INITIATION AND ULTRASTRUCTURE OF A REPTILIAN FIBROBLAST CELL LINE OBTAINED FROM CUTANEOUS FIBROPAPILLOMAS OF THE GREEN TURTLE, CHELONIA MYDAS

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SUMMARY

Two fibroblastic cell lines were established from explants of fibropapillomas of each of two different green turtles (Chelonia mydas). These cells, designated GTTP (Green Turtle Fibropapilloma), were subcultured approximately 30 times at 30°C in Eagle’s minimal essential media supplemented with 2 to 10% fetal bovine serum. The ultrastructural morphology of the cultured fibroblasts is described. The cells contained abundant rough endoplasmic reticulum, polysomes, and mitochondria; collagen fibrils were visible in the extracellular space. No viruss-like particles or evidence of other pathogenic agents could be demonstrated by electron microscopy in any of the cultured cells examined.

Key words: fibropapilloma; cell line; ultrastructure; green turtle; Chelonia mydas.

INTRODUCTION

Primary cell cultures of reptilian tissues have been described by several investigators. These include various components of tail regenerate of the American anole, Anolis carolinensis (1,13,14), cells propagated from embryos of the leopard gecko, Eublepharis macularius (8), cells from the skin of the green turtle, Chelonia mydas (6), cells from the heart of a box turtle, Terrapene carolina (2), spleen, kidney, liver, and testes from a rattlesnake, Crotalus horridus (10), kidney from a kingsnake, Lampropeltis getulus (10), and heart from a hognose snake, Heterodon platyrhinos (10). Cell cultures have also been established from several reptile neoplasms such as those derived from the spleen of a Russell’s viper, Vipera russelli, with a myxofibroma (18) and those derived from a fibroma of a timber rattlesnake, Crotalus horridus (9).

Green turtle fibropapilloma was first described in 1938 (7,15) and is currently a significant disease problem in green turtles in Florida and Hawaii. Although detailed histopathologic and molecular biological studies have been conducted, no specific etiologic agent has been identified (5). In this report we describe the ultrastructural appearance of fibroblasts derived from fibropapillomas of the green turtle, Chelonia mydas, and incubated at 30°C.

MATERIALS AND METHODS

Origin of the cell line. Papillomatous lesions on the neck and axillary regions of two adult green turtles from the Indian River Lagoon System, Florida, were removed under local anesthesia, and tissue from each turtle was washed and minced finely in Eagle’s minimal essential medium (MEM) containing 200 μg/ml gentamicin.

Part of the finely minced tissue of each turtle was put directly into plastic cell culture flasks containing 5 ml of MEM with 10% fetal bovine serum (FBS), 200 μg/ml gentamicin, and 2 μg/ml amphotericin B. The remainder was put in trypsinizing flasks containing a trypsin-EDTA solution in phosphate buffered saline and was stirred on a magnetic stirrer. The supernantant was collected 3 times at 20-min intervals and centrifuged at low speed. The resulting pellet of cells was seeded into cell culture flasks containing 5 cc MEM with 10% FBS, 200 μg/ml gentamicin, and 2 μg/ml amphotericin B. The flasks were incubated at 30°C and the growth medium was changed at 3-d intervals.

Electron microscopy. The monolayers of cells were put into suspension by scraping them from the bottoms of the flasks into the cell culture medium. The cells were centrifuged gently and the pellet resuspended in 3% glutaraldehyde. After further low speed centrifugation the pellet was resuspended in 3% glutaraldehyde and refrigerated for 15 min. The cells were then washed in a 0.1 M sodium cacodylate buffer. After postfixation in 1% osmium tetroxide, the cells were dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 10 electron microscope.

RESULTS

Cell line initiation. Adherence of cells from the tissue pieces to the cell culture flasks was slow. Trypsinized
Preparations of specimens from the first turtle produced a few small attached cell colonies after 5 d. These colonies were trypsinized at 7 d and allowed to resettle to encourage growth. A confluent monolayer was produced at 2 wk. Attachment of cells from the second turtle was slower and occurred only in the tissue that had been minced but not trypsinized. After 16 d cells began to attach and divide and produced a confluent monolayer after 4 wk.

Once initial monolayers were formed growth became much more rapid and the cells could be maintained in media containing 2% FBS. These cells were designated GTFP (Green Turtle Fibropapilloma) and each cell line was subcultured approximately 30 times.

**Ultrastructure.** The ultrastructural morphology of the cells from both turtles was that of fibroblasts. The cells had been harvested at Days 1, 4, and 7 after passage, and after 24 h (Day 1) collagen fibrils were present in the extracellular space in both cell lines. The cells contained neither desmosomes nor cytoplasmic tonofilaments, indicating they were not of epithelial origin.

**Nucleus.** The nuclei of both cell lines tended to be elongated and have irregular outlines (Fig. 1). Nucleoli were present in about 50% of the cells and occasionally two or three nucleoli were present in one nucleus.

**Endoplasmic reticulum.** At Day 1 the endoplasmic reticulum was extensive and the cisternal space was slightly dilated. By Day 4 the cisternae were dilated and could be seen to contain flocculent material. At Day 7 the cisternae of the endoplasmic reticulum containing the flocculent material became very dilated near the periphery of the cells (Fig. 2). Exocytotic vesicles were observed discharging contents into the extracellular space.

**Mitochondria.** Mitochondria were numerous at all stages of harvest.

**Golgi.** The golgi apparatus was prominent at all stages, but became more prominent, numerous, and well organized at Days 4 and 7 (Fig. 1).

**Ribosomes.** The cytoplasm contained abundant free and membrane-bound ribosomes. Large accumulations of free ribosomes were a feature of these cells, being present from Day 1 and becoming extensive by Day 8. They were usually seen near the periphery of the cells (Fig. 3).

**Autophagic vacuoles.** Autophagic vacuoles were present and seemed more numerous at Day 1 than Day 4 or 7. Membrane-bound vesicles containing lamellar membranous structures (myelin figures) were present, especially at Day 1.

**Collagen.** Unorganized collagen fibrils surrounded the cells in both cell lines (Fig. 3). By Day 7 the fibrils seemed more organized.

**Virus particles.** No virus particles were seen.

**Discussion**

The two cell lines (GTFP) of the present report were derived from fibropapillomas of green turtles from the Indian River Lagoon System, Florida. The light and electron microscopic features of this tumor have recently been described (5). The cells were grown in MEM and FBS because other reptilian cell lines have been initiated in this medium (16, 17). The cell monolayers were established in 10% FBS, but 2% FBS was found adequate for maintenance once the cells were well established. The
The fibroblastic cell cultures were established for the purpose of investigating the possibility of a viral etiology of green turtle fibropapilloma. In examining six fibroblast cell cultures from two affected turtles, no virus-like particles were seen. The cause of this neoplasm remains unknown.

REFERENCES


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