Cultured keratinocytes obtained from human hair follicles might be a useful tool to study mutagenicity in human epithelial cells. Human hair follicles possess a cytochrome P-450 dependent enzyme system which is capable to metabolize xenobiotics. The preservation of this enzyme in vitro is important for the application of hair follicle cell cultures in genotoxicity studies especially for promutagens and procarcinogens.

We studied the immunolocalization of cytochrome P-450 using monoclonal antibodies (K03 and K07) raised against two isoenzymes. The antigens were present in freshly plucked hair follicles, fibroblasts and the cell line SWK14. In the cultured keratinocytes no staining was observed by the antibodies. Since the cell line SWK14 shows a medium dependence on the antibodies, the absence of cytochrome P-450 in the hair follicle keratinocytes is ascribed to the culture conditions. Further studies on the relation between culture media and maintenance of cytochrome P-450 is required.

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EXPERIMENTAL

Reagents

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). Cytochrome P-450 antisera and antibodies were supplied by Dr. L. G. De Duve, Brussels. Immunoblotting and immunogold labeling were performed using the antibody conjugates for detection of cytochrome P-450 isoenzymes, following a method previously described.

RESULTS

Cytosolic and subcellular distribution of cytochrome P-450 in cultured mammalian keratinocytes

We studied the subcellular distribution of cytochrome P-450 in cultured hair follicle keratinocytes. The cytochrome P-450 content was determined using a colorimetric assay (0.42 pmol/mg protein) and a fluorometric assay (0.38 pmol/mg protein). The results were consistent with previous studies. The cytochrome P-450 content in the cytosolic fraction was significantly lower than in the subcellular fraction.

Immunocytochemical localization

Immunocytochemical localization of cytochrome P-450 was performed using a monoclonal antibody against rat liver cytochrome P-450. The antibody stained the cytosolic fraction of the cultured hair follicle keratinocytes.

DISCUSSION

The results of this study indicate that cultured hair follicle keratinocytes contain cytochrome P-450 isoenzymes, and that the cytochrome P-450 content is significantly lower than in the subcellular fraction. This is consistent with previous studies.

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