CELLULAR AND MOLECULAR STUDIES OF GROWTH, DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION OF HUMAN BRONCHIAL EPITHELIAL CELLS IN VITRO

Curtis C. Harris, Roger R. Reddel, Yang Ke, Andrea Pfeiffer, George Mark, Tohru Masui, George Yoakum, Brenda I. Gerwin, Paul Amstad and John F. Lechner

Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, Bethesda, Maryland 20892

INTRODUCTION

Normal human cells in vitro appear to retain many normal phenotypic properties, remain diploid, eventually undergo senescence and rarely, if ever "spontaneously" transform to malignant cells. Retained properties may include synthesis of classes of proteins associated with specific cell types such as collagens, keratins, or melanin; responsiveness to hormones; and antigenic specificity. In addition, human cells with abnormal phenotypes such as either enzymatic deficits or malignant properties frequently maintain these phenotype in vitro. Human cells cultured in vitro have thus proven to be extremely useful to scientists studying the molecular and biochemical aspects of human carcinogenesis. Such studies have been facilitated by the recent development of improved methods for culturing normal human epithelial tissues and cells. Chemically defined media have been developed for culturing many of these tissues and cells from normal organs, including those with a high rate of cancer in humans. Serum-free media have several advantages in studies of cultured human cells, including: (a) less experimental variability compared to serum-containing media; (b) selective growth conditions for normal cells of different types (e.g. epithelial versus fibroblastic) or for normal versus malignant cells; (c) ease of identification of growth factors, inhibitors of growth, and inducers of differentiation; and (d) ease of isolating and analyzing secreted cellular products. Advances in cell biology, including the delineation of biochemical and morphological markers of specific cell types, have also facilitated the identification of cells in vitro (including keratins as markers for epithelial cells and collagen types I and III for identifying fibroblasts). These advances have created experimental approaches to answering critical questions in human cell carcinogenesis.

This brief review will describe our recent studies concerning the molecular mechanisms controlling growth and squamous differentiation of normal human bronchial epithelial (NHBE) cells and the dysregulation of these controls during the multistage process of carcinogenesis.

GROWTH AND DIFFERENTIATION

The balance between growth and terminal differentiation is strictly
controlled in normal bronchial epithelial cells. Furthermore, carcinogenesis studies using murine epidermal cells suggest that defects in differentiation occur during tumor initiation and that selective clonal expansion of these initiated cells occurs during tumor promotion. Studies using human bronchial epithelial cells are producing results supporting this hypothetical sequence of aberrations in control of growth and differentiation.

The concept of autocrine production of growth factors has been proposed to explain the uncontrolled growth of some neoplastic cells. "Ectopic" hormones produced by carcinomas are candidates for autocrine growth factors. For example, gastrin-releasing peptide (the mammalian equivalent of bombesin) is secreted by most small cell carcinomas of the lung, and intracellular human chorionic gonadotropin is detected in many non-small cell carcinomas of the lung. A monoclonal antibody to bombesin blocks the binding of the hormone to cellular receptors and inhibits clonal growth of small cell carcinomas in vitro and their growth as xenografts in vivo. Both of these hormones enhance the growth of normal bronchial epithelial cells in vitro by binding to specific membrane receptors. A second example is that 12-0-tetradecanoylphorbol-13-acetate (TPA) inhibits the growth of normal human colonic epithelial cells and is mitogenic in cultures of epithelial cells from adenomatous polyps. Therefore, an imbalance between the pathways of growth and differentiation could provide a selective clonal expansion advantage for preneoplastic and neoplastic human cells, in the presence of an agent to which normal human epithelial cells respond by terminally differentiating.

Over the past few years, there has been considerable interest in the role that aberrant differentiation plays in carcinogenesis. It has been shown previously that NHBE cells, but not lung carcinoma cells, can be induced to undergo squamous differentiation by exposing them to serum, TPA or transforming growth factor beta (TGF-β). Because of their short culture passage life span (4-5 subculturings) it is often difficult to do repeated experiments with the same culture of normal cells. To overcome this problem we recently transformed NHBE cells by infection with adenovirus 12-SV40 (Ad12-SV40) SV40 virus, or by transfection of normal cells with a plasmid containing SV40 large T antigen gene. Ten different cultures of bronchial epithelial cells thus transformed have been studied for their ability to undergo squamous differentiation when exposed to TPA, TGF-β or serum (Ke et al., unpublished results) All ten T-antigen positive cell cultures were significantly inhibited and differentiated when exposed to serum or TGF-β. However, none differentiated when exposed to TPA. From one such cell line, two subclones have been isolated one of which is induced by serum to stop dividing and to differentiate, and a second that not only fails to undergo squamous differentiation but is mitogenically stimulated when exposed to serum. These phenotypically very different subclones provide a new in vitro model for delineating the mechanism(s) of human bronchial epithelial cell squamous differentiation.

NEOPLASTIC TRANSFORMATION

Human cells have been transformed to malignant cells by oncogenic viruses or transfected genetic elements of oncogenic DNA and RNA viruses. For example, non-tumorigenic human skin keratinocyte cell lines became malignant if transfected with Ha- or Ki-ras oncogene or if treated with 4-nitroquinoline-1-oxide and EBV "immortalized" human lymphocytes became malignant if treated with N-acetyoxy-2-acetylaminofluorene. In these cases, the transformed cells were apparently immortal, and aneuploid, and produced progressively growing carcinomas in the athymic nude mouse assay. In the case of Ha-ras transfected bronchial and epidermal epithelial cells, the malignant epithelial cells continued to synthesize keratin.