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Actin and Tubulin in Normal and Cystic Fibrosis Fibroblasts

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Actin and tubulin contents of early passage, confluent human fibroblast cultures have been determined. Actin comprised 5.87 ± 0.81% of the total protein of IMR-90 fibroblasts which was not significantly different than the actin contents of two cystic fibrosis fibroblast cultures GM0142 and GM1348 (5.64 ± 0.90% of total protein). However, a significant difference between the amount of tubulin in IMR-90 fibroblasts (7.17 ± 0.25% of total protein) and the amount of tubulin in cystic fibrosis fibroblasts (4.51 ± 0.64% of total protein) was found.

INTRODUCTION

One of the most common genetic diseases is cystic fibrosis. It is transmitted as an autosomal recessive trait and results primarily in exocrine gland dysfunction (1). Because of the genetic nature of the disease, cultured skin fibroblasts from subjects have been examined for abnormalities and many diverse differences between normal and cystic fibrosis (CF) fibroblasts have been reported. These include altered monosaccharide composition of membrane glycolipids (2), leakage of lysosomal enzymes into the medium (3) and premature senescence (4) in CF fibroblasts. Two properties associated with the cytoskeleton have also been reported to be altered in CF fibroblasts (5). Colchicine binding, which is a property of the protein tubulin, and the activity of the tubulin associated enzyme, tyrosyl tubulin ligase, are decreased in CF fibroblasts. Whether the decreased colchicine binding resulted from a decreased affinity of the tubulin for colchicine or from lower amounts of tubulin was not determined, but the results indicate that there may be alterations in the cytoskeleton in cystic fibrosis. Major elements of the cytoskeleton are microtubules formed from tubulin and microfilaments formed from actin.

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They are important in regulating general properties which are altered in cystic fibrosis. These include secretion (6), and properties of membranes involving transport (7). For these reasons the amounts of two major proteins of the cytoskeleton, actin and tubulin, were determined in normal and CF fibroblasts.

MATERIALS AND METHODS

Two fibroblasts cultures from individuals with cystic fibrosis were obtained from the Human Genetic Mutant Cell Repository, IMR, Camden, New Jersey. GM0142 was from a 14 year old male and GM1348 was from an 18 year old female. Both donors died in the year that the cells were obtained. The well characterized IMR-90 (8), obtained from the same source, was used as a normal human fibroblasts cell culture. All cells were grown on Falcon 75 cm^2 plastic flasks in Alpha Minimum Essential Medium supplemented with 10% fetal calf serum. Confluent cells were subcultivated at a split ratio of one to four.

For actin and tubulin determinations, confluent cultures were harvested by scraping in phosphate buffered saline (Ca^{++}, Mg^{++} free). The harvested cells were washed three times with 10 vol of this buffer and then heated for 2 min in a boiling water bath and cooled on ice. Double radioisotope labelling and peptide isolations were used as previously described, to determine the amount of actin (9) and tubulin (10) in acetone powders prepared from the boiled cells (11). This methodology permitted the determination of both actin and tubulin in the same samples.

RESULTS AND DISCUSSION

No apparent differences in population doubling times were noted in the three cell cultures. All were at an early passage level when obtained and became confluent within one week when subcultivated at a split ratio of one to four. Senescence as indicated by increased time for population doubling was therefore not apparent in these cells which were subcultivated no more than three times prior to harvesting.

Table 1 summarizes the results of actin determinations for the three fibroblast cultures. The number of estimates made for each culture is indicated. There was no significant difference between the actin content of IMR-90 fibroblasts (5.87 ± 0.81% of total protein) and the mean value obtained for cystic fibrosis fibroblasts (5.64 ± 0.90% of total protein). The present estimates of actin content are somewhat higher then a value previously reported for IMR-90 cells using similar methodology (9). Two factors could account for this. In the present study cells were harvested from confluent cultures of early passage cells. In the previous study no attempt was made

Table 1

Cell Type	Actin content (% $^{w}/_{w}$)
IMR-90	5.87 ± 0.81 (n = 3)
GM0142	5.94 ± 1.15 (n = 5)
GM1348	5.28 ± 0.28 (n = 4)

Actin contents of fibroblasts. Results are expressed as % $^{w}/_{w}$ of total protein.

to ensure that early passage confluent cultures were used. A second factor which may alter the amount of actin measured is the treatment of samples. Work in progress on the determination of actin and tubulin contents of human periferal lymphocytes has indicated that considerable proteolysis occurs in these cells if cells are not heated in a boiling water bath prior to the protein determinations. In the present study all fibroblasts were heat treated prior to analysis. In the previous study they were not.

The amounts of tubulin in the same samples used in the actin determinations are given in Table 2. A t test indicated that the tubulin content of CF fibroblasts (4.51 ± 0.64% of total protein), was significantly less ($p \leq 0.01$) than that of IMR-90 fibroblasts, (7.17 ± 0.25% of total protein). This finding demonstrates that the decreased colchicine binding of CF fibroblasts previously reported (5) is due to decreased amounts of tubulin in these cells. Whether the lower concentration of tubulin results in decreased amounts of microtubules in CF fibroblasts is not yet known. However, altered functional capabilities regarding secretion and transport are apparent in cystic fibrosis and the cytoskeleton is implicated in these processes. The present findings

Table 2

Cell Type	Tubulin content (% $^{w}/_{w}$)
IMR-90	7.17 ± 0.25 (n = 3)
GM0142	4.80 ± 0.53 (n = 5)
GM1348	4.15 ± 0.64 (n = 4)

Tubulin content of fibroblasts. Results are expressed as % $^{w}/_{w}$ of total protein.

suggest that the cytoskeleton may be altered in cystic fibrosis due to an imbalance of microfilaments and microtubules or to decreased number of microtubules. Since tubulin is a major component of the mitotic spindle, decreased tubulin content may also account for early senescence, as manifested by a loss in ability to undergo cell division, which has been observed in CF fibroblasts (4).

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