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İnguinal Herni Keselerinden Elde Edilen Doku Örneklerinin Kültüründen Düz Kas Hücreleri Türetilmiştir

Smooth Muscle Cells are Derived Predominantly from Tissue Explant of Inguinal Hernia Sac

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ÖZ

GİRİŞ ve AMAÇ: Processus vaginalisin (PV) kapanmasının, testisin inişini sağlamak için geçici olarak bulunan düz kas hücrelerinin, devamlılığına bağlı olduğu ileri sürülmüştür. Bu amaçla, inguinal herni keseleri, PV kapanması ve düz kas hücre varlığı arasındaki ilişkinin değerlendirilmesi için kültüre edildi. Bu çalışma, sadece inguinal herni keselerinde düz kas hücresi varlığını gösteren ek bilgiler vermekle kalmayıp, aynı zamanda inguinal herninin cerrahi dışı tedavisi için yapılacak çalışmalar için yeni fikirler sunmaktadır.

YÖNTEM ve GEREÇLER: Herni keseleri, yaşları 2 ay ile 5 yaş arasında değişen 11 çocuktan inguinal herni ameliyatı sırasında elde edildi. Örnekler uygun şekilde hazırlandı ve kültüre edildi. Hücrelerin morfolojik özellikleri ışık mikroskobu ile değerlendirildi. Hücrelerin hayatiyetleri, tripan blue exclusion metodu ile değerlendirildi. Gelişen hücreler, imminohistokimyasal olarak aktin ve miyozin ile boyandı.

BULGULAR: Işık mikroskobu incelemesi ile bu hücrelerin iğ şekilli olduğu ve santral yerleşimli yuvarlak çekirdeklerinin bulunduğu görülmüştür. Tüm flask üreyen hücrelerle dolduğunda, kontakt inhibisyon olmadığından, üst üste çoğalan hücreler tipik tepe-vadi görünümü oluşturmuştur. Hücrelerin canlılığı %95' in üzerinde bulunmuştur. Gelişen hücrelerin % 80 inin düz kas aktin ve düz kas myosin antikorları ile boyandığı saptanmıştır.

TARTIŞMA ve SONUÇ: Herni kesesi dokulardan gelişen hücrelerin büyük çoğunluğunu, düz kas hücreleri oluşturmuştur. Bu bulgu, PV'in inhibisyonu ile düz kas hücresi varlığı arasındaki ilişkiyi desteklemektedir. Bu bilgi inguinal herninin cerrahi dışı tedavisi üzerinde yapılacak çalışmalar için kullanılabilecektir.

Yayın hakları Güncel Pediatri'ye aittir.

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Anahtar Kelimeler: inguinal herni kesesi, düz kas hücresi, doku kültürü
Türkçe Kısa Başlık: İnguinal hernide doku kültürü

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ABSTRACT

INTRODUCTION: Obliteration of processus vaginalis (PV) has been proposed to result from persistence of smooth muscle which is presented transiently to propel the testis. Sacs associated with inguinal hernia were cultivated to define the cells that are going to proliferate for evaluating the association of inhibition of obliteration of PV and the presence of smooth muscle (SM). The present study does not only provide additional information about the presence of SM in sacs from boys with inguinal hernia, but also provides a new tool for researches directed to define the non- operative treatment of inguinal hernia.

METHODS: Hernia sacs were obtained from eleven boys with the ages ranging from two months to five years during operations for inguinal hernia. Samples were prepared and cultivated. Morphologic characteristics of cell populations were examined by light microscopy. Viability was estimated by trypan blue exclusion method. Growing cells were identified via immunohistochemical staining for smooth muscle actin and myosin.

RESULTS: Light microscopic images of growing cells displayed characteristic spindle shaped morphology with centrally located round nucleus. When the flasks reached confluence, a hill-valley appearance was observed because of absence of contact inhibition. Cell viability was found more than 95%. Approximately, 80% of growing cell populations was stained positive with actins and myosin antibodies.

DISCUSSION and CONCLUSION: In tissue explants of hernia sac, most commonly proliferating cell type is smooth muscle cells. This evidence supports the association of inhibition of PV and the presence of SM. The SM obtained from sacs associated with inguinal hernia may be used for researches directed to establish the non-operative treatment of inguinal hernia.

Keywords: inguinal hernia sac, smooth muscle cell, tissue culture

İngilizce Kısa Başlık: Tissue Engineering in Inguinal Hernia

Introduction:

Inguinal hernia is the most common pediatric surgical pathology occurring with a frequency from 1% to 3% at birth (1). The testis descends into the scrotum via procesus vaginalis (PV). Failed obliteration of the PV results in an inguinal hernia, hydrocele or hydrocele of the cord. The exact mechanism of obliteration of PV remains unexplained. According to one of the current explanations, smooth muscle (SM) and striated muscles are reported to develop from gubernaculum, which is the preceeding mesenchymal tissue. The testis is propelled through the PV via the propulsive force generated by the SM into the scrotum. After descending the testis, the SM undergoes programmed cell death. If the SM persists, it hinders the obliteration of PV (2).

If the SM is associated with the persistence of PV, the cultivation of sacs should also yield SM. Therefore sacs associated with inguinal hernia are cultivated to define the cells that are going to derive.

Materials and Methods:

Samples of sacs associated with inguinal hernia were obtained during operations from eleven boys with the ages ranging from 2 months to 5 years. Tissues were mechanically disrupted by surgical blades under laminar airflow and placed onto dishes. They were cultured under standard conditions using Dulbeco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum and 1 ml of solution that contains 100 U penicillin and 0.1 mg streptomycin. Culture medium was changed once in three days along 4-5 weeks until confluence was reached. Morphological examination of cell populations was performed using light microscopy. Culture flasks were treated with trypsin and cells were resuspended. Viability was estimated by trypan blue exclusion method. Cells were seeded onto slides and used for immunohistochemical study. At second passage, the isolated cells were identified by immunostaining for SM actin and myosin using monoclonal antibodies against these proteins (Sigma-Aldrich Co. LLC, Taufkirchen Germany). In this indirect immunoperoxidase procedure (Biogenex, Fremont CA, USA), the peroxidase activity was visualized by incubation in 3-amino-9-ethyl-carbazole. The slides were examined by light microscopy (Nikon, Tokyo, Japan). Control sections were incubated in the absence of primary antibody.

Results:

Emerging cells from explant tissue were detected following 3 to 4 days of seeding onto dishes. Light microscopic examination of cells from all samples has displayed cells that predominantly have characteristic spindle shaped morphology with centrally located round nucleus. Rare round or angular flat cell colonies and fusiform, firm conjugated cells with apically located round nucleus and fibril

elongations were also detected. These cells were identified as epithelial cells and fibroblasts, respectively. When the flasks reached confluence, a hill-valley appearance was observed because of absence of contact inhibition (Figure 1-2). Cell viability of populations was found to be more than 95%. Immunohistochemical studies have showed the expression of smooth muscle actin and myosin by 80% of the growing cells.

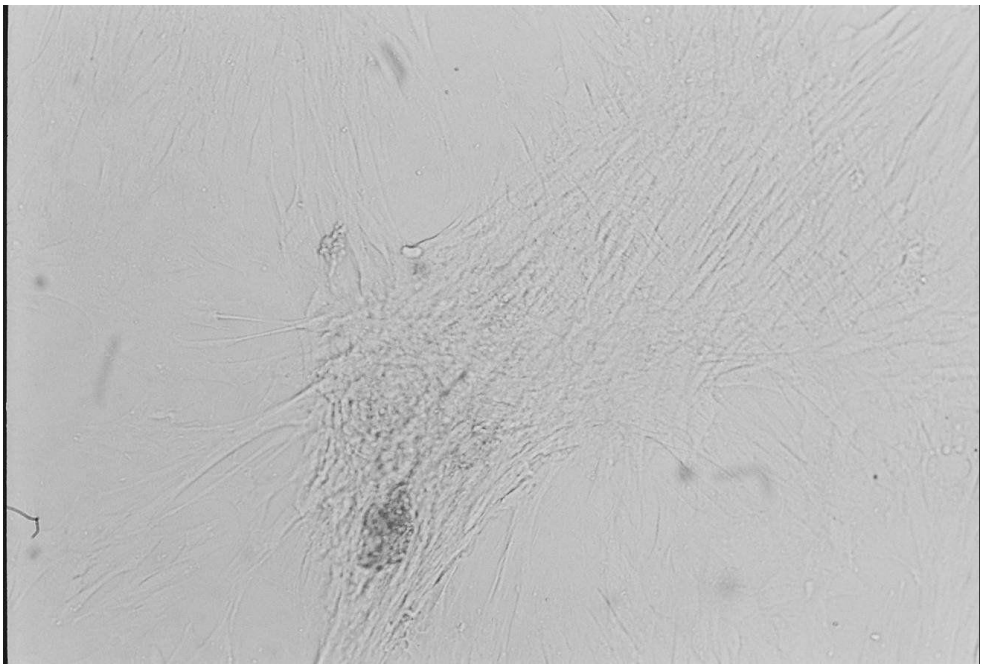

Figure 1: Hill and valley sign formed by growing cells.

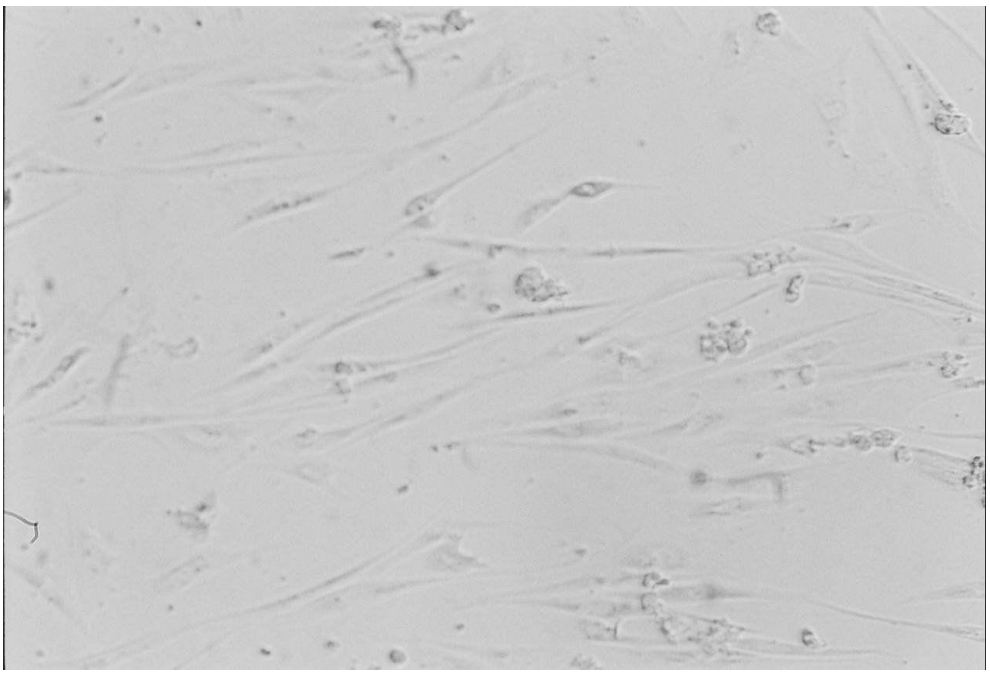

Figure 2: Separately proliferating cells

Discussion:

Inguinal hernia is the most common pediatric surgical diseases. It is resulted from failed closure of processus vaginalis that is described as a simple peritoneal extension through which testis descends. (3,4) Although close relationship between testicular descent and PV enlargement is clear, the cause of unclosure is still controversial (5).

The patency of PV that results in an inguinal hernia is suggested to associate with the presence of smooth muscle. Therefore the persistence of SM that should undergo programmed cell death after propelling the testis has been considered to hinder the obliteration (2).

The SM appears in the gubernaculum around the PV at the 26th week of fetal life. The testis is suggested to descend through the PV via the propulsive force generated by the SM (2). While sacs associated with inguinal hernia contain most, sacs associated with undescended testis contains least SM. The obliteration of PV has been suggested to occur through the programmed cell death of SM, which also involves a dedifferentiation into myofibroblasts (5). A decrease in sympathetic tonus has been suggested to be mandatory for the initiation of programmed cell death through the intrinsic pathway that involves the increase in cytosolic calcium (6,7). Therefore the decrease in sympathetic tonus after propulsion of testis into the scrotum has been proposed to be the physiologic mechanism of obliteration of PV through the programmed cell death of SM (4). If the mechanism is initiated before the descent of testis, the amount of SM decreases and the descent is not succeeded. If aberrations occur and the SM persists, the obliteration is inhibited (2).

The present study has been conducted to determine the cells that grow through cultivation of sacs associated with inguinal hernia. The cultivation of sacs from boys with inguinal hernia has also revealed the growth of SM. Furthermore, 80% of cells cultivated from sacs have been SM. In addition to SM, fibroblasts and epithelial cells were grown. We think that this growth is additional evidence for the association between the presence of SM and inhibition of obliteration of PV.

If the failed obliteration of PV results from persistence of SM, the failure of programmed cell death in SM should result in obliteration of PV. Therefore inducing apoptosis in the SM content of PV may result in non-operative treatment of inguinal hernia. The SM obtained through cultivation of sacs associated with inguinal hernia may represent an important tool for studies directed to succeed the non-operative treatment of inguinal hernia.

The present study does not only provide additional information about the presence of SM in sacs from boys with inguinal hernia, but also provides a new tool for researches directed to define the non- operative treatment of inguinal hernia.

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