A QUANTITATIVE, CLONAL ASSAY FOR CARCINOGEN-INDUCED ALTERATIONS OF RESPIRATORY EPITHELIAL CELLS IN CULTURE

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INTRODUCTION

The identification of environmental carcinogens and the assessment of the potential risks of these substances to humans require experimental systems to measure quantitatively the activity of carcinogens and promoters. Cell culture systems are potentially very useful experimental models for such studies. Short-term, inexpensive cell culture assays for carcinogens are available. These assays have a high predictive ability for the detection of known carcinogens with few false positive results (Barrett et al., 1980). The end point measured, preneoplastic or neoplastic transformation of the test cells, is relevant to the carcinogenic process and is not predicated on a theoretical correlation. These systems can also be used for mechanistic studies on the cellular and molecular basis of neoplastic development. The results with cells and tissues of different species (including human) can be compared and contrasted under similar experimental conditions.

The most relevant cell culture systems employ cells that are from the target tissues for environmental carcinogens, i.e., epithelial cells. Unfortunately, most quantitative cell transformation assays use fibroblasts, because methods for growing these cells in vitro have been available for a number of years (Barrett et al., 1980); however, malignant tumors of nonepithelial
cells account for only 10 to 20% of human malignant neoplasms. Recently, methods for growing epithelial cells in culture have been developed and a number of laboratories have demonstrated induction of neoplastic transformation of these cells in culture by various chemical carcinogens (Franks and Wigley, 1979; Harris, 1982). However, quantitation of the frequency of early, carcinogen-induced changes on a per-cell basis has not been achieved with epithelial cells. In this report we describe our recent results on the growth of rat tracheal epithelial (RTE) cells in culture and the use of these cells for quantitative assays of carcinogen-induced cytotoxicity and preneoplastic transformation.

Advantages of Carcinogenesis Studies with Rat Tracheal Epithelial Cells

Respiratory epithelium is an important target for environmental carcinogens. Lung cancer in men accounts for 34% of the cancer deaths in the U.S., and the incidence of this disease is clearly related to exposure to a number of environmental factors (American Cancer Society, 1980; Doll, 1978). Because of the need to understand factors that influence the etiology and pathogenesis of this prevalent human disease, model systems have been developed using tracheal epithelium as the target tissue. The development of both in vivo and in vitro models for carcinogenesis studies with tracheal epithelium (see below), combined with the environmental importance of this tissue, gives this system a unique advantage over other epithelial systems.

A number of experimental models in laboratory animals have been developed for studying carcinogenesis of respiratory epithelium (for review see Nettesheim and Griesemer, 1978). The best characterized models are the intratracheal instillation technique of Saffiotti et al. (1968) using the Syrian hamster, and the heterotopic tracheal graft model using Fisher strain 344 rats, originally described by Kendrick et al. (1974). These models have been characterized with respect to their target tissue's capacity to metabolize and activate polynuclear aromatic hydrocarbons to ultimate carcinogenic forms (Mass and Kaufman, 1982).

Nettesheim and co-workers, using the tracheal transplant model, have studied the influence of host and environmental factors on experimental respiratory carcinogenesis and the induction, characteristics, and sequential histological changes of carcinogen-induced preneoplastic lesions following controlled exposure of the tracheas to carcinogens and cocarcinogens