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ARAŞTIRMA MAKALESİ/**RESEARCH ARTICLE**

MICROTUBULE ORGANIZING CENTRE IN CULTURED FIBROBLAST CELLS

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ABSTRACT

Many cell types express an internal and external polarization of various cytoplasmic components as microtubule organizing centre (MTOC). It is believed that MTOC and increasing and decreasing of cytoplasmic microtubules are very important for direction of cell movement and cell polarity. In our experiments, the relationship between MTOC and cytoplasmic microtubules is examined by using immunofluorescence methods on 3T3 fibroblast cell lines. Our results demonstrated that MTOC has a central location in most cells. Moreover a coordination between microtubules and centrioles during formation of cytoplasmic microtubules was identified. It has been shown that the microtubule formation initiated at the cell centre in which centrioles were located.

Key Words: Fibroblast, Microtubule, Microtubule Organizing Centre, MTOC.

KÜLTÜR FİBROBLAST HÜCRELERİNDE MİKROTUBÜL OLUŞUM MERKEZİ

ÖZ

Hücre türlerinin çoğu, mikrotubül oluşum merkezi (MTOC) gibi çeşitli sitoplazmik bileşenlerin iç ve dış biçimlenmelerinin etkisi altındadır. MTOC ve oluşan ya da yıkılan sitoplazmik mikrotubüller, hücre hareket yönü ve hücre biçimlenmesinde çok önemlidir. Çalışmamızda, 3T3 fibroblast kültür hücrelerinde immunofluoresans yöntemi kullanarak, sitoplazmik mikrotubüller ve MTOC arasındaki ilişki araştırıldı. Bulgularımız, hücrelerin büyük çoğunluğunda, MTOC'un, hücre merkezine yakın konumda olduğunu açık olarak göstermiştir. Bunun yanısıra, sitoplazmik mikrotubüllerin oluşumu sırasında, sentrioller ile mikrotubüller arasında uyumlu bir ilişki olduğu görülmüştür. Çalışmamızda, mikrotubül oluşumunun sentriyollerin bulunduğu hücre merkezinde meydana geldiği ortaya konmuştur.

Anahtar Kelimeler: Fibroblast, Mikrotubül, Mikrotubül Oluşum Merkezi, MTOC.

1. INTRODUCTION

Cellular microtubules are often associated with special structures called microtubule organizing centre (MTOC) (Berhadsky and Vasiliev, 1988; Preston et al., 1990; Vogl et al., 1995). Microtubules of cytoskeleton are usually about 25 nm in diameter as tubes which are universal component of all eukaryotic cells except mammalian erythrocyte. Just 25 years ago, microtubules were discovered by organization of the first mitotic spindle of the embryo after duplication of the sperm centrosome. Microtubules play several important roles like transport of vesicles, chromosomes, etc., cell motility (wavy locomotion, cilia or flagellate locomotion, etc.) and changes in cell shape (from squamous to round, elongation, etc.).

Microtubules are formed from a single protein called "tubulin". Assembling of microtubules occur from positive terminal by polymerization of tubulin in the cytoplasm when a critical tubulin concentration is reached (Prescott et al., 1992). Microtubules originate from a single site which is MTOC, radiating out towards periphery of the cell. *In vitro* experiments show that MTOCs are not only constituted nuclear centres of microtubules, but also control the structure of these microtubules and their arrays. Many different morphological types of MTOCs are described like basal bodies (Novi-

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koff and Holtzman, 1970), centrioles (Gould,1975), centrosomes and kinetochores (Wheatley, 1982; Brinkley, 1985; Schliwa, 1991; Vogl et al., 1995; Salisbury et al., 1999a ve 1999b; Schiebel, 2000), around of nucleus or without nucleating centre (Valle, 1984; Henderson et al., 1995) in various animal and plant cells. One of nucleating centre of microtubules is the plasma membrane (Mogensen et al., 1989). Also, there is a functional relationship between microtubules and structure and position of the Golgi apparatus (Skoufias et al., 1990; Rambourg et al., 1996).

Although some cells do not contain centrioles (as *fungi*, high organisation of plants, *Drosophila* embryo and early mammals embryo), the most described MTOC structure is centrosome. Centrosomes consist of one or a pair of centrioles surrounded by pericentriolar materials (PCM) which are proteins. In animal cells, several centrosomal proteins have been identified (Kuriyama and Borisy, 1985; Frash et al., 1986; Toriyama et al., 1988; Buendia et al., 1990; Moudjou et al., 1991; Chevrier et al., 1992). So, it is necessary to understand where MTOCs are, and what MTOCs are.

This experiment was planned to explain the relationship between MTOC and cytoplasmic microtubules and to show the formation of MTOC.

2. MATERIALS AND METHODS

3T3 (mouse fibroblast) cell lines were cultured in Dulbecco's Modification of Eagles Medium (DMEM, Gibco) supplemented in 10 % Foetal Calf Serum (FCS, Gibco) with 24 ml Nocodazole (Aldrich) in 0.125 % DMSO (Sigma) for 1hour. Then the medium was changed with fresh medium without Nocodazole for 5, 10 and 30 minutes. The cells were grown in an atmosphere of 90 % air, 10 % carbon dioxide at 37 °C. After culturing, cells were washed with Phosphate Buffer Saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.3) 3 times and then fixed with 90% methanol in PBS at (-20)°C for 5 minutes. After fixing, the cells were extracted with acetone (-20)°C for 30 seconds, and then washed in 3 changes of PBS. Cells were processed according to standard immunofluorescence procedures. They were incubated first with rabbit serum for 1 hour then YL 1/2 antibody (Sera-Lab.) against tyrosinated tubulin for 1 hour and finally Fluorescein Iso Thiocyanate (FTIC) rabbit anti rat serum (Dako) for 1 hour. Stained cells for fluorescence microscopy were washed in PBS then examined using Olympus BX50 epifluorescence microscope (Zeytinoğlu et al., 1996).

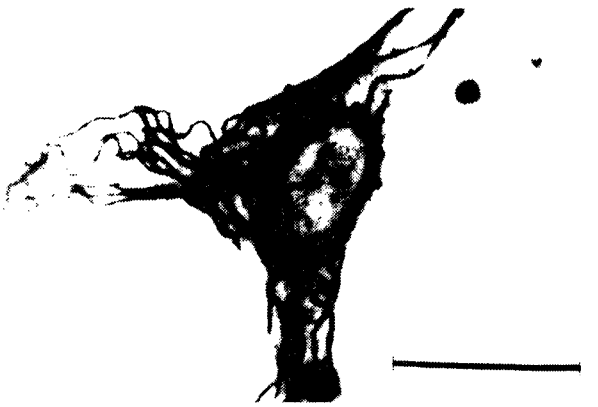

Figure 1. Organization of Microtubules In 3T3 Cell Line. Microtubules Stained by Fluorescent Labelled Anti-Tubulin Antibody. Scale Bar = 25 μ

3. RESULTS

Microtubules were visible in the cytoplasm of an interphase cell by labelling with fluorescent anti-tubulin antibodies after the cells have been fixed (Figure 1). Usually the microtubules are present in high density in the cell and radiate out from the cell centre to periphery.

In this experiment, fibroblasts were first treated with nocodazole for 1 hour, then nocodazole containing medium was washed for regrowing of the microtubules in the cells. After nocodazole treatment, the cells were examined under the fluorescence microscope. All the microtubules were disappeared in the cell body. There was not any polymerized tubulin. Cytoplasm of the cells was appeared as blurry (Fig. 2). After removal of the nocodazole from the medium, regrowth of the microtubules was very rapid in the cells.

After 5 minutes removal of nocodazole from the medium of the cells, two types of microtubules started to regrow in the cell body. One type of microtubules regrowed at the periphery of the cells. These types of microtubules were twisted on itself. The other types of

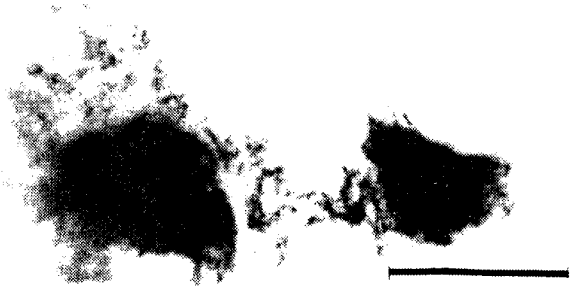

Figure 2. 3T3 Cell Lines Treated With Nocodazole For 1 Hour. Distribution of Depolymerized Microtubules by Fluorescent Labelled Anti-Tubulin Antibody Staining. Scale Bar = 25 μ

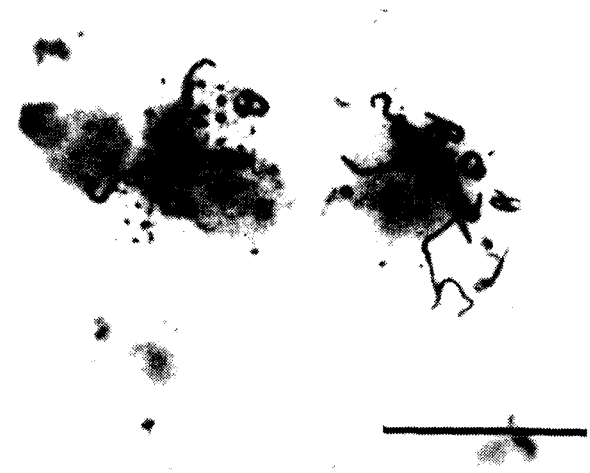

Figure 3. Immunofluorescence Labelled Distribution of Microtubules of 3T3 Cell Line After 5 Minutes Removal of Nocodazole From The Medium. Scale Bar = 25 μ

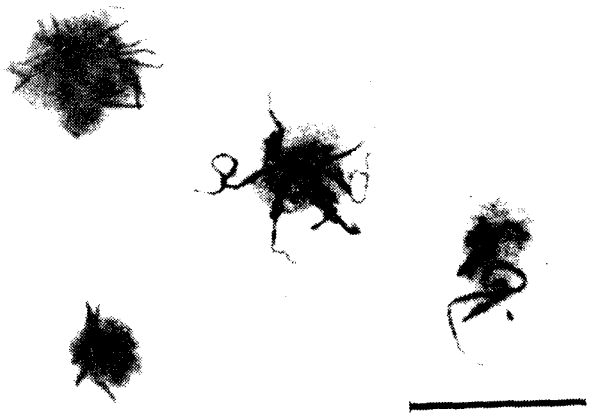

Figure 4. Immunofluorescence Labelled Distribution of Microtubules of 3T3 Cell Line After 10 Minutes Removal of Nocodazole From The Medium. Scale Bar = 25 μ

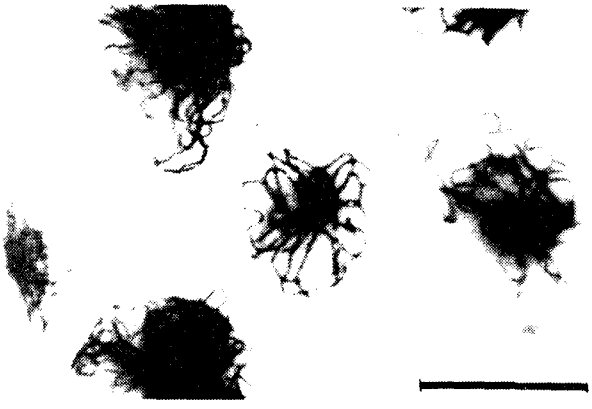

Figure 5. Immunofluorescence Labelled Distribution of Microtubules of 3T3 Cell Line After 30 Minutes Removal of Nocodazole From The Medium. Scale Bar = 25 μ

microtubules associated with a nucleating centre (Fig. 3). Second type of microtubule regrowth occurred from a nucleating centre towards cytoplasmic periphery as starlike structures. Also, this type of microtubules was seen more elongated than first type of microtubules.

We examined microtubule changes after 10 minutes removal of nocodazole. Single free microtubules which were centred microtubules appeared very early peripherally located in the cells (Fig. 4). Most microtubules were established as a group which were nucleated from same single centre (Fig. 4). Single centred microtubules were seen more elongated than 5 minutes microtubules. Also, a pretty big blurred area was seen at the microtubule nucleating centre.

After 30 minutes, the observation of the cells showed that there were no free microtubules. The blurred area was quite small at the microtubule nucleating centre and the cells were filled by numerous of elongated hairlike microtubules (Fig. 5). Centre of microtubules was not seen clearly for abundance of microtubules.

4. DISCUSSION

This study has shown that nocodazole treatment for 1hour was very effective on polymerized microtubules in cells. After nocodazole treatment, blurred areas were seen in cells. It has been suggested that the appearance of blurred cell cytoplasm must be tubulin monomers occurring of the depolymerization of microtubules after nocodazole treatment. This suggestion is supported by the following results in this study. The blurred area in the cells were dramatically reduced after removing nocodazole. Therefore the elongation of microtubules by polymerization of tubulines were increased in the cells. The blurred areas in the cells were not the centre of the cells, which were nucleating centre of microtubules. This result conflicts with previous investigations, reporting. Jashi and Schiebel reported that tubulines polymerized at positive ends of microtubules which was opposite from the nucleating centre of microtubules and also it was at free terminal of microtubules.

The microtubules were allowed to regrowth for various periods of time in the nocodazole free medium. It was shown that there were two kinds of regrowth of cytoplasmic microtubules. Most of microtubules have had a same nucleating centres which was reported as MTOC (Joshi, 1994; Schiebel, 2000). There was not any reported nucleating centres about some of microtubules which are called free microtubules. This second type of microtubules were demonstrated in some researches (Chrgtien et al., 1995). It was thought that MTOC might not be the only one object or structure

and/or locations of MTOCs, and could be different in the same cells. In classical text books about biology it is explained that microtubules of the cells in mitosis have two nucleating centres which each one of double centrioles are at opposite location. Also many researchers reported that centrioles were MTOCs (Van-Der et al., 1995). Numerous free microtubules at the cell periphery were observed in this work independent MTOCs. Also, some researchers reported similar microtubules (Prescott et al., 1992). Suggesting that MTOCs could be different structures during the beginning of elongation of microtubules. But, after few seconds main MTOC which were nucleating centre of starlike organized microtubules, were appeared to suppress on nucleating centres of peripheral free microtubules. According to our results, suppressor main MTOC can be the centrioles.

The notion that microtubule organization was controlled by factors are distributed throughout the unit cytoplast of the cell as the centrosome, centrosomal proteins, Golgi apparatus, cell membrane and nuclear surface (Tucker et al., 1992; Schmit et al., 1994; Vogl et al., 1995; Rambourg et al., 1996; Salisbury et al., 1999; Schiebel, 2000). Each of them could be recessive MTOC for free microtubules. However, if the centrioles are absent in the cell, any and/or each of those can be dominate MTOC. It is possible that there is an interaction among them. Also, it is possible to hypothetize that formation of microtubules from different structure of MTOC can serve for specific functional purposes.

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