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β-Carotene promotes the development of NNK-induced small airway-derived lung adenocarcinoma ☆

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ABSTRACT

Aim: β-Carotene has shown cancer-preventive effects in preclinical studies while increasing lung cancer mortality in clinical trials. We have shown that β-carotene stimulates cAMP signalling *in vitro*. Here, we have tested the hypothesis that β-carotene promotes the development of pulmonary adenocarcinoma (PAC) *in vivo* via cAMP signalling.
Methods: PAC was induced in hamsters with the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), followed by β-carotene for 1.5 years. Incidence, multiplicity and size of lung tumours were recorded, and phosphorylated CREB and ERK1/2 in tumour cells were determined by Western blots. Cyclic AMP in blood cells was analysed by immunoassays, retinoids in serum and lungs by HPLC.
Results: β-Carotene increased lung tumour multiplicity, lung tumour size, blood cell cAMP, serum and lung levels of retinoids and induced p-CREB and p-ERK1/2 in lung tumours.
Conclusions: Our data suggest that β-carotene promotes the development of PAC via increased cAMP signalling.

1. Introduction

Lung cancer is the leading cause of cancer death in the industrialised countries, and smoking is a major risk factor that is estimated to cause about 80% of all lung cancers.[1] Pulmonary adenocarcinoma (PAC) is the predominating histological type of lung cancer today. The prognosis of PAC is poor, with 5-year survivals below 20%. Novel strategies for the prevention of these cancers in populations at risk are therefore urgently needed.

The pro-vitamin A, β-carotene, vitamin A (retinol), and its metabolites such as all-trans-retinoic acid (ATRA), 9-cis-retinoic acid (9-cis-RA) and 13-cis-retinoic acid (13-cis-RA) have shown cancer-preventive effects in the preclinical studies.[2] However, the alpha-tocopherol β-carotene supplementation trial (ATBC), and the β-carotene and retinoid efficacy trial (CARET) in smokers and former smokers had to be stopped due to a significant increase in mortality from lung cancer.[3,4] In order to arrive at the identification of better cancer-preventive agents, it is important to understand the reasons for which these trials failed.

Most PACs in people are thought to arise from the epithelial cells of small airways in the lung periphery.[5] This epithelium consists mostly of conciliated Clara cells.[6] PAC induced in Syrian golden hamsters by the nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a model for human small airway-derived PAC.[7] Similar to human PAC,[8] these tumours in hamsters express activating

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point mutations in K-ras[9] while over-expressing cyclooxygenase-2 (COX-2) and the epidermal growth factor receptor (EGFR).[10] We have shown that human PAC cell lines that express the Clara cell-specific CC10 protein, immortalised human small airway epithelial cell line HPL1D (CC10 positive) and the NNK-induced PACs in hamsters (CC10 positive) are under growth control by $G\alpha_s$-coupled β-1 and β-2-adrenoreceptors that increase intracellular cAMP via activation of adenylyl cyclase. Classic agonists for β-1 and β-2-ARs as well as NNK initiated mitogenic signalling in these cells, via cAMP-induced activation of PKA, CREB and the PKA-dependent transactivation of the EGFR.[11,12] Specific inhibitors of adenylyl cyclase or PKA blocked all these downstream responses.[12] These findings are consistent with the 'classic' concept of β-AR signalling via $G\alpha_s$ adenylyl cyclase cAMP PKA CREB and transactivation of the EGFR.[13] The β-AR agonist epinephrine or the phosphodiesterase inhibitor theophylline each promoted the development of NNK-induced PAC, whereas the β-blocker propranolol significantly inhibited tumour development.[14,15] P-CREB and p-ERK1/2 were overexpressed in the NNK-induced PACs and in hyperplastic lesions.[10,16] These data identify cAMP-mediated signalling as an important regulator of small airway-derived PAC.

Vitamin A deficiency causes squamous cell metaplasia in large airways of hamsters, an effect reversed by the treatment with retinol.[17] Numerous preclinical studies have shown genomic effects of high concentrations of retinol via nuclear retinoid receptors that suggested this agent and its metabolites as cancer-preventive agents.[2] Based on these preclinical results, the alpha-tocopherol, β-carotene supplementation trial (ATBC), and the β-carotene and retinoid efficacy trial (CARET) in smokers and former smokers were conducted. Both the trials had to be stopped because of significant increases in lung cancer mortality in the β-carotene or retinoid-treated groups.[3,4]

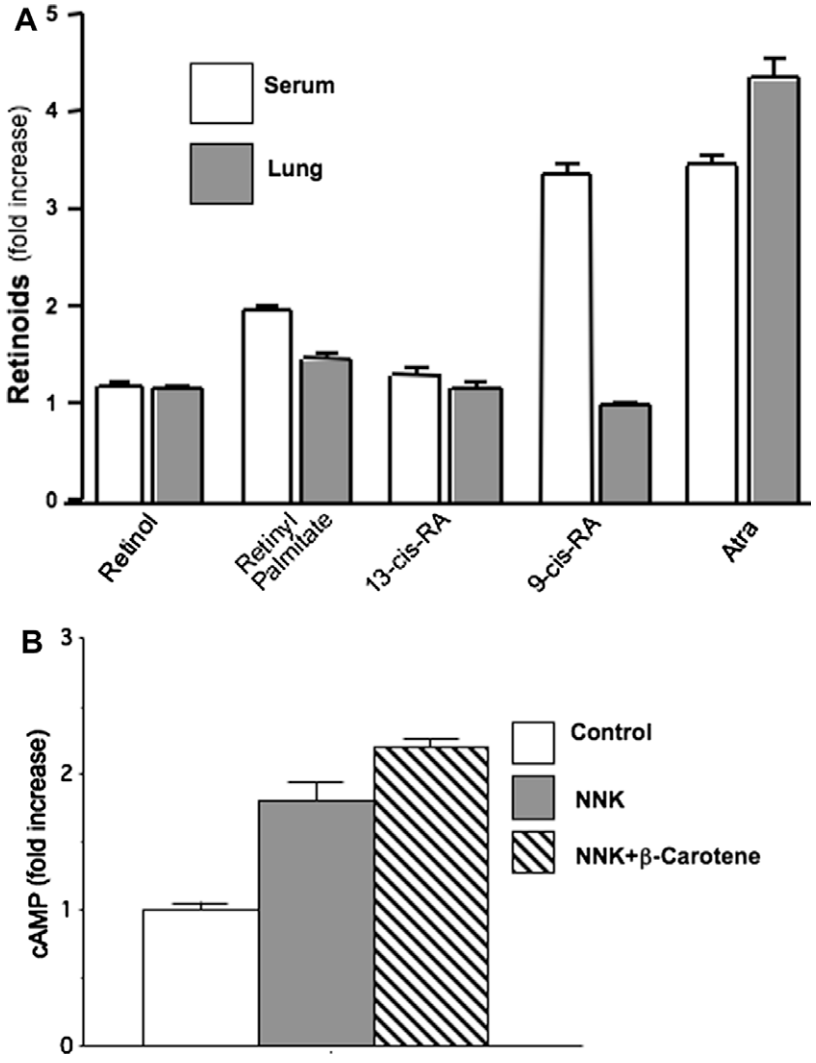

Fig. 1 – (A) Modulation of retinoid levels in serum and lung tissue. Retinyl palmitate and ATRA were the predominating metabolites, and their increase over controls was highly significant ($p < 0.001$). Mean values and standard errors of samples from 5 hamsters per group expressed as fold increase over controls. (B) Modulation of systemic cAMP levels in blood cells. NNK significantly ($p < 0.001$) increased systemic cAMP levels over controls. Animals treated with NNK+β-carotene showed an additional significant ($p < 0.001$) increase in systemic cAMP. Mean values and standard errors from 5 hamsters/group expressed as fold increase over controls.

In vitro studies in human small airway-derived PAC cells and small airway epithelia showed that low concentrations of 1 pM to 2 μM of β-carotene, retinol, ATRA, 9-cis-RA or 13-cis-RA increased intracellular cAMP, activating PKA CREB and ERK1/2.[18,19] These findings suggest that β-carotene may promote small airway-derived PAC *in vivo*. To test this hypothesis, we have investigated the effects of β-carotene on the development of small airway-derived NNK-induced PAC in hamsters.

2. Materials and methods

2.1. Bioassay experiment in hamsters

The main dependent variables of this study were tumour number per animal (lung tumour multiplicity) and tumour size. Survival was not used as end-point, because animals showing any respiratory problems were euthanised prior to the end of the study. Under these conditions, the survival of NNK-treated animals versus NNK+β-carotene treated animals was not significantly different. The animal experiment was conducted in compliance with the United States (US) Public Health Service Policy on Humane Care and Use of Laboratory Animals, and was approved by the Institutional Animal Care and Use Committee. Male, outbred Syrian golden hamsters (6 weeks old, Charles River) were randomly assigned to three treatment groups (20 hamsters per group), and were housed under standard laboratory conditions with free access to tap water and food (Purina lab chow). The development of PAC derived from small airway epithelial cells was induced in two treatment groups, as previously described[7,10,16] by subcutaneous injections with NNK (2.5 mg/100 g bodyweight in 0.2 ml sterile water 3X per week for 10 weeks). One week after the last NNK injection, injections with β-carotene (Sigma) started in one group (5 μM β-carotene in 0.2 ml sesame oil subcutaneously 2X/week until the end of the study). Sesame oil by itself modulates neither NNK-induced small airway epithelial cell proliferation nor NNK-induced PAC development.[20] The control group received subcutaneous injections with sterile water. The animals were observed for 1.5 years

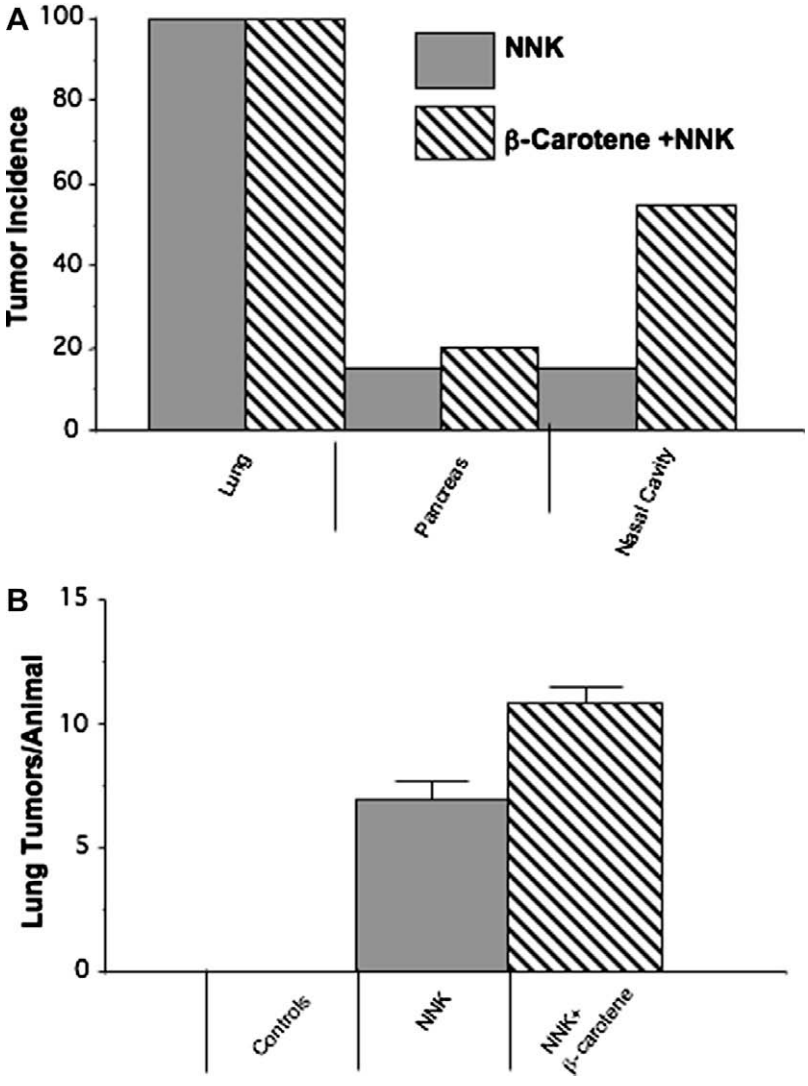

Fig. 2 – Tumour incidences in lungs, pancreas and nasal cavity (A) and lung tumour multiplicity (B). β-Carotene significantly ($p < 0.001$) increased lung tumour multiplicity. Data in both graphs are from 20 animals per group.

unless humane euthanasia by anaesthetic overdose (telazal, 50 mg/kg by intraperitoneal injection) was indicated at an earlier time in animals showing symptoms of disease. Blood samples were harvested from each animal followed by full necropsies. All major organs were fixed in 70% ethanol v/v, and processed for histopathology evaluation from paraffin-embedded tissue sections that are stained by haematoxylin/eosin. The histopathology diagnosis of lung adenoma versus lung adenocarcinoma was in accordance with the histopathology classification of neoplastic and preneoplastic lung lesions in the mouse.[21] Prior to tissue fixation, each lobe of the lungs was dissected into two halves by a longitudinal cut along its main lobar bronchus to allow for counting of lung tumours originating from airways. Each half of a lung lobe was embedded, and the resulting stained sections were scanned onto a Macintosh computer for the assessment of tumour sizes by image analysis (area measurements of the entire tumour surfaces by NIH Scion image analysis software). Statistical analysis of data from lung tumour multiplicity (number of lung tumours per animal) and tumour size (number of pixels per measured tumour area) was conducted by one-way analysis of variance (ANOVA), unpaired *t*-test and Mann–Whitney test. The cellular fraction of the blood samples were used for the determination of systemic cAMP levels. Snap frozen samples of serum and lung tissue from 4 randomly chosen control hamsters and hamsters that are treated with NNK+β-carotene were sent to a commercial laboratory (Craft Technologies) for the analysis of retinoids by HPLC.

2.2. *Determination of systemic cAMP levels*

The cellular fraction of blood samples comprising erythrocytes, lymphocytes, granulocytes and thrombocytes was used. These cells express β-adrenergic receptors, and lymphocytes are routinely used in human patients for the assessment of systemic cAMP-dependent signalling activity.[22] The levels of cAMP were determined with an enzyme immunoassay kit according to the manufacturers' instructions (Assay Designs Inc, Ann Arbor, MI). Data are expressed as mean values and standard errors of triplicate samples per treatment group. Statistical analysis of data was by ANOVA, Tukey-Kramer multiple comparisons test and two-tailed unpaired *t*-test.

2.3. *Analysis of signalling proteins in lung cells*

A PixCell IIe Laser Capture Microdissection system (Arcturus) was used to harvest lung tumour cells from hamsters treated with NNK or NNK+β-carotene and small airway epithelial cells from control hamsters, as previously described.[16] Protein was determined using the BCA Protein Assay (Pierce, Rockford, IL). Protein samples were treated with loading buffer at 100 °C for five minutes and electrophoresed in 12% w/v poly-

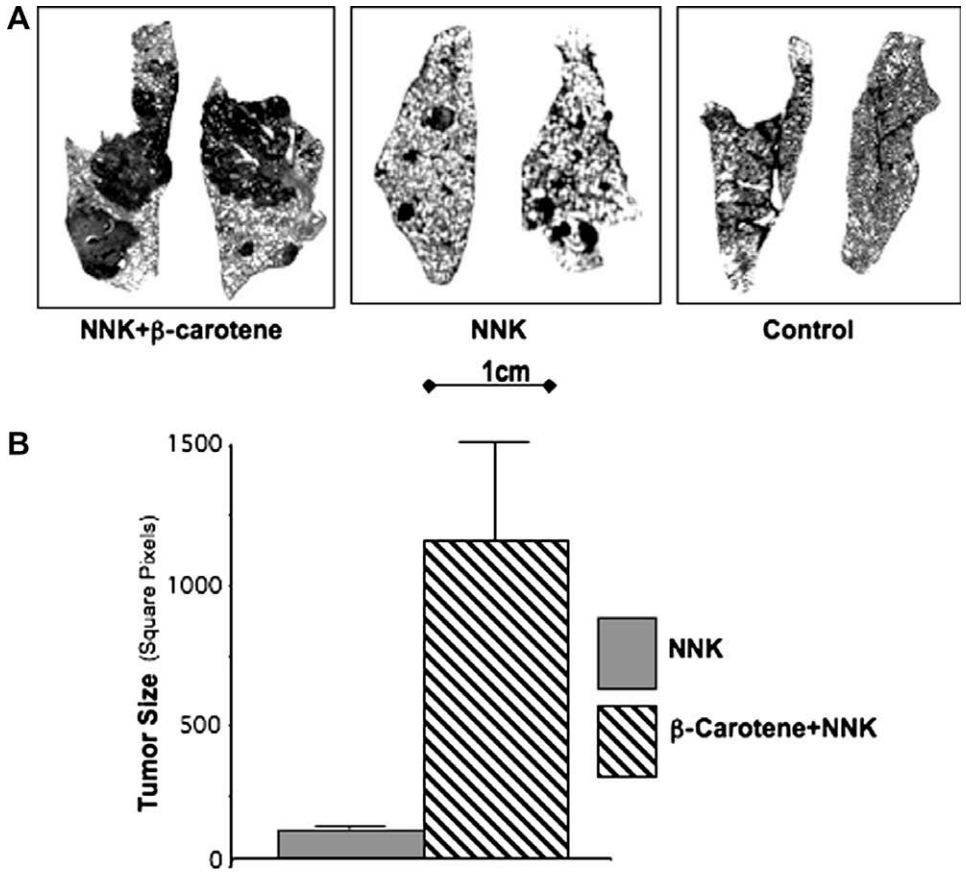

Fig. 3 – Modulation of NNK-induced lung tumour sizes by β-carotene. The graph in B shows the results of lung tumour area measurements. The lung tumours in animals treated with NNK+β-carotene were significantly ($p < 0.001$) larger than in the hamsters treated with NNK alone.

acrylamide gel, transferred onto nitrocellulose membranes in transfer buffer at 100 mV for 1 h, treated with blocking buffer (5% v/v non-fat dry milk in tris–buffered saline with Tween 20, TBST) for 1 h, and incubated with primary antibody overnight at 4 °C. The primary antibodies used were anti-total CREB (Upstate Biotechnology, Lake Placic, NY, USA), anti-p-CREB, anti-p-ERK1/2 and anti-ERK1/2 (Cell Signalling). After incubation with horseradish peroxidase-labelled secondary antibody (goat anti-mouse or goat anti-rabbit, Cell Signalling) for 1 h, immunoreactive bands were detected using a chemiluminescent reaction (ECL, Amersham Biosciences, Piscataway, NJ) via autoradiography on Kodak Bio-Max film. Three separate Western blots were conducted for each antibody per sample and yielded similar data. Relative densities of the bands were determined by image analysis using NIH SCION image analysis software. Mean values and standard errors from five densitometric readings per band were analysed by ANOVA and Tukey-Kramer multiple comparison test.

3. Results

The absorption of dietary β-carotene by the gastrointestinal tract of laboratory rodents is very poor, necessitating the use of a high-fat diet to yield significant levels of retinol and its metabolites that are formed from this provitamin in the lungs.[20,23] However, a high-fat diet can promote the development of PAC in people[24] and in laboratory rodents.[25] We therefore used subcutaneous injections of β-carotene in a carrier (sesame oil) that facilitates β-carotene absorption while neither promoting NNK-induced proliferation of small airway epithelial cells nor developing NNK-induced PAC.[20] We found that β-carotene induced increases of retinol, retinyl palmitate and retinoic acids in serum and lung tissue (Fig. 1A). All trans-retinoic acid (ATRA) and 9-cis-RA were the predominating metabolites in serum (9-Cis-RA: 3.45-fold, $p < 0.001$; ATRA: 3.5-fold, $p < 0.001$), whereas in lung tissue retinyl palmitate (RP) and ATRA predominated (RP: 1.5-fold, $p < 0.01$; ATRA: 4.4-fold, $p < 0.001$). These findings suggest that the observed modulation of NNK-induced carcinogenesis was predominantly caused by ATRA, 9-cis-RA and RP.

Analysis of cAMP in the cellular fraction of blood samples by immunoassay showed a 1.8-fold increase ($p < 0.001$) in the NNK-treated animals (Fig. 1B). The levels of blood cell cAMP were further increased (2.3-fold, $p < 0.001$) in the hamsters treated with β-carotene after the discontinuation of NNK treatments (Fig. 2). The observed increase of cAMP in the β-carotene-treated hamsters was significantly ($p < 0.001$) higher than in animals treated with NNK alone. These findings are suggestive of a generalised, systemic increase in cAMP-dependent signalling induced by NNK that was significantly enhanced further by β-carotene.

All the control hamsters survived until the end of the experiment (1.5 years), and none of them developed tumours. By contrast, none of the animals that are treated with NNK alone or with NNK+β-carotene survived beyond 12 months after the start of the NNK treatment with no significant difference in survival times among these two groups. All hamsters treated with NNK or NNK+β-carotene developed multiple airway-derived adenomas and adenocarcinomas, yielding a lung tumour incidence of 100% in both the treatment groups (Fig. 2A). In addition, the hamsters treated with NNK alone developed a low incidence (three of 20 hamsters) each of pancreatic ductal adenocarcinomas and olfactory adenocarcinomas of the nasal cavity, respectively (Fig. 2A). The incidence of the pancreatic neoplasms was slightly increased by β-carotene treatment (from 3 to 4 of 20 hamsters), whereas the incidence of olfactory adenocarcinomas almost quadrupled (from 3 to 11 of 20 hamsters).

The multiplicity of NNK-induced lung tumours (Fig. 2B) was significantly ($p < 0.001$) increased by β-carotene (NNK alone, mean number of tumours per animal: 6.9 ± 0.8; NNK+β-carotene, mean number of tumours per animal: 11.0 ± 0.7). Area measurements of tumour tissue by image analysis revealed that the lung tumours induced by NNK alone were significantly ($p < 0.001$) smaller (106 ± 12.3 square pixels; Figs. 3A and B and 4A), and most of them were diagnosed as adenomas due to their good demarcation from the surrounding normal tissue (Fig. 4A). By contrast, each of the hamsters treated with NNK+β-carotene had developed several very large adenocarcinomas that had replaced large portions of pulmonary lobes (Figs. 3A and B and 4B), yielding a 13.2-fold increase in mean lung tumour size (1401 ± 423 square pixels, $p < 0.001$). In addition, these animals had multiple small adenomas similar in size and appearance to those induced by NNK alone (Figs. 3A and B and 4B). Taken together,

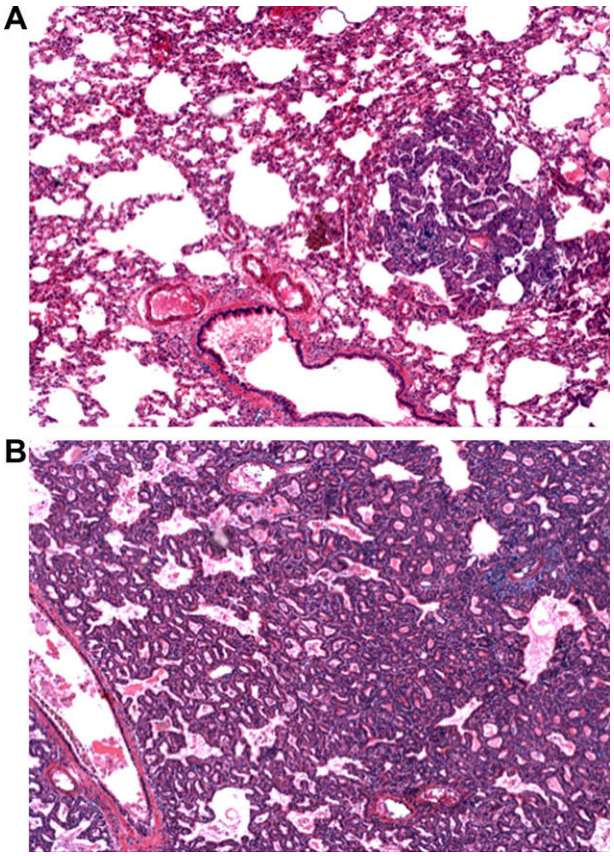

Fig. 4 – Histopathology of lung tumour induced in a hamster by NNK alone (A) or by NNK+β-carotene (B). Hematoxylin/eosin stain; X 40.

these data indicate that β-carotene not only promoted the development of NNK-induced lung tumours but also additionally accelerated their progression.

We have shown that NNK activates cAMP-dependent signalling via p-CREB and p-ERK1/2 in human PAC cells *in vitro*[12] and in NNK-induced PACs in hamsters *in vivo*.[10,16] We therefore investigated the expression levels of these two phosphorylated proteins in small airway epithelial cells from control hamsters in comparison to cells harvested from lung tumours of hamsters treated with NNK alone or with NNK+β-carotene. Our data show that p-ERK1/2 and p-CREB were significantly ($p < 0.001$) increased in lung tumour cells of hamsters treated with NNK alone (p-ERK1/2: 3.4-fold; p-CREB: 2.3-fold) over small airway epithelial cells of control hamsters (Fig. 5A and B). The expression levels of both these proteins were further enhanced significantly ($p < 0.001$) in tumours from animals given NNK+β-carotene (p-ERK1/2: 4.5-fold; p-CREB: 3.4-fold; Fig. 5A and B). These findings suggest that enhanced signalling via p-ERK and p-CREB contributed to the tumour-promoting effects of β-carotene on NNK-induced lung carcinogenesis.

4. Discussion

The classic action of retinoids is thought to involve the activation of nuclear retinoid receptors.[26] Our data show, for the first time, that β-carotene significantly promoted and accelerated the development of small airway-derived PAC induced by NNK *in vivo* by mechanisms that involved non-classical cellular signalling via p-ERK1/2 and p-CREB. The observed fourfold increase in the incidence of nasal cavity tumours may have been caused by similar mechanisms, even though regulatory signal transduction in this type of cancer has not been studied to date. Having harvested serum and lung tissues for HPLC analysis of retinoids 2 h after the last β-carotene injection, we were able to identify ATRA, 9-cis-RA and RP as the predomi-

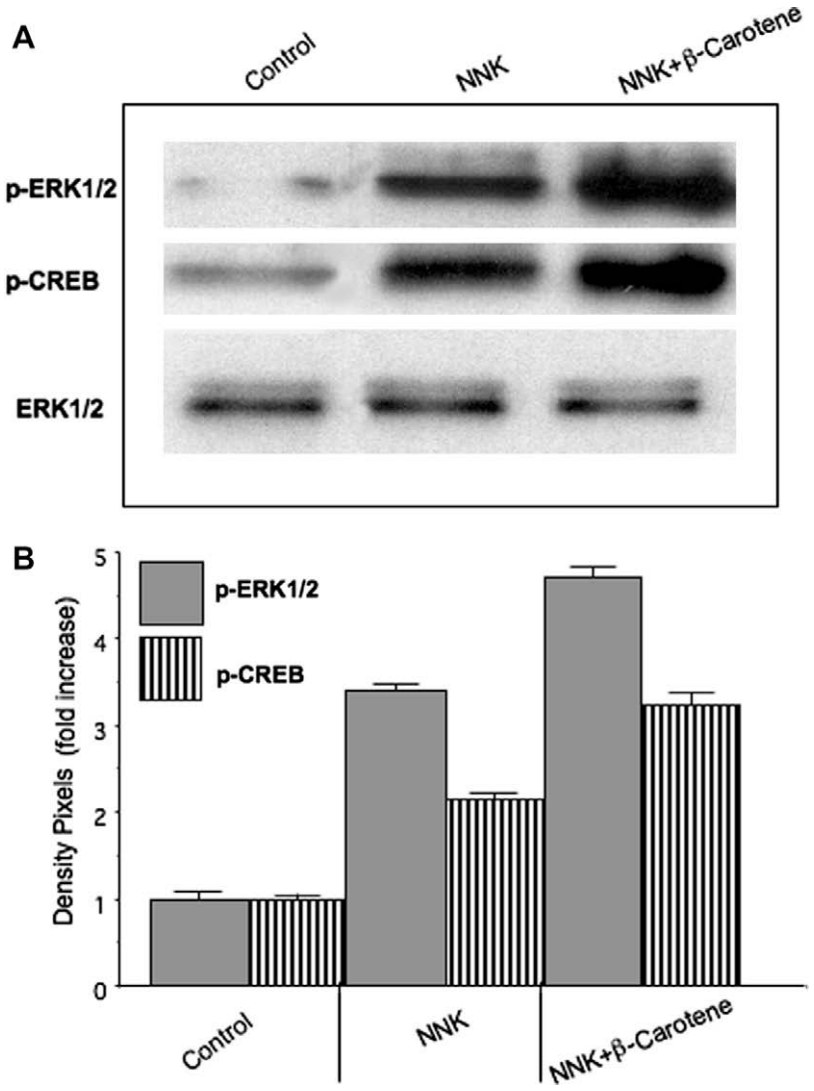

Fig. 5 – Western blots (A) and densitometry values (B) showing levels of p-ERK1/2 and p-CREB in small airway epithelial cells and lung tumour cells. Protein induction by NNK ($p < 0.001$) was significantly ($p < 0.001$) increased by β-carotene. Bars in the graph are mean values and standard errors of five densitometric measurements per band expressed as ratio of p-ERK1/2 or p-CREB over ERK1/2.

nating retinol metabolites in serum and lungs. These findings are in accord with the reports that have described non-genomic signalling induced by β-carotene, retinol or its metabolites in human PAC cells of small airway epithelial phenotype, in immortalised normal human small airway epithelial cells and in human bronchial epithelial cells.[18,19,27] While signalling via cAMP PKA CREB and PKA-dependent transactivation of ERK1/2 downstream of the EGFR stimulated the proliferation of small airway-derived PAC cells and its normal cells of origin, the same signalling cascade inhibited the proliferation of bronchial epithelial cells or small cell lung cancer cells.[18,19] In conjunction with our current findings, these data suggest that the non-classical signalling cascade stimulated by β-carotene and its metabolites modulates the regulation of lung cells with different outcomes for different types of cells. Small airway epithelia and the PACs derived from them are stimulated in their growth, whereas large airway epithelia and SCLC derived from them are inhibited. Since PAC was the predominating lung cancer type in the CARET trial,[28] the stimulation of non-genomic signalling in the current study may therefore justify the hypothesis that increased cAMP signalling may have contributed to the unfortunate outcome of this and similar clinical trials.

Immunoassays showed a significant increase in the intracellular cAMP in blood cells from hamsters treated with NNK that was further increased by β-carotene treatment. These findings suggest that hyperactive cAMP signalling in response to NNK and β-carotene is a systemic event that can be detected by a simple blood test. This interpretation is in accord with the reported increase in lung cancer deaths in the CARET trial.[4] In analogy to the assessment of cardiovascular function by analysis of cAMP in blood cells,[22] it may hence be possible to identify individuals with elevated systemic cAMP, who would respond with lung cancer promotion to β-carotene or retinoid treatment. On the other hand, individuals with below normal cAMP levels in blood cells may benefit from the selective cancer-preventive effects of these agents.

It has been shown that vitamin A deficiency alone or in combination with benzo (a)-pyrene caused squamous metaplasia, a precursor of squamous cell carcinoma, in the epithelium of the trachea and stem bronchi in hamsters *in vivo* and in organ culture.[17,29,30] Treatments with β-carotene, retinol or other retinoids reversed or reduced this response.[30,31] The hamster trachea and stem bronchi are coated by a pseudostratified respiratory epithelium found in large airways (trachea, stem bronchi, lobar bronchi and segmental bronchi) of humans,[32] and comprised basal cells, mucous cells and ciliated cells. In accord with the cited data in hamsters, the recent studies with immortalised human large airway epithelial cells have shown a significant retinoid-induced inhibition of cell proliferation involving a cAMP-dependent inhibition of ERK1/2 phosphorylation.[19] Dietary β-carotene administered to A/J mice enhanced bronchial epithelial cell proliferation in NNK-treated animals, but did not promote the NNK-induced PACs.[20] The mouse lung is a model for the human lung periphery, with all intrapulmonary airways being coated by the simple respiratory epithelium comprising non-ciliated Clara cells and sparse ciliated cells that are restricted to small airways (bronchioles) in humans.[32] The stimulation of bronchial epithelial cell proliferation in mice is thus equivalent to the stimulation of human small airway epithelial cells in vitro by β-carotene and retinoids.[18,19] However, spontaneous and NNK-induced lung tumours in the mouse are derived from alveolar type II cells,[33] and not from small airway epithelia as most human PACs[5] or the PACs induced in the hamster by NNK.[7] In turn, human PAC cell lines with features of alveolar type II cells are not stimulated in their growth by agents that increase intracellular cAMP.[34] The observed β-carotene induced proliferation of bronchial epithelial cells in mice, therefore did not promote the development of PAC.

Collectively, our data in NNK-induced small airway-derived PAC and the *in vitro* responses of human PAC cells of this phenotype[18,19] suggest that β-carotene and retinoids promote the development of this PAC type.

Conflict of interest statement

None declared.

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REFERENCES

1. Ezzati M, Henley SJ, Lopez AD, Thun MJ. Role of smoking in global and regional cancer epidemiology: current patterns and data needs. *Int J Cancer* 2005;**116**:963–71.
2. Sporn MB, Suh N. Chemoprevention: an essential approach to controlling cancer. *Nat Rev Cancer* 2002;**2**:537–43.
3. Group TA-TB-CCPS. The effects of vitamin E and beta-carotene on the incidence of lung cancer and other organs in male smokers. *New Engl J Med* 1994;**330**:1029–35.
4. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New Engl J Med* 1996;**334**:1150–5.
5. Garber ME, Troyanskaya OG, Schluens K, et al. Diversity of gene expression in adenocarcinoma of the lung. *Proc Natl Acad Sci USA* 2001;**98**:13784–9.
6. Plopper CG, Hill LH, Mariassy AT. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung. III. A study of man with comparison of 15 mammalian species. *Exp Lung Res* 1980;**1**:171–80.
7. Schuller HM, Witschi HP, Nylen E, et al. Pathobiology of lung tumors induced in hamsters by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the modulating effect of hyperoxia. *Cancer Res* 1990;**50**:1960–5.
8. Mitsudomi T, Viallet J, Mulshine JL, et al. Mutations of ras genes distinguish a subset of non-small-cell lung cancer cell lines from small-cell lung cancer cell lines. *Oncogene* 1991;**6**:1353–62.
9. Oreffo VI, Lin HW, Padmanabhan R, Witschi H. K-ras and p53 point mutations in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced hamster lung tumors. *Carcinogenesis* 1993;**14**:451–5.
10. Schuller HM, Cekanova M. NNK-induced hamster lung adenocarcinomas over-express beta2-adrenergic and EGFR signaling pathways. *Lung Cancer* 2005;**49**:35–45.

11. Schuller HM, Tithof PK, Williams M, Plummer 3rd H. The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a beta-adrenergic agonist and stimulates DNA synthesis in lung adenocarcinoma via beta-adrenergic receptor-mediated release of arachidonic acid. *Cancer Res* 1999;**59**:4510–5.
12. Laag E, Majidi M, Cekanova M, et al. NNK activates ERK1/2 and CREB/ATF-1 via beta-1-AR and EGFR signaling in human lung adenocarcinoma and small airway epithelial cells. *Int J Cancer* 2006;**119**:1547–52.
13. Maudsley S, Pierce KL, Zamah AM, et al. The beta(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. *J Biol Chem* 2000;**275**:9572–80.
14. Schuller HM, Porter B, Riechert A. Beta-adrenergic modulation of NNK-induced lung carcinogenesis in hamsters. *J Cancer Res Clin Oncol* 2000;**126**:624–30.
15. Schuller HM, Porter B, Riechert A, Walker K, Schmoyer R. Neuroendocrine lung carcinogenesis in hamsters is inhibited by green tea or theophylline while the development of adenocarcinomas is promoted: implications for chemoprevention in smokers. *Lung Cancer* 2004;**45**:11–8.
16. Cekanova M, Majidy M, Masi T, Al-Wadei HA, Schuller HM. Overexpressed Raf-1 and phosphorylated cyclic adenosine 3′-5′-monophosphatate response element-binding protein are early markers for lung adenocarcinoma. *Cancer* 2007;**109**:1164–73.
17. Harris CC, Sporn MB, Kaufman DG, et al. Histogenesis of squamous metaplasia in the hamster tracheal epithelium caused by vitamin A deficiency or benzo[*a*]pyrene-ferric oxide. *J Natl Cancer Inst* 1972;**48**:743–61.
18. Al-Wadei HA, Takahashi T, Schuller HM. Growth stimulation of human pulmonary adenocarcinoma cells and small airway epithelial cells by beta-carotene via activation of cAMP, PKA, CREB and ERK1/2. *Int J Cancer* 2006;**118**:1370–80.
19. Al-Wadei HA, Schuller HM. Cyclic adenosine monophosphate-dependent cell type-specific modulation of mitogenic signaling by retinoids in normal and neoplastic lung cells. *Cancer Detect Prev* 2006;**30**:403–11.
20. Goralczyk R, Wertz K, Lenz B, et al. Beta-carotene interaction with NNK in the AJ-mouse model: effects on cell proliferation, tumor formation and retinoic acid responsive genes. *Biochim Biophys Acta* 2005;**1740**:179–88.
21. Nikitin AY, Alcaraz A, Anver MR, et al. Classification of proliferative pulmonary lesions of the mouse: recommendations of the mouse models of human cancers consortium. *Cancer Res* 2004;**64**:2307–16.
22. Dizimiri N, Basco C, Moorji A, Afrane B, Al-Halees Z. Characterization of lymphocyte beta-2-adrenoreceptor signaling in patients with left ventricular volume overload disease. *Clin Exp Pharmacol Physiol* 2002;**29**:181–8.
23. Wang XD, Liu C, Bronson RT, et al. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J Natl Cancer Inst* 1999;**91**:60–6.
24. Alavanja MC, Brown CC, Swanson C, Brownson RC. Saturated fat intake and lung cancer risk among nonsmoking women in Missouri. *J Natl Cancer Inst* 1993;**85**:1906–16.
25. El-Bayoumy K, Iatropoulos M, Amin S, Hoffmann D, Wynder EL. Increased expression of cyclooxygenase-2 in rat lung tumors induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanone: the impact of a high-fat diet. *Cancer Res* 1999;**59**:1400–3.
26. Davidovici BB, Tuzun Y, Wolf R. Retinoid receptors. *Dermatol Clin* 2007;**25**:525–530, viii.
27. Aggarwal S, Kim SW, Cheon K, et al. Nonclassical action of retinoic acid on the activation of the cAMP response element-binding protein in normal human bronchial epithelial cells. *Mol Biol Cell* 2006;**17**:566–75.
28. Brodkin CA, McCullough J, Stover B, et al. Lobe of origin and histologic type of lung cancer associated with asbestos exposure in the Carotene and Retinol Efficacy Trial (CARET). *Am J Ind Med* 1997;**32**:582–91.
29. McDowell EM, DeSanti AM, Newkirk C, Strum JM. Effects of vitamin A-deficiency and inflammation on the conducting airway epithelium of Syrian golden hamsters. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1990;**59**:231–42.
30. Chopra DP. Retinoid reversal of squamous metaplasia in organ cultures of tracheas derived from hamsters fed on vitamin A-deficient diet. *Eur J Cancer Clin Oncol* 1983;**19**: 847–57.
31. Clamon GH, Sporn MB, Smith JM, Saffiotti U. Alpha- and beta-retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture. *Nature* 1974;**250**:64–6.
32. Reznik-Schuller H, Reznik G. Experimental pulmonary carcinogenesis. *Int Rev Exp Pathol* 1979;**20**:211–81.
33. Belinsky SA, Devereux TR, Foley JF, Maronpot RR, Anderson MW. Role of the alveolar type II cell in the development and progression of pulmonary tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the A/J mouse. *Cancer Res* 1992;**52**:3164–73.
34. Adissu HA, Schuller HM. Antagonistic growth regulation of cell lines derived from human lung adenocarcinomas of Clara cell and aveolar type II cell lineage: implications for chemoprevention. *Int J Oncol* 2004;**24**:1467–72.