion in OSCC cells and normal keratinocytes

After demonstrating that normal keratinocytes and OSCC cells are able to secrete norepinephrine, we investigated whether the NNK carcinogen could stimulate hormone secretion. For this analysis, the cells were treated with NNK at different concentrations (100 nM, 1 μM and 10 μM) for 6 h. After this period, the supernatant from each cell line was collected and tested for norepinephrine levels. Our results indicated that NNK at 100 nM and 10 μM

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

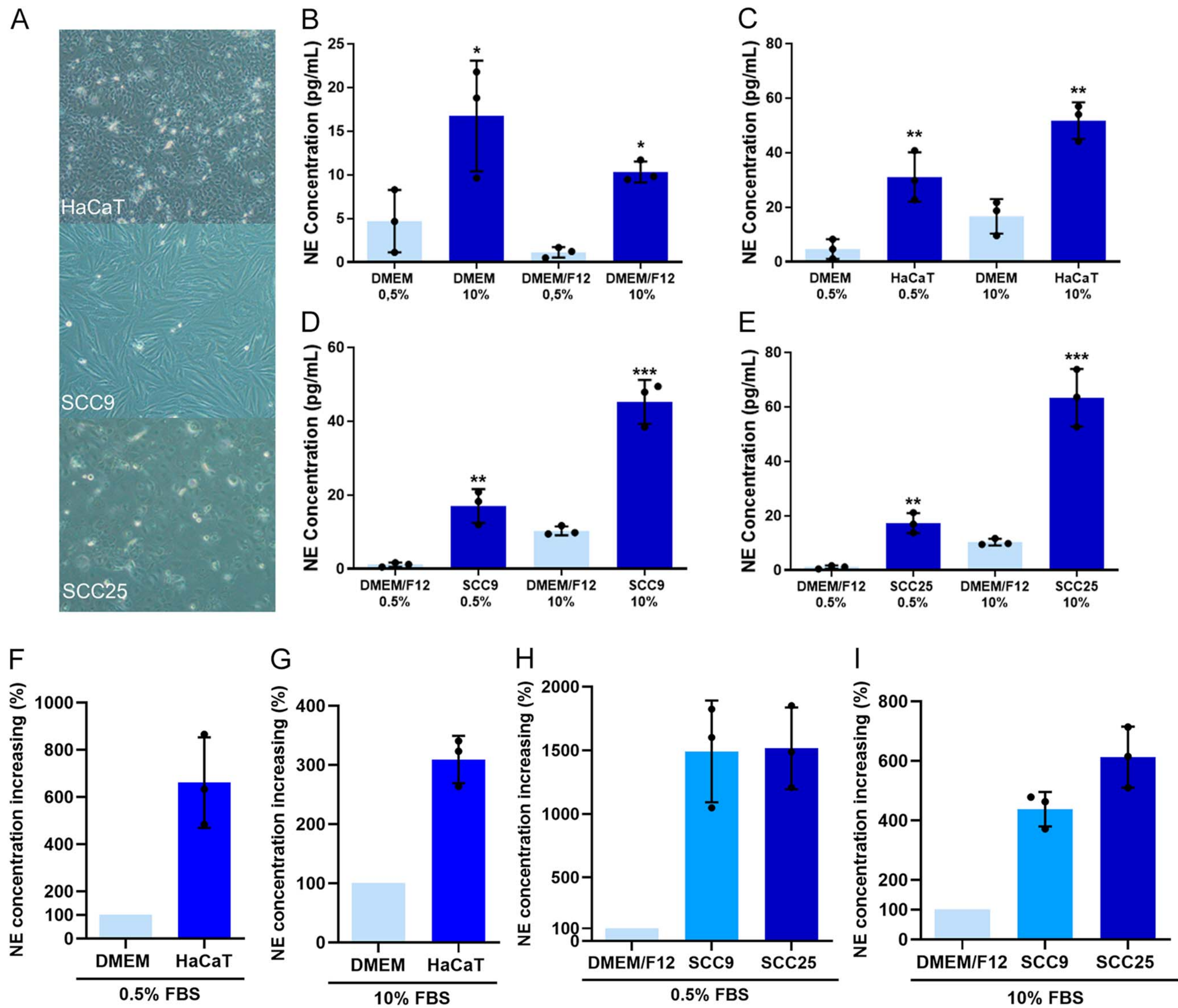

Figure 1

Oral cancer cells and keratinocytes secrete the stress-related neurotransmitter norepinephrine. Microscopic aspects of the cell lines used in the study: HaCaT, SCC9 and SCC25 (A). DMEM and DMEM/F12 media supplemented with FBS had higher norepinephrine levels in comparison with the respective media without supplementation (B). Norepinephrine secretion by the HaCaT (C), SCC9 (D) and SCC25 cells (E). Norepinephrine levels were measured in the supernatants of HaCaT and OSCC cells, in normal concentration of FBS (10%) or in reduced concentration (0.5%). Cell supernatants from the three cell lines tested showed higher norepinephrine concentrations compared to culture medium without cells (adjusted for normal or reduced FBS concentration). (F, G, H and I) The data represent the percentage increase in norepinephrine levels in the cell supernatants from each cell line compared to their respective culture media without cells with FBS at 0.5% (F and H) or 10% (G and I). Each value represents the mean ± SEM of the assays, $n = 3$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Abbreviations: NE, norepinephrine.

stimulated increased norepinephrine secretion by the HaCaT cells when compared to unstimulated cells ($P < 0.05$), with the 10 μM concentration eliciting the most robust norepinephrine increase (Fig. 2A). NNK at 10 μM induced significant norepinephrine secretion by SCC25 cells ($P < 0.05$) (Fig. 2C). Treatments with 100 nM and 10 μM NNK also increased norepinephrine secretion by SCC9 cells, but this result did not reach statistical significance ($P > 0.05$) (Fig. 2B).

NNK increases keratinocytes and OSCC cells proliferation

To evaluate the effects of NNK on keratinocyte and OSCC cell proliferation, the cells were treated with different concentrations of NNK. The results showed that the highest concentration of NNK tested (10 μM) induced a significant increase in cellular proliferation in all cell lines tested (Fig. 3A, B, C). All NNK concentrations

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

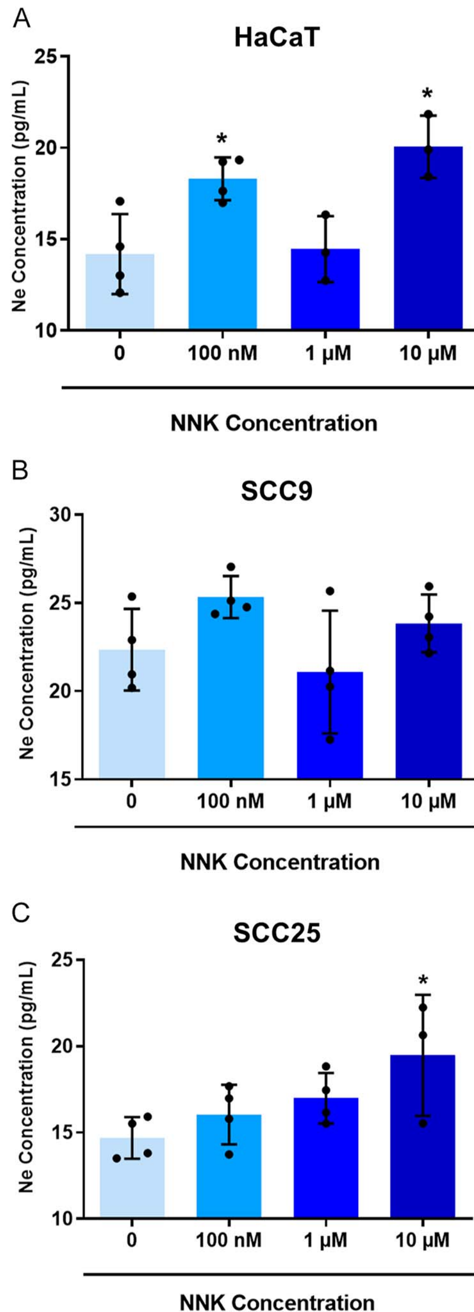

Figure 2

NNK promotes norepinephrine secretion in HaCaT and SCC25 cells. HaCaT (A), SCC9 (B) and SCC25 (C) cells were cultivated for 24 h in reduced FBS concentration (0.5%), and then they were stimulated with different concentrations of NNK (100 nM, 1 μM and 10 μM) for 6 h. (A) NNK (100 nM and 10 μM) induced increased norepinephrine secretion by HaCaT cells compared to the control (non-stimulated cells). (B) NNK did not significantly induce norepinephrine secretion by SCC9 cells. (C) NNK (10 μM) induced higher norepinephrine secretion by SCC25 cells compared to the control. Each value represents the mean ± SEM of the assays (n = 4 per group). *Indicates statistically significant differences between cells stimulated with NNK and non-stimulated cells (P < 0.05). Abbreviations: NE, norepinephrine.

increased the proliferation rates of HaCaT cells in a dose-dependent manner, but only 10 μM NNK induced a significant increase in cell proliferation relative to untreated cells (P < 0.05) (Fig. 3A). The results for SCC9 cells demonstrated that 1 and 10 μM NNK increased cell proliferation rates by 70 and 151% compared to unstimulated cells, respectively (P < 0.05) (Fig. 3B). NNK at different concentrations induced increased proliferation levels of SCC25 cells, but only 10 μM NNK reached statistical significance, increasing the cell proliferation rate by 41% (P < 0.05) (Fig. 3C). NNK at 10 nM reduced SCC25 cell proliferation, but this effect was not statistically significant (P > 0.05) (Fig. 3C).

Proliferation assays after treatment with NNK in combination with β-adrenergic and nicotinic receptor antagonists

To evaluate whether the effects of NNK on HaCaT, SCC9 and SCC25 cells are dependent on β1- and β2-adrenergic receptors and α4- and α7-AChRs, we carried out cell proliferation assays with NNK-treated cells in the absence or presence of antagonists for these receptors. For this purpose, the cells were stimulated with 10 μM NNK with or without the following inhibitors: α-bungarotoxin (inhibitor of nAChR-α7 receptor), dihydro-β-Erythroidine (inhibitor of nAChR-α4 receptor) and propranolol (inhibitor of β1- and β2-adrenergic receptors). The concentration of 10 μM NNK was selected because it induced the most intense stimulatory effects on the proliferation rates in the tested cells. The results showed that none of the three antagonists tested had a significant effect on HaCaT NNK-induced cell proliferation (P > 0.05) (Fig. 4A). Nevertheless, propranolol (a nonspecific β1- and β2-adrenergic receptor inhibitor) completely blocked the effects caused by NNK on SCC9 and SCC25 cell proliferation (Fig. 4B and C). Dihydro-β-erythroidine completely inhibited the NNK effect on SCC9 cell proliferation (P < 0.05) and partly inhibited NNK-induced SCC25 cell proliferation, but in this cell line, the result did not reach statistical significance (P > 0.05) (Fig. 4B and C). Interestingly, α-bungarotoxin (BTX) had no effect on NNK-induced proliferation in any of the OSCC cell lines (P > 0.05) (Fig. 4B and C). These results suggest that the effect of NNK on OSCC cell proliferation is dependent on β-adrenergic and nAChR-α4 receptor activation.

Discussion

In this study, our results revealed the ability of oral cancer cells to secrete the neurotransmitter norepinephrine in an autocrine manner, which may play a role in an autocrine catecholamine loop. The higher levels of norepinephrine secreted by OSCC cell lines compared to non-malignant cells may represent

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

https://doi.org/10.1530/EO-24-0076

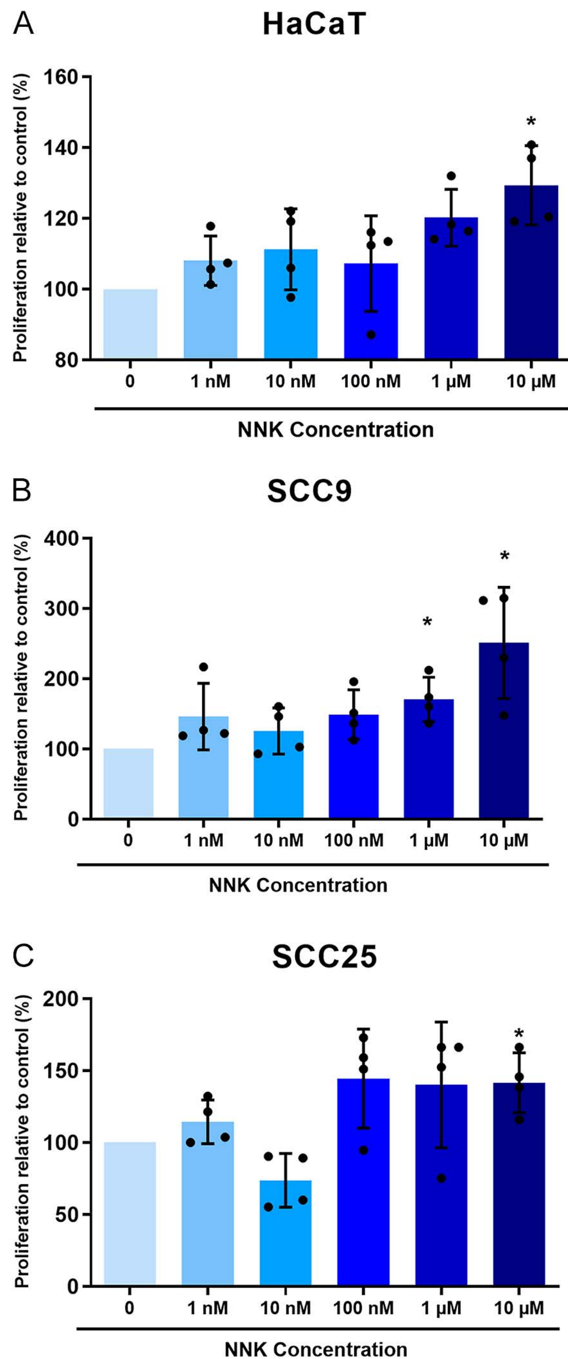

Figure 3

NNK effects on the proliferation of keratinocytes and OSCC cells. HaCaT, SCC9 and SCC25 cells were cultivated for 24 h in reduced FBS concentration (0.5%) and then stimulated with 1 nM, 10 nM, 100 nM, 1 μM and 10 μM NNK for 48 h. NNK at 10 μM increased the proliferation of HaCaT (A), SCC9 (B) and SCC25 cells (C) compared to control (non-stimulated cells). NNK at 1 μM also induced SCC9 cell proliferation (B). Each value represents the mean ± SEM of the assays. (SCC9 cells, $n = 4$ per group; SCC25 cells, $n = 5$ per group). *$P < 0.05$.

adrenergic hyperactivation in cancer cells. Non-tumorigenic and tumorigenic human breast cells (Amaro *et al.* 2020), pancreatic cancer cells and normal pancreatic ductal epithelial cells (Al-Wadei *et al.* 2012*a*), and immortalized human small airway epithelial cell lines (Al-Wadei *et al.* 2010) can produce norepinephrine without any stimulation. Nonetheless, this is the first time that this ability has been described in OSCC cells.

Our results indicate that 10% FBS supplementation provides a higher concentration of norepinephrine in the culture medium in comparison to 0.5%, indicating that the concentrations of norepinephrine in culture media are dependent on FBS concentration. Previous studies indicate that catecholamines can stimulate norepinephrine secretion in some cell lines (Luo *et al.* 2017) and enhance the viability and proliferation of oral cancer cell lines (Bernabé *et al.* 2011, Kwon *et al.* 2021). To prevent interference from norepinephrine in FBS, we performed all experiments with the lowest possible concentration of FBS. Furthermore, norepinephrine levels in the supernatant of HaCaT and OSCC cells were higher than in culture media without cells, demonstrating that these cell lines were able to produce and secrete norepinephrine.

NNK is a highly specific tobacco carcinogen nitrosamine, but the mechanisms involved in oral carcinogenesis and the proliferation of oral cancer cells induced by NNK are poorly understood. Our results revealed that the highest concentration of NNK (10 μM) induced a significant increase in cellular proliferation in keratinocytes (HaCaT) and OSCC cells (SCC9 and SCC25). This concentration is similar to that found in a pack of cigarettes (Proulx *et al.* 2005). An interesting finding in our study is that a 1 μM dose of NNK has less effect than a lower dose in HaCaT and SCC9 proliferation rates. A nonlinear dose–response could explain this finding, as many toxic and carcinogenic compounds exhibit hormetic effects, where lower doses elicit a stronger response than higher doses due to adaptive cellular mechanisms. In addition, biphasic effects may be at play, where low doses induce subtle epigenetic or proliferative changes, while higher doses activate protective pathways that mitigate the impact. To clarify this unexpected dose–response relationship, further studies should include intermediate concentrations to better define the effect, as well as analyses of apoptosis and stress markers to determine whether compensatory mechanisms are involved (Park *et al.* 2017).

NNK is an irritant chemical that can promote oxidative stress and contribute to cell proliferation (Therriault *et al.* 2003, Xue *et al.* 2014). In a previous study by our group, we showed that norepinephrine combined with NNK inhibited the apoptosis of NOK-SI cells by reducing the activity of caspases 3 and 7, allowing the uncontrolled

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

Endocrine Oncology (2025) **5** e240076
https://doi.org/10.1530/EO-24-0076

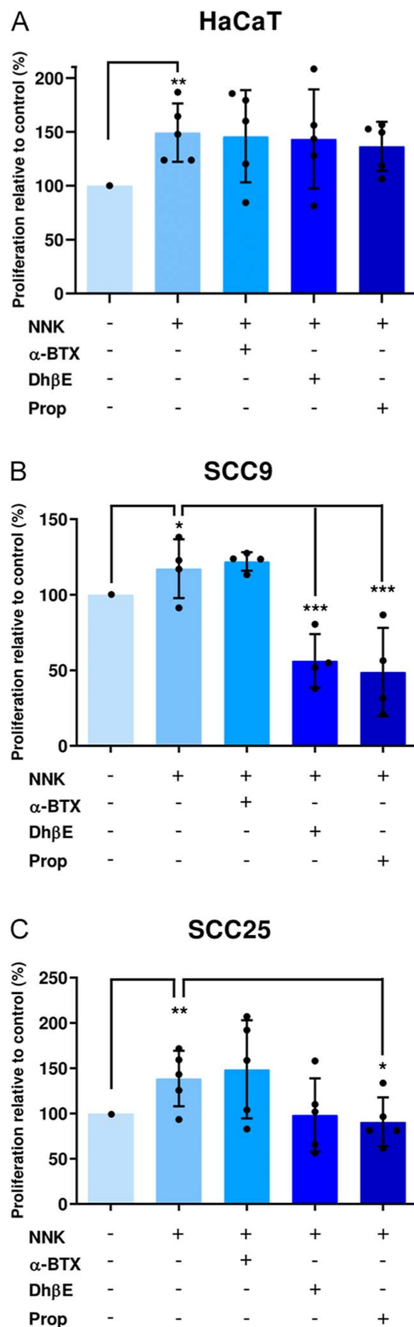

Figure 4

Role of β-adrenergic receptors and nicotinic acetylcholine receptors (nAChR) α4 and α7 in NNK-induced OSCC cell proliferation. (A) In HaCaT cells, NNK-induced proliferation was not inhibited by any of the antagonists evaluated. (B) In SCC9 cells, NNK-induced cell proliferation was significantly inhibited by DhβE and propranolol (a non-selective β1- and β2-adrenergic receptor antagonist). (C) In SCC25 cells, propranolol blocked NNK-induced proliferation, while the α4-nAChR antagonist DhβE reduced it, though this effect did not reach statistical significance ($P > 0.05$). Each value represents the mean ± SEM of the assays. (HaCaT cells, $n = 5$ per group; SCC9 cells, $n = 4$ per group; SCC25 cells, $n = 5$ per group). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

proliferation of damaged keratinocytes (Valente *et al.* 2021). Moreover, as described by Kim and colleagues (2018), treatment of Ishikawa cells with NNK induces cell cycle progression and proliferation by upregulating cyclin E and cyclin D expression and downregulating p21 and p27 (Kim *et al.* 2018). NNK can induce proliferation in other cell lines, including colorectal cancer (Jiang *et al.* 2024), oral cancer (SCC-15, HSC-13, OSC-19 and UM1 cell lines) (Guo *et al.* 2020), and endometrial adenocarcinoma (Kim *et al.* 2018). Non-neoplastic alveolar epithelial cells subjected to prolonged exposure to NNK acquired increasingly malignant properties, such as enhanced proliferation and cell motility (Mennecier *et al.* 2014).

NNK has a high affinity for β-adrenergic receptors due to its structural resemblance to classical beta-adrenergic agonists (Schuller 2007*a*). When β1- and β2-AR were blocked in esophageal cancer cells, a reversal of the oncogenic effects of NNK was observed (Kun *et al.* 2009). Activation of β-adrenergic receptors in response to NNK has been demonstrated in the growth of pancreatic carcinoma (Weddle *et al.* 2001), colon cancer (Wu *et al.* 2005) and esophageal squamous cell carcinoma (Zhang *et al.* 2015) cell lines. In our study, we conducted cell proliferation assays in NNK-treated cells in the presence of inhibitors of β-adrenergic receptor types 1 and 2. Our results showed that propranolol blocked the effects caused by NNK on the proliferation of SCC9 and SCC25 cells, but not in HaCaT cells. These results suggest that tobacco-related nitrosamines may influence OSCC progression via beta-adrenergic activation, a recognized stress-associated pathway. Previously, we demonstrated that propranolol reduces oral cancer cell viability in a dose- and time-dependent manner (Shibuya *et al.* 2022). β-adrenergic receptors are also involved in the proliferation of various types of cancer, including pancreas (Shin *et al.* 2008), colorectum (Wu *et al.* 2005), stomach (Shin *et al.* 2006) and lung (Schuller *et al.* 1999). The effects on cell proliferation in these studies were blocked by propranolol.

After demonstrating that keratinocytes and oral SCC cells are capable of producing NE, we investigated whether the carcinogen NNK stimulates hormone production. Our preliminary studies indicate that lower NNK concentrations do not significantly affect secretion levels. Consequently, these concentrations were excluded to optimize experimental efficiency and focus on doses that elicit biologically relevant effects. In addition, very low concentrations may fall within the range of normal biological fluctuations, making it difficult to distinguish NNK-specific effects from baseline secretion variability. This suggests that only higher concentrations of NNK may effectively disrupt cellular signaling pathways involved in NE secretion, potentially by activating stress-related or oncogenic mechanisms that enhance hormone release. In our findings, NNK induced SCC25 and HaCaT cells to

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

secrete norepinephrine. The same was not observed in SCC9 cells, indicating that this cell line may have already reached a maximum limit of neurotransmitter secretion under basal conditions without NNK stimulation. Norepinephrine secretion from human small airway epithelial cells is regulated by nAChRs, and there is a greater increase in receptor responsiveness when stimulated with NNK (Egleton *et al.* 2009). Pancreatic cancer cells and normal pancreatic ductal epithelial cells display an autocrine catecholamine loop that stimulates their proliferation via β-ARs and nAChRs-α3, -α5 and -α7 (Al-Wadei *et al.* 2012*b*). Al-Wadei and colleagues reported that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and its synthesizing enzyme, glutamate decarboxylase 65 (GAD65), were suppressed in NNK-induced small airway-derived pulmonary adenocarcinoma cells and in pancreatic ductal adenocarcinoma (Al-Wadei & Schuller 2009). Despite the suppression of GABA, protein expression of nAChR-α4 and -α7 was upregulated (Al-Wadei & Schuller 2009).

This study showed that the tested antagonists of nAChR-α4 and nAChR-α7 had no significant effects on NNK-induced proliferation in HaCaT cells. However, dihydro-β-erythroidine inhibited the NNK effect on SCC9 cell proliferation and, in part, its effect on the NNK-induced proliferation of SCC25 cells. α-bungarotoxin had no effect on cell proliferation induced by NNK in any cancer cell line. These results indicate that the NNK effect on the proliferation of OSCC cells can be dependent on nAChR-α4. Schuller and colleagues (2007) discussed how human small cell lung cancer exposure to NNK increased the expression of nAChR-α7 and caused an influx of Ca^{2+}. This cascade induces the activation of PKC, Raf-1, ERK1/2 and c-myc, increasing cell proliferation (Schuller 2007*b*). Nitrosamines in tobacco, such as NNK, can promote the activation of the serine/threonine kinase Akt and contribute to the carcinogenesis of normal human airway through nAChRs-α3, -α4 and -α7, respectively (Al-Wadei *et al.* 2010).

The present study has some limitations. Although the manufacturer does not indicate the presence of catecholamines in the original formulation of culture media without FBS, these were not tested for detectable levels of norepinephrine. Second, norepinephrine levels were measured using a specific kit through ELISA and not by a more sensitive method, such as high-performance liquid chromatography. Moreover, we did not analyze the effects of an NNK antagonist on norepinephrine production and cell proliferation. Therefore, we cannot determine whether norepinephrine release is secondary to NNK stimulation or a by-product of NNK-induced proliferation. Finally, the effects of β-adrenergic, nAChR-α4 and nAChR-α7 antagonists associated with NNK stimulation on norepinephrine secretion have not been evaluated.

In the present study, we demonstrated for the first time that OSCC cells secrete stress-related norepinephrine, and this event can be further regulated by the tobacco carcinogen NNK. NNK also increases the proliferation of HaCaT and OSCC cells, mainly through β-adrenergic receptors, the same receptors activated by norepinephrine. These findings together suggest that OSCC development may be related to an imbalance in norepinephrine secretion and activation of its receptors, directly inducing changes associated with the malignant phenotype. In conclusion, this study reveals that OSCC cells secrete the stress-related neurotransmitter norepinephrine, and the tobacco carcinogen NNK induces increased cell proliferation through a stress-related cellular adrenergic pathway.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

Funding

This study was supported by the São Paulo State Research Foundation (FAPESP) grant 2013/24661-6, designated to Flávia Alves Verza.

Author contribution statement

Flávia Alves Verza contributed to writing the original draft, methodology, data curation and formal analysis. Ana Lívia Santos Sousa contributed to writing the original draft. Sandra Helena Penha de Oliveira was responsible for writing, review and editing. Daniel Galera Bernabé contributed to conceptualization, resources, formal analysis, supervision, funding acquisition, project administration, writing, review and editing. The paper was critically reviewed and approved by all authors.

References

Al-Wadei H & Schuller HM 2009 Nicotinic receptor-associated modulation of stimulatory and inhibitory neurotransmitters in NNK-induced adenocarcinoma of the lungs and pancreas. *J Pathol* **218** 437–445. (https://doi.org/10.1002/path.2542)

Al-Wadei HAN, Al-Wadei MH, Masi T, *et al.* 2010 Chronic exposure to estrogen and the tobacco carcinogen NNK cooperatively modulates nicotinic receptors in small airway epithelial cells. *Lung Cancer* **69** 33–39. (https://doi.org/10.1016/j.lungcan.2009.09.011)

Al-Wadei HAN, Plummer HK, Ullah MF, *et al.* 2012*a* Social stress promotes and γ-aminobutyric acid inhibits tumor growth in mouse models of non-small cell lung cancer. *Cancer Prev Res* **5** 189–196. (https://doi.org/10.1158/1940-6207.capr-11-0177)

Al-Wadei MH, Al-Wadei HAN & Schuller HM 2012*b* Pancreatic cancer cells and normal pancreatic duct epithelial cells express an autocrine catecholamine loop that is activated by nicotinic acetylcholine receptors α3, α5, and α7. *Mol Cancer Res* **10** 239–249. (https://doi.org/10.1158/1541-7786.mcr-11-0332)

Amaro F, Silva D, Reguengo H, *et al.* 2020 Β-adrenoceptor activation in breast Mcf-10a cells induces a pattern of catecholamine production similar to that of tumorigenic Mcf-7 cells. *Int J Mol Sci* **21** 7968. (https://doi.org/10.3390/ijms21217968)

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

Antoni MH, Lutgendorf SK, Cole SW, *et al.* 2006 The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat Rev Cancer* **6** 240–248. (https://doi.org/10.1038/nrc1820)

Armaiz-Pena GN, Cole SW, Lutgendorf SK, *et al.* 2013 Neuroendocrine influences on cancer progression. *Brain Behav Immun* **30** S19–S25. (https://doi.org/10.1016/j.bbi.2012.06.005)

Bastos DB, Sarafim-Silva BAM, Sundefeld MLMM, *et al.* 2018 Circulating catecholamines are associated with biobehavioral factors and anxiety symptoms in head and neck cancer patients. *PLoS One* **13** e0202515. (https://doi.org/10.1371/journal.pone.0202515)

Bernabé DG 2021 Catecholamines mediate psychologic stress–induced cancer progression. *Cancer Res* **81** 5144–5146. (https://doi.org/10.1158/0008-5472.can-21-3077)

Bernabé DG, Tamae AC, Biasoli ÉR, *et al.* 2011 Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinoma cells. *Brain Behav Immun* **25** 574–583. (https://doi.org/10.1016/j.bbi.2010.12.012)

Bray F, Laversanne M, Sung H, *et al.* 2024 Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **74** 229–263. (https://doi.org/10.3322/caac.21834)

Chen Y, Wang Q, Cao L, *et al.* 2021 Nicotine-derived NNK induces the stemness enrichment of CRC cells through regulating the balance of DUSP4-ERK1/2 feedback loop. *Ecotoxicol Environ Saf* **214** 112057. (https://doi.org/10.1016/j.ecoenv.2021.112057)

Danaei G, Vander Hoorn S, Lopez AD, *et al.* 2005 Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *Lancet* **366** 1784–1793. (https://doi.org/10.1016/s0140-6736(05)67725-2)

Egleton RD, Brown KC & Dasgupta P 2009 Angiogenic activity of nicotinic acetylcholine receptors: implications in tobacco-related vascular diseases. *Pharmacol Ther* **121** 205–223. (https://doi.org/10.1016/j.pharmthera.2008.10.007)

Grando SA 2014 Connections of nicotine to cancer. *Nat Rev Cancer* **14** 419–429. (https://doi.org/10.1038/nrc3725)

Guo J, Zhou S, Huang P, *et al.* 2020 NNK-mediated upregulation of DEPDC1 stimulates the progression of oral squamous cell carcinoma by inhibiting CYP27B1 expression. *Am J Cancer Res* **10** 1745–1760.

Hecht SS 2003 Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* **3** 733–744. (https://doi.org/10.1038/nrc1190)

Schuller HM 2013 Regulatory role of the α7nAChR in cancer. *Curr Drug Targets* **13** 680–687. (https://doi.org/10.2174/138945012800398883)

Itoi K & Sugimoto N 2010 The brainstem noradrenergic systems in stress, anxiety and depression. *J Neuroendocrinol* **22** 355–361. (https://doi.org/10.1111/j.1365-2826.2010.01988.x)

Jethwa AR & Khariwala SS 2017 Tobacco-related carcinogenesis in head and neck cancer. *Cancer Metastasis Rev* **36** 411–423. (https://doi.org/10.1007/s10555-017-9689-6)

Jiang M, Zhang X, Huang H, *et al.* 2024 A novel four gene biomarker for tobacco smoking-induced colorectal cancer progression. *Nicotine Tob Res* **26** 1286–1295. (https://doi.org/10.1093/ntr/ntae077)

Kalantari-Dehaghi M, Bernard H-U & Grando SA 2012 Reciprocal effects of NNK and SLURP-1 on oncogene expression in target epithelial cells. *Life Sci* **91** 1122–1125. (https://doi.org/10.1016/j.lfs.2012.02.004)

Kim S-M, Hwang K-A, Choi D-W, *et al.* 2018 The cigarette smoke components induced the cell proliferation and epithelial to mesenchymal transition via production of reactive oxygen species in endometrial adenocarcinoma cells. *Food Chem Toxicol* **121** 657–665. (https://doi.org/10.1016/j.fct.2018.09.023)

Kun G, Ma Q, Wang L, *et al.* 2009 Norepinephrine-induced invasion by pancreatic cancer cells is inhibited by propranolol. *Oncol Rep* **22** 1265–1270. (https://doi.org/10.3892/or_00000505)

Kwon S, Chun K, Kil H, *et al.* 2021 β2-adrenergic receptor expression and the effects of norepinephrine and propranolol on various head and neck cancer subtypes. *Oncol Lett* **22** 804. (https://doi.org/10.3892/ol.2021.13065)

Laag E, Majidi M, Cekanova M, *et al.* 2006 NNK activates ERK1/2 and CREB/ATF-1 via β-1-AR and EGFR signaling in human lung adenocarcinoma and small airway epithelial cells. *Int J Cancer* **119** 1547–1552. (https://doi.org/10.1002/ijc.21987)

Lin C, Lin L, Chen T, *et al.* 2023 The expression of nicotinic acetylcholine receptor subunits and their associations with local immune cells and prognosis in oral squamous cell carcinoma. *Cancer Med* **12** 18918–18930. (https://doi.org/10.1002/cam4.6482)

Luo Y, Liu B, Yang X, *et al.* 2017 Myeloid adrenergic signaling via CaMKII forms a feedforward loop of catecholamine biosynthesis. *J Mol Cell Biol* **9** 422–434. (https://doi.org/10.1093/jmcb/mjx046)

Lutgendorf SK & Andersen BL 2015 Biobehavioral approaches to cancer progression and survival: mechanisms and interventions. *Am Psychol* **70** 186–197. (https://doi.org/10.1037/a0035730)

Mennecier G, Torres LN, Cogliati B, *et al.* 2014 Chronic exposure of lung alveolar epithelial type II cells to tobacco-specific carcinogen NNK results in malignant transformation: a new in vitro lung carcinogenesis model. *Mol Carcinog* **53** 392–402. (https://doi.org/10.1002/mc.21987)

Moreno-Smith M, Lutgendorf SK & Sood AK 2010 Impact of stress on cancer metastasis. *Future Oncol* **6** 1863–1881. (https://doi.org/10.2217/fon.10.142)

Park JE, Jang YL & Jang C-Y 2017 The tobacco carcinogen NNK disturbs mitotic chromosome alignment by interrupting p53 targeting to the centrosome. *Toxicol Lett* **281** 110–118. (https://doi.org/10.1016/j.toxlet.2017.09.019)

Phillips DH 2002 Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis* **23** 1979–2004. (https://doi.org/10.1093/carcin/23.12.1979)

Proulx LI, Gaudreault M, Turmel V, *et al.* 2005 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, a component of tobacco smoke, modulates mediator release from human bronchial and alveolar epithelial cells. *Clin Exp Immunol* **140** 46–53. (https://doi.org/10.1111/j.1365-2249.2005.02739.x)

Reitsma MB, Flor LS, Mullany EC, *et al.* 2021 Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and initiation among young people in 204 countries and territories, 1990–2019. *Lancet Public Health* **6** e472–e481. (https://doi.org/10.1016/s2468-2667(21)00102-x)

Santos-Sousa AL, Kayahara GM, Bastos DB, *et al.* 2024 Expression of β1- and β2-adrenergic receptors in oral squamous cell carcinoma and their association with psychological and clinical factors. *Arch Oral Biol* **162** 105939. (https://doi.org/10.1016/j.archoralbio.2024.105939)

Schuller HM 2007*a* Neurotransmitter receptor-mediated signaling pathways as modulators of carcinogenesis. *Prog Exp Tumor Res* **39** 45–63. (https://doi.org/10.1159/000100045)

Schuller HM 2007*b* Nitrosamines as nicotinic receptor ligands. *Life Sci* **80** 2274–2280. (https://doi.org/10.1016/j.lfs.2007.03.006)

Schuller HM, Tithof PK, Williams M, *et al.* 1999 The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone is a β-adrenergic agonist and stimulates DNA synthesis in lung adenocarcinoma via β-adrenergic receptor-mediated release of arachidonic acid. *Cancer Res* **59** 4510–4515.

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/

Shi M, Liu D, Duan H, *et al.* 2011 The β2-adrenergic receptor and Her2 comprise a positive feedback loop in human breast cancer cells. *Breast Cancer Res Treat* **125** 351–362. (https://doi.org/10.1007/s10549-010-0822-2)

Shibuya CM, Tjioe KC, Oliveira SHP, *et al.* 2022 Propranolol inhibits cell viability and expression of the pro-tumorigenic proteins Akt, NF-κB, and VEGF in oral squamous cell carcinoma. *Arch Oral Biol* **136** 105383. (https://doi.org/10.1016/j.archoralbio.2022.105383)

Shin VY, Wu WKK, Chu KM, *et al.* 2006 Functional role of-adrenergic receptors in the mitogenic action of nicotine on gastric cancer cells. *Toxicol Sci* **96** 21–29. (https://doi.org/10.1093/toxsci/kfl118)

Shin VY, Jin HC, Ng EKO, *et al.* 2008 Nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone induce cyclooxygenase-2 activity in human gastric cancer cells: involvement of nicotinic acetylcholine receptor (nAChR) and β-adrenergic receptor signaling pathways. *Toxicol Appl Pharmacol* **233** 254–261. (https://doi.org/10.1016/j.taap.2008.08.012)

Thaker PH, Han LY, Kamat AA, *et al.* 2006 Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* **12** 939–944. (https://doi.org/10.1038/nm1447)

Therriault M-J, Proulx L-I, Castonguay A, *et al.* 2003 Immunomodulatory effects of the tobacco-specific carcinogen, NNK, on alveolar macrophages. *Clin Exp Immunol* **132** 232–238. (https://doi.org/10.1046/j.1365-2249.2003.02142.x)

Tjioe KC, Cardoso DM, Oliveira SHP, *et al.* 2022 Stress hormone norepinephrine incites resistance of oral cancer cells to chemotherapy. *Endocr Relat Cancer* **29** 201–212. (https://doi.org/10.1530/erc-20-0460)

Valente VB, de Melo Cardoso D, Kayahara GM, *et al.* 2021 Stress hormones promote DNA damage in human oral keratinocytes. *Sci Rep* **11** 19701. (https://doi.org/10.1038/s41598-021-99224-w)

Weddle DL, Tithoff P, Williams M, *et al.* 2001 β-Adrenergic growth regulation of human cancer cell lines derived from pancreatic ductal carcinomas. *Carcinogenesis* **22** 473–479. (https://doi.org/10.1093/carcin/22.3.473)

Wong HPS, Yu L, Lam EKY, *et al.* 2007 Nicotine promotes cell proliferation via α7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated pathway in human colon adenocarcinoma HT-29 cells. *Toxicol Appl Pharmacol* **221** 261–267. (https://doi.org/10.1016/j.taap.2007.04.002)

Wu WKK, Wong HPS, Luo SW, *et al.* 2005 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone from cigarette smoke stimulates colon cancer growth via β-adrenoceptors. *Cancer Res* **65** 5272–5277. (https://doi.org/10.1158/0008-5472.can-05-0205)

Xie H, Li C, He Y, *et al.* 2015 Chronic stress promotes oral cancer growth and angiogenesis with increased circulating catecholamine and glucocorticoid levels in a mouse model. *Oral Oncol* **51** 991–997. (https://doi.org/10.1016/j.oraloncology.2015.08.007)

Xue J, Yang S & Seng S 2014 Mechanisms of cancer induction by tobacco-specific NNK and NNN. *Cancers* **6** 1138–1156. (https://doi.org/10.3390/cancers6021138)

Yamazaki H, Inui Y, Yun CH, *et al.* 1992 Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis* **13** 1789–1794. (https://doi.org/10.1093/carcin/13.10.1789)

Yang EV, Kim S-j, Donovan EL, *et al.* 2009 Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. *Brain Behav Immun* **23** 267–275. (https://doi.org/10.1016/j.bbi.2008.10.005)

Zhang N, Sun X, Sun M, *et al.* 2015 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone promotes esophageal squamous cell carcinoma growth via beta-adrenoceptors in vitro and in vivo. *PLoS One* **10** e0118845. (https://doi.org/10.1371/journal.pone.0118845)

Zoli M, Pucci S, Vilella A, *et al.* 2018 Neuronal and extraneuronal nicotinic acetylcholine receptors. *Curr Neuropharmacol* **16** 338–349. (https://doi.org/10.2174/1570159x15666170912110450)

Downloaded from Bioscientifica.com at 04/27/2025 03:37:02PM
via Open Access. This work is licensed under a Creative Commons
Attribution-NonCommercial 4.0 International License.
https://creativecommons.org/licenses/by-nc/4.0/