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ARAŞTIRMA MAKALESİ/**RESEARCH ARTICLE**

STUDY OF MORPHOLOGICAL CELL MOTILITIES ON CULTURED FIBROBLASTS AND MYOBLAST CELLS IN VITRO

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ABSTRACT

Cell movement plays an important role in many biological events, including embryonic development, wound healing, immunological responses, and metastasis. In this study, we isolated fibroblasts and myoblast cells from chick embryos, and cultured by using cell culture method. We compared cell morphology and motility between cultured fibroblasts and myoblast cells by means of cytochemical stains in vitro. In this study, it has been shown that there were some different cell morphology and movements after 48 h between these cells derived from mesoderm, and the reasons of these differences were discussed. We believe that our data may give contribution to cell motilities on cultured fibroblasts and myoblast cells in vitro.

Key Words: Fibroblasts, Myoblast, Chick embryo, Cell motility.

KÜLTÜRÜ YAPILMIŞ FİBROBLAST VE MİYOBLAST HÜCRELERİNDEKİ MORFOLOJİK HÜCRE HAREKETLERİNİN İN VİTRO İNCELENMESİ

ÖZ

Hücre hareketleri embriyonik gelişim, yara iyileşmesi, immunolojik cevap ve metastas gibi biyolojik pek çok olayın gerçekleşmesinde önemli rol oynamaktadır. Bu çalışmada tavuk embryosundan izole edilen fibroblast ve myoblast hücreleri hücre kültürü yöntemi kullanılarak kültüre edildiler. Fibroblast ve myoblast hücreleri arasındaki hücre morfolojisi ve hareketleri bakımdan farklılıkları sitokimyasal boyalar yardımıyla karşılaştırıldı. Çalışmamızda mezoderm kökenli iki farklı hücre çeşitinde farklı hücre morfolojisi ve hareketleri olduğunu görülmüş ve bu farklılıkların nedenleri tartışılmıştır. Bu çalışmada elde edilen verilerin in vitro ortamda kültürü yapılmış fibroblast ve myoblast hücre hareketlerinin incelenmesi ile ilgili çalışmalara katkıda bulunacağı düşünülmektedir.

Anahtar Kelimeler: Fibroblast, Miyoblast, Tavuk embriyosu, Hücre hareketi.

1. INTRODUCTION

Movement is a fundamental property of living things that distinguish from non living things (Lazarides and Revel, 1979). Cell movement plays an important role in many biological events (Raucher and Sheetz, 2000). For example, the most common function of motility among the prokaryotes seems to allow the organisms to move toward or away from environmental stimuli, therefore, there are some different cell movement types in prokaryotes, such as gliding motility, swarming motility, twitching motility, and swimming motility (Balows et al, 1992). Lymphocytes, macrophages and white blood cells as eukaryotic cells in the blood migrate into an open wound and infection area (Raucher and Sheetz, 2000). Fibroblasts migrate into a wound to take part in the healing (Raffetto et al, 2001). Cells also display a wide variety of internal movements, such as streaming of the cytoplasm, secretion of cell products from vesicle, endocytosis, exocytosis, contraction of muscle and separation of the paired chromosomes in the course of cell division (Taylor, 1986; Young and Heath, 2000). During

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development, embryonic cells migrate often over large distances to reach their final destinations. Cell movement can also have fatal results. Individual cancer cells sometimes break loose from a tumor and go to other parts of the body to establish new tumors called metastasis (Cavallaro and Christofori, 2001).

Cell motility is a complex function of many cellular components. It requires the interaction of the cell membrane and membrane proteins, the cytosol, the extracellular matrix. Cytoskeleton is composed of structural elements of three main types microflaments, microtubules, and intermediate filaments (Parker et al, 2002). The fact that certain cells move has been known since Anton van Leeuwenhoek, who observed swimming sperm cells in his primitive microscope in the 17 th century, but the study of the mechanisms of cell motility has been facilitated by the development of new methods (Berns, 1983). Tissue culture is one of the fundamental new methods for the study of cell motility. To understand the molecular mechanisms of cell motility, investigators have studied cells moving in tissue culture where conditions permit observations at the highest possible magnification and resolution. As fibroblasts are easy to grow in laboratory condition for cell culture, they have been studied the most commonly by researchers of the cytoskeleton and cell motility (Browder et al, 1991).

Fibroblasts are the dominant cell types of connective tissue family (Fawcett and Jensh 1997). They are derived from undifferentiated mesenchymal cells (Gartner and Hiatt, 1997). These cells are responsible for the synthesis of collagen, reticular and elastic fibbers, and amorphous ground substance of connective tissue. Therefore, they are rich in organelles, such as Golgi apparatus, rough endoplasmic reticulum, and mitochondria (Young and Heath, 2000; Gartner and Hiatt, 1994). Fibroblasts also seem to be the most versatile of connective tissue cells, displaying a remarkable capacity to differentiate into other members of the family, such as adipose cells, osteoblasts, chondrocytes. Fibroblasts are non motile for long periods in connective tissue in vivo but they are capable of orientation and preferential migration, and play important role in wound healing process (Wheater et al, 1987; Raffetto et al, 2001).

Development of muscle cells provide an excellent experimental system enabling to study the molecular events involved in fusion and cell differentiation (Browder et al, 1991). Muscle cells are capable of contraction. Organisms harness the contraction of muscle cells to arrangement of the extracellular components of muscle permitting locomotion, contraction, pumping and other propulsive movement. Skeletal muscle cells display characteristic alterations of light and dark cross bonds, therefore they are called striated or skeletal muscle (Gartner and Hiatt, 1997). Skeletal muscle develop from the mesoderm. Muscle growth is the result of fusion owing to myoblasts and myotubes (Moore and Persaud, 2002).

Fibroblasts are multipotent dominant cell types of connective tissue on the other hand myoblast cells are differentiated cells in muscular system. The aim of this study was to obtain fibroblasts and myoblast cells that were isolated from living embryonic tissue, and to compare different cell motility between these cells by means of special cytochemical staining in cell culture.

2. MATERIALS AND METHODS

2.1 Preparation Of Cells Cultures

Fertile chicken eggs incubated in humidified atmosphere of 95% O2, and 5% CO2 at 38 0C. These eggs turn through 1800 daily. Cultured fibroblasts were obtained from 8 day old chicken embryo skin, and cultured myoblast cells were obtained from 12 day old chicken embryo limb by means of primary explant technique as described in previously Fresney (Fresney, 1994). The cultures were grown in 21 cm petri dishes containing 5 ml Dulbecco's modified Eagle's medium (DMEM) with 10% Foetal Calf Serum (FCS), 1% penicillin/streptomycin solutions (10000U/ml penicillin, 10 mg/ml streptomycin). All reagents were purchased from Sigma Chemical Co. (St. Louis, MO). The medium was changed daily by carefully removing and adding new medium. All petri dishes were incubated in 95% O2, and 5% CO2 at 38 0C for 48 h. After 48 h, the explant pieces were removed by forceps. The medium was removed and rinsed the cell layers with Dulbecco's phosphate buffered saline (PBS), and the cell layer is trypsinized. 3 ml 0,25% trypsin-ethyleneamintetraacetic acit solution (0,25% trypsin-EDTA) was added each petri dish and were incubated at 38 0C until the cells have detached and separated from one another. In general, 10 min incubation was sufficient. This process was monitored under the microscope. Finally, these released cells seed into 21 cm petri dishes containing 10 ml DMEM with 10% FCS and 1% penicillin/streptomycin. All petries were incubated same condition at 48 h (Jakoby and Paston, 1979).

2.2 Staining Of Cells

The medium was removed and the fibroblasts and myoblast cells layers were washed three times with PBS, and fixed overnight in 4% paraformaldehyde then the cells were washed by distilled water. All staining methods were modified for suitable stained of cultured

cells. Primary fibroblasts and myoblast cells were stained with modified **Giemsa stain;** (0,8 g Giemsa stain in 100 ml of a mixture of equal volumes of glycerol and methanol; Clark, 1981), **Harris' hematoxylin stain;** (1 g hematoxylin in 10 ml 99% absolute ethyl alcohol, was added potassium sulphate 20 g and 0,5 g mercuric oxide before used, was added 0,8 ml glacial acetic acid 0,8 ml, distilled water 190 ml; Smith and Bruton, 1979; British Drug House, 1960; Clayden, 1962), **Papanicolaou stain;** (1- Harris's hematoxylin, 2- orange-G; 0,6 g orange-G, 0,015 g. phosphotungstic acid, 100 ml 99 % absolute ethyl alcohol, 3-10 ml. Bismarck brown (0,5% alcoholic solution), 0,2 g phosphotungustic acid, 1 drop lithium carbonate; Clayden, 1962; Clark, 1981; Bancroft and Stevens, 1980), **Orcein stain;** (1 g orcein in 100 ml 70% ethyl alcohol and added 1 ml hydrochloric acid.), and **Altmann's stain;** (1-aniline 5 g, acid fuchsine 15 g, distilled water 100 ml, 2-saturated picric acid add 40 ml 30% absolute ethyl alcohol; Bancroft and Stevens, 1980).

Cultured cells were examined under the inverted microscope (Olympus BX50), and photos were taken by Olympus PM-30 attachment.

3.RESULTS

3.1 Cell Produce From Tissue

Primary extract pieces were examined daily by inverted microscope. Cultured fibroblasts and myoblast cells developed from primary explants basal layers. Cells were seen migrating radically from the explants. Since these cells had high dense around the explants, it was difficult to distinguish cell morphology and motility. We have observed that these cells always divided monolayer cell lines (Fig.1-2).

3.2 Primary Culture Cells

Both primary fibroblasts and myoblast cultured cells were removed for isolation from explants into new petri dishes. After trypsinating, the cells reattached very easily within 24 h. Cells were spread out randomly on the substrate, and cells were stained with Orcein stain. These conditions permit to observe cell morphology and movement (Fig.3-4).

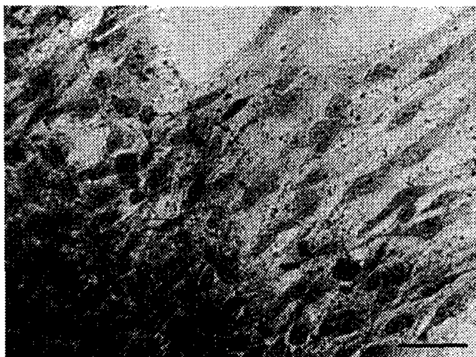


Figure 1. **Pioneer cultured fibroblasts from primary exract pieces obtained from embryos. Arrow indicated that primary extract pieces Scale Bar=20μm**

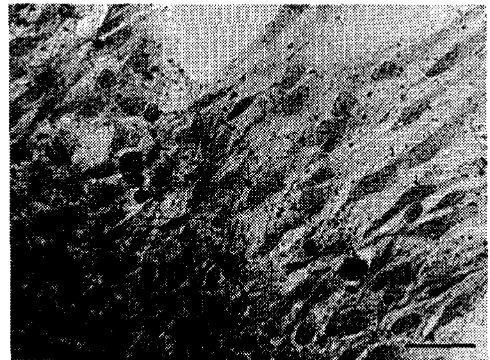


Figure 2. **Pioneer cultured myoblast cells from primary extract pieces obtained from embryos Scale Bar= 20μm**

Figure 3. **After tripsinization, fibroblasts extended into the substrate. Scale Bar = 20 μm**

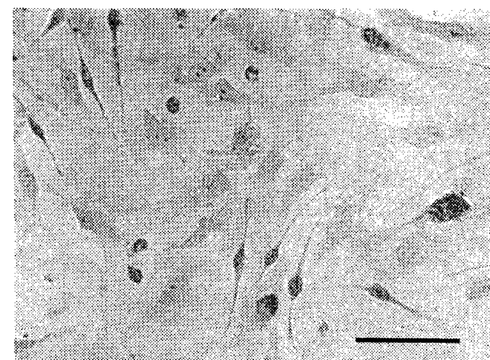


Figure 4. **After tripsinization, myoblast cells extended into the substrate. Scale Bar = 20 μm**

3.3 Staining

We used different staining methods to examine differences in morphology and movements between cultured fibroblasts and myoblast cells by means of different magnification degrees by inverted microscope at 24 and 48 h.

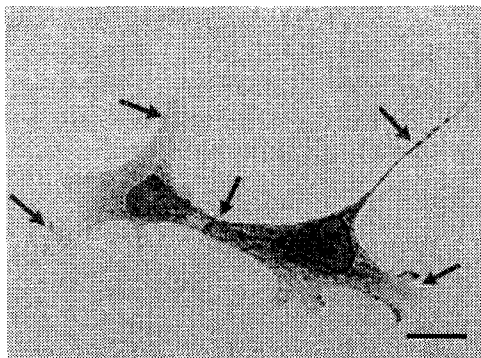


Figure 5. General image of fibroblasts after 48 h. Arrows; cytoplasmic protrusions. Scale Bar = 20 µm

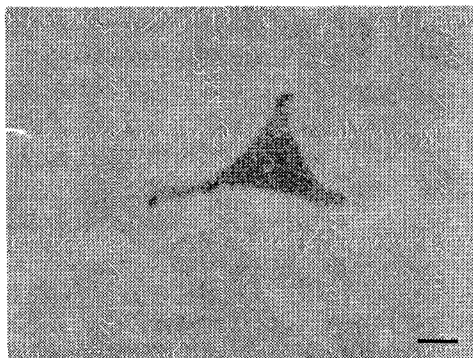


Figure 7. Homogen dispersed granules in fibroblasts. Scale Bar = 20 µm

Cultured fibroblasts and myoblast cells had similar morphological properties after 24 h. When fibroblasts and myoblast cells were stained with Giemsa stain, the cells had large unique oval nucleus centrally located, and contained darker-stained and well-defined one or two nucleoli (Fig. 5-6). After 48 h, fibroblasts were seen usually long, wide, and also flat, extending into the surrounding area.

In contrast, myoblast cells were seen thin, long, and ovoid cell shape 48 h later (Fig 3-4).

Lots of granules in fibroblasts and myoblast cells in the cytoplasm were observed with Altmann's stain. Although these granules were determined homogeny dispersed in fibroblasts. They were determined usually dense, surrounding of nucleus in myoblast cells (Fig 7-8).

Extensions of cell membrane both fibroblasts and myoblast cells were examined by Orcein and Harris' hematoxylin stain, and two different extensions during fibroblasts migration. One of them was seen as sheet-like extensions of the cell membrane. The other extensions of the cell membrane were thin, long (Fig. 9). Cell membrane protrusion that located every two tips of the myoblast cells was observed. These extensions were examined short and extensive (Fig. 10).

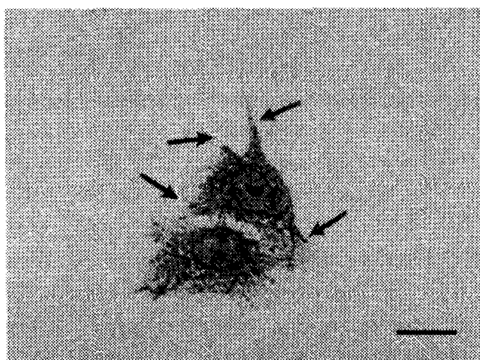


Figure 6. General image of myoblast cells after 48 h. Arrows; cytoplasmic protrusions. Scale Bar = 20 µm

Fibroblasts moved away from areas of high cell density towards determinated regions where there were fewer cells. We examined a triangle shape due to protrusions and long tail at the posterior of the cell during fibroblasts movement. These protrusions were predominantly located in front of the moving fibroblasts. Fibroblasts detached on substratum by means of these extensions were noticed. Fibroblasts gradually began to pull forward and extended to limits of plasma membrane. Long tail occurred cause extension. We showed that tail usually snap and remained on substrate, the cell moved forwards. We noticed lots of motile fibroblasts with Harris' hematoxylin stain (Fig.9). On the other hand, it was observed that myoblast cells moved towards each other to fuse. It is examined that myoblast cells fused each other by means of short and extensive cytoplasmic extensions in these tips 48 h later. We noticed that many myoblast cells fused like this with Harris' hematoxylin stain (Fig. 9). It is observed that mitotic activities of fibroblasts were much more than that of myoblast cells after 48 h.

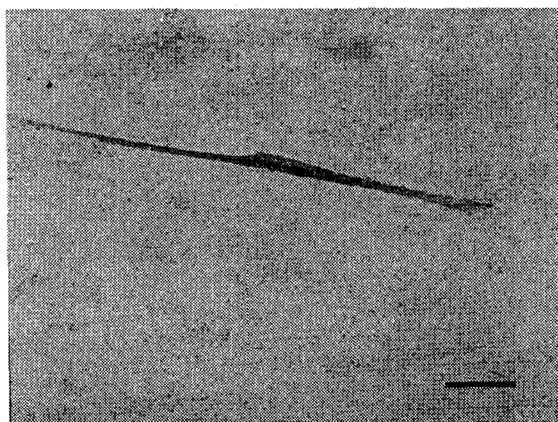


Figure 8. Granules surrounding nucleus in myoblast cells. Scale Bar = 20 um

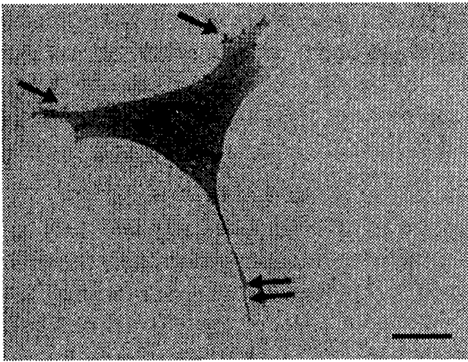


Figure 9. Motile fibroblast cells, Single arrow; membranes ruffles, double arrow, thin and long vell extension. Scale Bar = 20μm

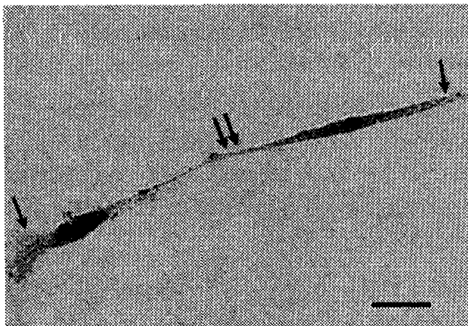


Figure 10. Fused myoblast cells. Single arrow; cell protrusion in two against tips, double arrow, fusion area. Scale Bar= 20 μm.

4. DISCUSSION

Organisms consist of different cells. These cells show lots of different movements that play important roles in numerous biological events. Therefore researchers examine these different movements to understand mechanisms of biological events. Although it is difficult to observe cell motility in vivo, some methods were improved in vitro as cell culture techniques.

Fibroblasts and myoblast cells were examined by histological methods in connective tissue but we didn't find any study comparing the differences of cell movements between cultured cells in vitro. In this study, we used and grew two different cell types in culture conditions from chicken embryo. Then, we analyzed both cell movements and morphological differences between fibroblasts and myoblast cells.

First of all, we realized that fibroblasts and myoblast cells had similar shapes after 24 h. In contrast to fibroblasts, shape difference was observed in myoblast cells after 48 h. Myoblast cells were capable of highly differentiation. We suggested that myoblast cells in culture didn't start differentiation therefore we observed similar shape both fibroblasts and myoblast cells.

Skeletal muscle of differentiation is characterized by at least four stages during embryological development. In the first step, mesenchymal cells differentiate into long, mononuclear skeletal muscle precursor called myoblast cells (Moore and Persaud, 2002). Secondly, myoblast cells proliferate by a number of mitotic divisions (Carlson, 1996). In the third step, the myoblast cells begin to fuse with one another to form elongated, multinucleated, cylindrical structures called myotubes (Torgan and Daniels, 2001). In the last step, myotubes are converted into muscle fibers and syntheses muscle-specific proteins. It was possible to see all the stages of myogenesis in our studies that myoblast cells fuse for myotube formation. Myoblast cells need to change cell shape for differentiation. Therefore their shapes are different from fibroblasts. Our data were parallel to the previous studies supported to knowledge of articles (Wheater et al, 1987). These stages are controlled and stimulated by muscle specific genes, K channels, calcium, calmodulin, cell adhesion molecules, and growth factors (Suzuki et al, 2000; Cooper, 2001, Lougheed et al, 2001).

We showed that two different cell membrane protrusions on fibroblasts. One of them was membrane ruffles that was sheet like extension of the cell membrane. This protrusion is called lamellipodia (Scaife and Langdon, 2000; Tsuji et al, 2002). The other extension of cell membrane was long and thin extension different form lamellipodia. We noticed that these protrusions predominantly located around the periphery of the cell. Beningo et al are reported that motile Goldfish fin fibroblasts determinated direction of cell migration via lamellipodia. This extension contact with substrate adhesion molecules (Beningo et al, 2001). Further study showed that Rat1 fibroblasts got longer and longer until it is so stretched that the back or trailing. The tail snaps cell moved forward. During the movement this triangle looks like the shape of a fibroblast cell (Arthur and Burridge, 2001). Our results supported these studies. We also showed that myoblast cells had different shape from fibroblasts after 48 h. Muscle differentiation causes an alteration of the cell shape (Cooper, 2001). We noticed that differentiation of muscle precursor cells had long, ovoid cell shape, protrusions short and large extension of the cell membrane. Myoblast cells were in bipolar shapes because of the extensions.

In this study, our results showed that many fused myoblast cells by means of protrusions located at two tips. These extensions played an important role to recognize each other to fusing myoblast cells. Myogenesis is essential to muscle tissue development but this mechanism poorly understood. Researchers still

investigate how muscle cells come together and fuse (Charlton et al, 1997). Myoblast cells come together via these protrusions that are located at two tips then fuse each other. These tips of the myoblast cells may support some special molecules for identification and fusion. Some researchers observed that some cell surface adhesion molecules localized at these tips. Other studies showed that multiple proteins were required for migration, and fusion of myoblast cells, such as integrins and the other cell surface adhesion molecules, metalloproteas, phosholipasess (Horsley et al, 2001). In addition to these molecules, increase in the intracellular calcium, several calcium-dependent molecules and potassium channels were also required (Lougheed et al, 2001).

Our data showed that fibroblasts migrated away from areas of high cell density toward regions where there were fewer cells. Recent studies reported that various factors, such as scatter factor (SF), plateled growth factor (PDGF), epidermal growth factor (EGF) effect on both fibroblasts and myoblast cells motiliy (Delehedde et al, 2001; Scall et al, 1999). Especially, SF is a protein usually released by fibroblats that cause dispersal of epithelial cell colonies and disruption of intercellular junctions, as well as an alteration of morphology with ruffling and rapid extension and movement of pseudopodia (Warn and Dowrick, 1989; Gherardi, 1991). Meanwhile this factor is required for movement and control of directing myogenic cells migration (Scall et al, 1999).

We showed that fibroblasts had more mitotic activity than myoblast cells. It is possible that differences in fibroblasts play an important role in wound healing process in connective tissue. Fibroblasts scatter and migrate across the wound surface and proliferate injured area (Warn et al, 2001). In contrast to myoblast cells, differentiated in muscular system, these cells have excessive mitotic activity. Our results suggest that after myoblast fusion, especially the rate of mitotic activity decreased. During development of skeletal muscle tissue, myoblast cells differentiate into muscle cell and lose division capability due to genetic regulation, intracellular calcium levels, several calcium dependent molecules and potassium channels (Horsley et al, 2001). These processes involved in many complex mechanisms that are not well delineated and still examine.

It was the purpose of the present article to estimate some different cell motility between cultured fibroblasts and myoblast cells. We compared different cell morphology and motility between fibroblasts and myoblast cells due to cytochemical staining methods. We showed that different movements in two different cells. Further studies are needed to identify which signals and molecules regulate these movements thus explaining molecular mechanisms.

REFERENCES

Arthur, W.T. and Burridge, K. (2001). RhoA inactivation by p190RhoGAP regulates cell spreading and migration by promoting membrane protrusion and polarity. *The Molecular Biology of the Cell,* 12, 2711-2720.

Balows, A., Trüper, H.G., Dwaekin, M., Harder, W., and Schleifer, K.H. (1992).*The Prokaryotes, Volume* I, pp. 62-64, Springer-Verlag.

Bancroft, J.D, and Stevens, A. (1980). Theory and Practice of Histological Techniques, pp. 436, *Churchill Living Stone,* Edinburg.

Beningo, K.A., Dembo, M., Kaverina, I., Small, V., and Wang, Y.L. (2001). Nascent focal adhesion are responsible for the generation of strong propulsive forces in migrating fibroblasts. *The Journal of Cell Biology* 153(4), 881-887.

Berns, W.M. (1983). Cell, Second Edition, pp.256, *Saunders Collage Publish,* New York.

British Drug House. (1960). Biological Stains and Staining Methods, pp. 34, *The British Drug House Ltd,* Dorset.

Browder, L.W., Ericson, C.A., and Jeffery, W.R. (1991), Developmental Biology, Third Edition, pp. 330-332, *Saunders College Publishing*

Carlson, B.M. (1996). Patten's Foundations of Embryology, Sixth Edition, pp. 34-35, *McGraw-Hill,* New York.

Charlton, C.A., Mohler, W.A., Radice, G.L., Hynes, R.O., and Blau, M. (1997). Fusion competence of myoblasts rendered genetically null for N-cadherin in culture. *The Journal of Cell Biology,* 138(2), 331-336.

Clark, G. (1981). Staining procedures, Fourth Edition, pp. 173-174, 106, 208, 123-124, *Williams& Wilkins,* Baltimore.

Clayden, C.E. (1962). Practical Section Cutting and Staining, Fourth Edition, pp. 198, J. and A. *Churchill Ltd,* London.

Cavallaro, U. and Christofori, G. (2001). Cell adhesion in tumour invasion and metastasis: loss of the glue is not enough. *Biochimica et Biophysica Acta,* 1552(1), 39-45.

Cooper, E. (2001). A new role for ion channels in myoblast fusion. *The Journal of Cell Biology,* 153(4), 9-11.

Delehedde, M., Sergeant, N., Lyon, M., Rudland, P.S., and Fernig, D.G. (2001). Hepatocyte growth factor/scatter factor stimulates migration of rat mammary fibroblasts through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways. *European Journal of Biochemistry* 269, 4423-4429.

Fawcett, D.W. and Jensh. R.P. (1997). Bloom&Fawcett Concise Histology, pp.47-48, *Chapman&Hall,* New York.

Freshney, I.R. (1994). Culture of Animal Cells, Thirds Edition, pp. 129-136, *John Wiley and Sons Inc Publication,* New York

Gartner, L.P. and Hiatt, J.L. (1997). Color Textbook of Histology, pp.93-95,131, *W.B.Saunders Company,* Philadelphia.

Gartner, L.P and Hiatt, J.L. (1994). Color Atlas of Histology, Second Edition, pp.43-52, *Williams&Wilkins,* Baltimore.

Gherardi, E. (1991). Growth factors and cell movement. *European Journal of Cancer,* 27(4), 405-407.

Horsley, V., Friday, B.B., Matteson, S., Kegley, K.M., Gephart, J., and Pavlath, G.K. (2001). Regulation of the growth of multinucleated muscle cells by an NFATC2-dependent pathway. *The Journal of Cell Biology,* 153(2), 329-338.

Jakoby, B.W and Paston, H.I. (1979). Cell Culture, pp. 511-527, *Academics Press Limited,* San Diego.

Lazarides, E. and Revel. J.P. (1979). The molecular basis of cell movement. *Scientific American,* 12(4), 88-100.

Lougheed, J.F., Liu, J.H., Espinos, E., Mordasini, D., Bader, C.R., Belin, D., and Bernheim, L. (2001). Human myoblast fusion requires expressin of functional ınward rectifier Kir2.1 channels. *The Journal of Cell Biology,* 153(4), 677-685.

Moore, K.L. and Persaud, T.V.N. (2002). Kilinik Yönleri ile İnsan Embriyolojisi, (Çev, Yıldırım, M), Altıncı Baskı, pp. 426-427, *Nobel Tıp Kitapevleri,* İstanbul.

Parker, K.K., Brock, A.I., Brangwynne, C., Mannix, R.J., Wang, N., Ostuni, E., Geisse, N.A., Adams, J.C., Whitesides, G.M., and Ingber, D.E. (2002). Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *The Faseb Journal,* 16(10), 1195-1204.

Raffetto, J.D., Mendez, M.V., Marien, B.J., Byers, R., Phillips, T.J., Park, H.Y., and Menzoian, J.O. (2001). Changes in cellular motility and cytoskeletal actin in fibroblast from patients with chronic venous insufficiency and in neonatal fibroblasts in the presence of chronic wound fluid. *The Journal of Vascular Surgery* 33(6), 1233-1241.

Raucher, D and Sheetz, M.P. (2000). Cell spreading and lamellipodial extension rate is regulated by membrane tension. *The Journal of Cell Biology* 148(1), 127-136.

Scall, M., Bonafede, A., Dathe, V., Sachs, M., Cann, G., Christ, B., and Saberi, B.B. (1999). SF/HGF is a mediator between limb patterning and muscle development. *Development,* 126, 4885-4893.

Scaife, R.M. and Langdon, W.Y. (2000). c-Cbl localizes to action lamellae and regulates lamellipodia formation and cell morphology. *The Journal of Cell Science,* 113, 215-226.

Smith, A. and Bruton.J. (1979). Histological Staining Techniques, pp. 193, *Wolfe Medical Publications Ltd,* London.

Suzuki, J., Yamazaki, Y., Guang, L., Kaziro, Y., and Koide, H. (2000). Involvement of Ras and Ral in chemotatic migration of skeletal myoblasts. *Molecular and Cellular Biology,* 20(13), 4658-4665.

Taylor, W.E. (1986). Cell motility, *Journal of Cell Science* Supplement, 4, 89-102.

Torgan, C.E. and Daniels, M.P. (2001). Regulation of myosin heavy chain expression during rat skeletal muscle development in vitro. *The Journal of Molecular Biology of the Cell,* 12, 1499-1508.

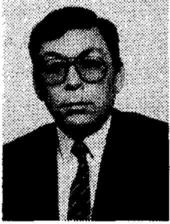
Tsuji, T., Ishizaki, T., Okamoto, M., Higashida,C., Kimura, K., Furuyashiki, T., Arakawa, Y., Birge, R.B., Nakamoto, T., Hirai, H., and Norumiya, S. (2002). Rock and mDia1 antagonise in Rho-dependent Rac activation in Swiss 3T3 fibroblasts. *The Journal of Cell Biology* 157(5), 819-830.

Warn, R.M. and Dowrick, P. (1989). Motility factors on the march. *Nature,* 340, 186-187.

Warn, R., Harvey, P., Warn, A., Comer, A.F., Heldin, P., Versnel, M., Arakaki, N., Daikuhara, Y., Laurent, G.J., Herrick, S.E., and Mutsaers, S.E. (2001). HGF/SF induces mesothelial cell migration and proliferation by autocrine and paracrine pathways. *Experimental Cell Research,* 267, 258-266.

Wheater, P.R., Burkitt, H.G.,and Daniels, V.G. (1987). Functional Histology a Text and Colour Atlas, Second Edition, pp. 52-54, *Churchill Livingstone,* Edinburgh.

Young, B. and Heath, J.W. (2000). Wheater's and Functional Histology a Text and Colour Atlas, Fourth Edition, pp. 27, 69-71, *Harcourt Publishers Limited,* Edinburg.



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