ELSEVIER

European Journal of Cancer 41 (2005) 2745–2753

European Journal of Cancer

www.ejconline.com

PKA-dependent growth stimulation of cells derived from human pulmonary adenocarcinoma and small airway epithelium by dexamethasone

H.A.N. Al-Wadei [a], T. Takahasi [b], H.M. Schuller [c,*]

[a] *Experimental Oncology Laboratory, College of Veterinary Medicine, University of Tennessee, Knoxville, TH, USA*
[b] *Department of Molecular Carcinogenesis, Center for Neurological Diseases, Nagoya University, Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA*

Received 8 April 2005; received in revised form 15 August 2005; accepted 2 September 2005
Available online 18 October 2005

Abstract

Smoking is a risk factor for lung cancer, chronic obstructive pulmonary disease, chronic bronchitis and asthma. The chronic lung diseases are also a predisposing factor for the development of lung cancer. Glucocorticoids are used for the management of chronic lung diseases because of their anti-inflammatory activity. These drugs also have anti-tumourigenic effects in mouse models of lung cancer. Glucocorticoids are frequently used as co-treatment with cancer therapy. Using the human pulmonary adenocarcinoma (PAC) cell line NCI-H322 with features of bronchiolar Clara cells, and immortalised human small airway epithelial cells, our data show that the glucocorticoid dexamethasone increased cell proliferation in MTT assays in a PKA-dependent manner. Dexamethasone significantly increased intracellular cAMP in direct immunoassays. Immunoblot analysis revealed increased phosphorylation of ERK1/2 and of the transcription factor CREB in response to dexamethasone. These data suggest that glucocorticoids could have tumour promoting activity on a sub-set of human PAC.

Keywords: Lung adenocarcinoma; Dexamethasone; cAMP; PKA; CREB; ERK1/2; Cell proliferation

1. Introduction

Lung cancer ranks second as a cause of death after cardiovascular disease and the death rate for lung cancer exceeds the combined total for cancer of the breast, prostate and colon in developed countries [1]. Among the four major histological lung cancer types (adenocarcinoma, small-cell carcinoma, squamous cell carcinoma, large-cell carcinoma), peripheral adenocarcinoma (PAC) has increased dramatically over the last 20 years and is the leading type of lung cancer today in both male and female smokers and non-smokers [2–4].

Smoking is a documented risk factor for the development of all histological lung cancer types and is believed to account for 70–90% of all lung cancer cases [5,6]. Pre-existing non-neoplastic pulmonary diseases such as chronic obstructive pulmonary disease (COPD), bronchitis and asthma additionally increase the risk for the development of lung cancer in smokers and non-smokers [7–10]. However, few reports have addressed the effects of individual members of this chronic disease family on defined histological lung cancer types [11–13]. To our knowledge, the potential effects of therapeutics used for the long-term management of chronic non-neoplastic pulmonary disease on the development of lung cancer have also not been investigated to date.

Glucocorticoids are widely used for the long-term management of COPD, chronic bronchitis and asthma

* Corresponding author. Tel.: +1 865 974 8217; fax: +1 865 974 5616.
E-mail address: hmsch@utk.edu (H.M. Schuller).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.09.001

because of their anti-inflammatory actions and documented synergy with β_2-adrenergic receptor agonist bronchodilators [14,15]. They are also frequently prescribed to minimise the side effects of cancer chemotherapy and radiation therapy [16]. A significant population of lung cancer patients is therefore exposed to glucocorticoids either before the diagnosis of lung cancer or during cancer therapy. Studies in mouse models of lung cancer have shown significant cancer preventive effects of glucocorticoids [17], leading to recent strategies to propose this family of drugs for lung cancer prevention in smokers and ex-smokers. In addition, glucocorticoids have been shown to inhibit cell proliferation in a number of non-pulmonary cancers and embryonic fibroblasts, an effect involving the cyclin-dependent kinase inhibitors p21 and p27 [18,19].

Studies conducted in our laboratory have identified a novel β-adrenergic receptor-initiated growth-stimulating pathway in cell lines derived from human PAC of Clara cell phenotype [20]; whereas the same signalling pathway was growth-inhibiting in cell lines derived from human PAC with phenotypic features of alveolar type II cells [21]. Moreover, we have identified the tobacco-specific carcinogenic nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) as a high affinity agonist for β_1- and β_2-adrenergic receptors in Chinese hamster ovary (CHO) cells stably overexpressing the human β_1- or β_2-adrenergic receptor [20]. In cell lines derived from human PAC of Clara cell phenotype, NNK stimulated the release of arachidonic acid (AA) and DNA synthesis via binding to β_1- and β_2-adrenergic receptors [20], an observation in line with the documented overexpression of AA-metabolising enzymes in human PAC [22]. In support of these in vitro observations, bioassay experiments in a hamster model of NNK-induced PAC derived from bronchiolar Clara cells have documented strong tumour promoting effects of the β-adrenergic agonist epinephrine while the β-adrenergic antagonist propanolol inhibited tumour development [23]. More recently, we have also shown that theophylline, which increases the levels of the β-adrenergic receptor second messenger cAMP via inhibition of phosphodiesterase, significantly promoted the development of NNK- induced PAC in hamsters [24].

Because of the documented synergy of glucocorticoids with β-adrenergic agonists in the treatment of non-neoplastic pulmonary disease [15], our current study has tested the hypothesis that glucocorticoids may stimulate the β-adrenergic growth-regulating pathway in human PAC of Clara cell phenotype and in their putative cells of origin, small airway epithelial cells (SAECs). In support of this hypothesis, our data reported in this study, from NCI-H322 cells derived from a human PAC of Clara cell phenotype and in immortalised human small airway epithelial cells expressing the Clara cell specific CC10 antigen, demonstrates that dexamethasone caused PKA-dependent stimulation of cell proliferation. It was also accompanied by a significant increase in intracellular cAMP and activity of the cAMP-dependent protein kinase A (PKA), the transcription factor cAMP response element (CREB) and of the mitogen-activated protein kinases ERK1/2.

2. Materials and Methods

2.1. Cell lines and tissue culture

The human PAC cell line with characteristics of Clara cells, NCI-H322 (Center for Applied Microbiology and Research, ECACC, Salisbury, Wiltshire, UK) was maintained in RPMI-1640 medium, containing 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine, 4500 mg/l glucose, 1500 mg/l sodium bicarbonate and supplemented with 10% fetal bovine serum (American Type Culture Collection, Manassas, VA). The Simian virus 40 (SV40)-immortalised human peripheral airway cell line HPL1D was established and characterised by Dr. Takahashi's laboratory [25] and is referred to in this publication as small airway epithelial cells (SAECs). This cell line was maintained in F-12 nutrient mixture (HAM) with L-glutamine (Gibco) medium, buffered with 15 mM HEPES (Ph 7.3) and supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 10^{-7} M hydrocortisone, 2×10^{-10} M triiodothyronine (Cambrex, Walkersville, MD) and 1% fetal calf serum (ATCC).

2.2. Assessment of cell numbers by MTT assay

The effects of dexamethasone (Sigma, St. Louis, MO, USA) on cell proliferation were assessed by the colorimetric 3-(4,5-dimethyle thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (**2**) (Sigma). Briefly, the MTT test is based on the NADH-dependent enzymatic reduction of the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide] in metabolically active cells but not in dead cells. Cells were seeded into 6-well tissue culture plates (Falcon, Franklin Lakes, NJ, USA) at a density of 50 000 cells per well for NCI-H322, and 50 000 cells per well for SAEC cells. The cells were left to grow in complete media at 37 °C with 5% CO_2 for 5 h to attach. The cells were then switched to fresh low serum media and dexamethasone was added at the concentrations specified in the figure legend and incubated for 24, 48, or 72 h. Fifty microliters of 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (0.5 mg/ml) was dissolved in phenol-free RPMI-1640 medium (Gibco) and then added to the cells for 2–3 h to allow metabolic conversion of the MTT substrate to blue formazan. After 2–3 h, the media were replaced with isopropanol, and optical density at 570 nm was determined using an ELISA reader. Data are expressed as mean values and standard errors of four