SERUM-FREE CULTURE CONDITIONS FOR THE GROWTH OF NORMAL RAT MAMMARY EPITHELIAL CELLS IN PRIMARY CULTURE

STEPHEN P. ETHIER

Department of Chemical Carcinogenesis, The Michigan Cancer Foundation, 110 E. Warren Avenue, Detroit, MI 48201

(Received 15 November 1985; accepted 14 March 1986)

SUMMARY

A monolayer culture system has recently been developed for the extended growth and serial passage of normal rat mammary epithelial (RME) cells. In this system the cells undergo greater than 20 population doublings when grown on type I collagen-coated tissue culture dishes in Ham's F12 medium supplemented with insulin, hydrocortisone, epidermal growth factor, prolactin, progesterone, cholera toxin, and 5% fetal bovine serum (FBS). The purpose of the present studies was to define additional growth factors that would allow equivalent RME cell proliferation in serum-free medium. Ethanolamine (EA) was effective at reducing the FBS requirements for RME cell proliferation and at its optimum concentration did so by greater than 20-fold. Even with optimum levels of EA there was essentially no cell proliferation in the absence of FBS. However, addition of bovine serum albumin (BSA) to the hormone, growth factor, and EA-supplemented medium resulted in substantial proliferation in the absence of serum, and the further addition of transferrin (T) potentiated this effect. Thus, in this culture system, replacement of FBS with EA, BSA, and T resulted in RME cell proliferation in primary culture which was equivalent to that obtained in the 5% FBS-containing medium.

Key words: mammary epithelial; serum-free medium.

INTRODUCTION

In vitro culture systems that allow extended growth of mammalian cells are a powerful tool for the study of the control of cell proliferation. Recent work in this area has focused on the role of specific hormones and growth factors in initiating a sequence of events that ultimately results in cell division. To evaluate the mechanisms by which growth factors exert their effect, culture systems that allow cell proliferation in chemically defined media are required. The recent work with fibroblastic (1) and hemopoetic cells (7,13) has provided most of the available data on the cellular responses to growth factors. The influence of platelet-derived growth factor, epidermal growth factor, and somatomedin-C on proliferation of 3T3 cells has been extensively investigated (12,14,17), as has the role of the so-called transforming growth factors on proliferation of fibroblasts in both monolayer and suspension culture (2,16,18).

Reports of extended proliferation of a variety of epithelial cell types of both rodent and human origin have now been published. Among the epithelia that have been successfully propagated in vitro are human and mouse mammary (5,9), rat esophageal and tracheal (3,8), and human and mouse epidermis (10,15). The culture media that support growth of these cell types are invariably supplemented with several specific hormones and growth factors, although other undefined components such as feeder layers, serum, or bovine pituitary extract are also required for extended growth of these cells. Recently a culture system was developed in this laboratory for the extended growth of normal rat mammary epithelial (RME) cells in monolayer culture (6). In this system the cells proliferate through greater than 20 population doublings in a medium supplemented with insulin (IN), hydrocortisone (HC), epidermal growth factor (EGF), prolactin (M), progesterone (P), cholera toxin (CT), and 5% fetal bovine serum (FBS). In this report, the results of experiments aimed at eliminating the FBS from the culture medium are described. The data indicate that addition of ethanolamine (EA), bovine serum albumin (BSA), and transferrin (T) to the hormone and growth factor-supplemented medium results in RME cell proliferation in serum-free primary culture that is equivalent to that obtained with the 5% FBS-containing medium.

MATERIALS AND METHODS

The methods for the preparation of rat mammary epithelial cells for primary culture have been described previously (6). Briefly, the inguinal mammary glands of 45 to 50-d-old inbred virgin female Lewis rats are removed, minced, and incubated overnight in type III collagenase (200 μm/ml) at 37°C in a gyratory water bath. The collagenase dissociation is followed by a 45-min incubation in 0.05% Pronase at 37°C. The cells are washed and the epithelial cells, as small aggregates, are
FIG. 1. Derivation of a low-serum medium for RME cell proliferation. Cells were seeded at $2 \times 10^6$ cells/dish in medium supplemented with 5% FBS and switched 24 h later to the experimental medium. At the end of primary culture, nuclei were isolated and counted. Each point represents the mean ± SD for at least three dishes per point and each experiment was done at least twice. A, influence of FBS concentration on RME cell proliferation; B, influence of PE, EA, and T on RME cell proliferation in medium supplemented with either 1 or 2% FBS; C, influence of EA concentration on RME cell proliferation in medium supplemented with 2% FBS. Bar indicates growth for the control group grown in 5% FBS containing medium; D, influence of FBS concentration on RME cell proliferation in medium supplemented with 5 μM EA. Bar indicates growth for the control groups grown in 5% FBS containing medium without EA.