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Cancer Letters 246 (2007) 24–33
CANCER Letters
www.elsevier.com/locate/canlet

Smoking is associated with increased telomerase activity in short-term cultures of human bronchial epithelial cells

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Received 23 November 2005; received in revised form 17 January 2006; accepted 24 January 2006

Abstract

Telomerase plays an important role in the maintenance of telomere ends in normal and tumor cells and ectopic expression can immortalize human bronchial epithelial (HBE) cells. We assessed telomerase activation, growth properties and methylation status in the *hTERT* promoter in a panel of HBE cell cultures in relation to smoking and previous lung cancer history. HBE cells were obtained from a total of 26 subjects, six of whom were lifelong non-smokers, while 20 subjects had a smoking history, including seven who had lung carcinoma. Telomerase activity was determined using the telomeric repeat amplification protocol (TRAP). Maximum passage number and time to senescence were also determined through extended culturing. The distribution of the telomerase activity between ever-smokers and never-smokers was significantly different ($P=0.03$, F-test), and there was a strong correlation between telomerase activity and the number of pack-years smoked ($P=0.0012$, F-test for slope). A small difference in telomerase activity was observed according to lung cancer status ($P=0.02$, F-test). Telomerase activity was not correlated with maximum passage number after extended culturing or with time to senescence. None of the HBE cultures demonstrated methylation of the *hTERT* promoter. Our results indicate an association between tobacco carcinogen exposure and telomerase activity in normal bronchial epithelium, although a causative role of tobacco smoking in the (re)activation of telomerase can not be proven. An increase in telomerase activity in normal bronchial epithelium might extend the lifespan of cells at risk for malignant transformation, and thus contribute to lung carcinogenesis.

Keywords: Telomerase activity; Bronchial epithelial cells; Smoking

1. Introduction

The majority of human lung cancers develop from the stepwise accumulation of genetic and epigenetic alterations in bronchial epithelial cells in response to the carcinogenic effects of cigarette smoking. DNA

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doi:10.1016/j.canlet.2006.01.023

adducts and abnormalities of p16, p53, and K-ras have been described in lung cancers from chronic smokers and to a lesser extent in those from non-smokers [1–2]. K-ras activation, p16 promoter hypermethylation and p53 mutation can occur in chronic smokers before any clinical evidence of disease [3–4]. Normal human somatic cells have a finite replicative lifespan due to shortening of telomeres during normal replication. The acquisition of unlimited replicative properties is a critical step in the development of cancerous cells. The requirements for immortalization are different for cells from different tissues of origin [5]. Telomerase activation provides an unlimited replicative potential in some cells [6,7], while inactivation of the p16 tumor suppressor gene in combination with newly acquired telomerase activity can immortalize epithelial cells efficiently [8]. Because the primary cause of lung cancer is exposure to carcinogens in tobacco smoke, we hypothesized that there may be a difference in telomerase activity between bronchial epithelial cells from smokers and non-smokers. Dysregulation of telomerase occurs early in the multistage pathogenesis of bronchogenic lung carcinomas [9,10], but the extent of telomerase (re)activation by smoking is not known.

To study the relationship between telomerase activity and smoking, we determined the telomerase activity in primary human bronchial epithelial (HBE) cell cultures during serial passages using a telomeric repeat amplification protocol (TRAP) assay. We adapted this assay to increase the sensitivity so we could detect the low levels of telomerase activity in normal HBE cells. We analyzed telomerase activity in lifetime non-smoking patients, in smoking patients and in patients who had surgery for lung cancer, most of whom had a history of smoking. In addition, we investigated the methylation status of *hTERT* gene to investigate the relationship between telomerase activation and promoter region hypermethylation.

2. Materials and methods

2.1. Patients and sample collection

For this study, a total of 26 HBE cultures from 26 subjects were included, eight of the HBE cultures were obtained during surgery for lung carcinoma and 18 were obtained from excess lung transplant donor tissue. Collection and procedures for HBE cell cultures have been described in detail elsewhere [11]. Twenty-three of the 26 HBE cultures reported here were also included in a prior study on chromosomal abnormalities, while three additional HBE cultures were used in the present study. All procedures were approved by the Institutional Review Boards of the participating institutions.

Six patients were lifelong non-smokers while 20 patients had some history of smoking (Table 1), including seven patients with a diagnosis of lung cancer (one patient undergoing lung cancer surgery had a benign tumor as a final pathologic diagnosis). The control cell lines BEAS-2B, HT-29, RKO and Calu-1 were directly obtained from the American Type Culture Collection (Manassas, VA).

2.2. Culture methods and determination of time to senescence

HBE cells were cultured on culture dishes coated with rat-tail collagen type I (Invitrogen, Carlsbad, CA). The primary

Table 1
Characteristics of human bronchial epithelial (HBE) cells and subjects

Variables	Never-smoker ($N=6$)	Ever-smoker no lung cancer ($N=13$)	Lung cancer ($N=7$)	Total ($N=26$)
Age[a]	34.7±23.3	50.6±16.5	65.7±7.5	50.9±19.3
Sex				
Male	4	7	3	14
Female	2	6	4	12
Tissue source				
Transplant	6	12	0	18
Lung surgery	0	1[b]	7	8
Initial culture status				
Fresh	2	3	4	9
Frozen	4	10	3	17
Smoking[c]	N/A[d]	22.5±16.7	50.0±28.7	25.0±26.7

[a] Average age with standard deviation. The groups have significantly different ages ($P=0.004$, Kruskal–Wallis test), non-smokers are younger than lung cancer patients ($P=0.02$, Wilcoxcon–Mann–Whitney test); ever-smokers are younger than lung cancer patients ($P=0.006$, Wilcoxcon–Mann–Whitney test); non-smokers are not significantly younger than ever-smokers ($P=0.19$, Wilcoxcon-Mann-Whitney test).
[b] Final pathology demonstrated a benign tumor in one surgical patient.
[c] Average number of pack-years with standard deviation. Ever-smokers versus lung cancer patients ($P=0.04$, Wilcoxcon–Mann–Whitney test).
[d] N/A, not applicable.