Altering basal medium components increases the in vitro lifespan of normal human keratinocytes. Shiwei Li1, Laurel M. Donahue1, Ann K. Shipley1, Kurt A. Droms1, Yuzhong Lin1, Paul W. Cook2 and Gary D. Shipley1. 1 Cascade Biologics, Inc., Portland, Oregon. 2 Department of Dermatology and Division of Molecular Medicine, Oregon Health Sciences University, Portland, Oregon.

Abstract

We are interested in the regulation of cellular senescence. Typically, normal human epidermal keratinocytes senesce without serum or fibroblast feeder layers. We have shown that replacing serum and fibroblast feeder layers is included in the culture environment, implicating humoral factors, and/or extracellular matrix components is contributing to cellular senescence. However, by manipulating only the composition of the basal medium, which contains no supplements or extraneous reagents, we can culture HEK for ~50 population doublings without serum or fibroblast feeder layers. Our medium requires only the same protein and growth factor supplements that are added to basal media that allow only 20 population doublings. Cells cultured in our medium (1) secrete sufficient cytokines, such as the appearance of human growth factor released by fibroblasts during terminal keratinocyte differentiation, (2) maintain normal epithelial morphology until senescence, and (3) can be expressed to induce the differentiation marker involucrin and involucrin under similar conditions (removal of EGF, insulin, etc.). We are currently investigating the roles of the medium components in the process of lifespan extension. This medium formulation should be very useful for understanding the underlying mechanisms of in vitro cellular aging.

Introduction

It has been nearly 30 years since replicating in vitro cultures of human epidermal keratinocytes were first established (see [1]). Standard keratinocyte cultures are grown in serum-containing media supplemented with EGF, insulin, etc.). We are currently investigating the role(s) of the medium components in the process of lifespan extension. This medium formulation should be very useful for understanding the underlying mechanisms of in vitro cellular aging.

Methods and Materials

Primary isolation of HEK. Neonatal foreskins from which skin was obtained were minced in a sterile Environment (MBE), 1982. 110:219-229.

Table 1. Common Components of Basal Media for Human Keratinocytes

A. Major (7)      Phosphate
B. Amino Acids (20)      Phenol red
C. Vitamins (9)      M154
D. Other organic compounds (5)
E. Trace elements (14)

• glycine
• glutamine
• cysteine
• sulfate
• niacinamide
• biotin
• ethanolamine
• choline
• acetate
• vitamin B12
• riboflavin
• pyridoxine
• phosphoethanolamine
• phenol red
• carbon dioxide
• nickel
• copper
• vanadium
• silicon

Cell culture and calculation of population doubling. All determinations were made in triplicate. Cell cultures were maintained in 10-cm tissue culture flasks in the indicated media. The cultures were maintained at 37 degrees C in a humidified atmosphere of 95% air/5% CO2. Cells cultured in the absence of EGF were harvested using trypsin-EDTA and counted in the Coulter Counter. The number of basal medium. These culture conditions have been shown to be the optimal conditions for studying cellular aging mechanisms. In addition, the new medium should be a useful tool for scientists requiring large banks of keratinocytes for research, diagnostic or therapeutic purposes.

References