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Differences in the Development of the Processus Vaginalis between Children with Undescended Testis and Inguinal Hernia

Dimitrios Sfoungaris, Vassilios Mouravas, Triantafyllia Koletsa[1], Vassilios Lambropoulos, Ioannis Kostopoulos[1], Ioannis Spyridakis

Second Department of Pediatric Surgery, Aristotle University of Thessaloniki, General Hospital Papageorgiou, Nea Efkarpia, 56403, [1]Department of Pathology, Aristotle University of Thessaloniki, School of Medicine, University Campus, 54124 Thessaloniki, Greece

ABSTRACT

Background: It has been shown in several investigations that smooth muscle cells (SMCs) are present on the patent processus vaginalis (PV) peritonei in cases of inguinal hernia (IH) preventing its obliteration. The PV fails to obliterate in cases of undescended testis (UT) as well, but without causing herniation. **Materials and Methods:** We conducted a case control study in order to compare the status of the SMCs present on the PV in UT and IH cases and correlate it with the clinical outcome of herniation. Specimens were harvested from the hernia sacs of 26 boys with IH aged from 2 days to 16 years (mean 44.31 months) and the PV of 14 children with UT, aged from 13 months to 13 years (mean 30.28 months). They were examined histologically and immunohistochemically for markers of mature SMC such as smooth muscle actin (SMA), desmin and h-caldesmon as well as for vimentin, an immature SMC marker. **Results:** The expression of SMA, desmin, and h-caldesmon was present in all cases of IH and UT. Vimentin was expressed in 13 out of 14 (93%) UT specimens and in 12 out of 26 IH sacs (46.1%), (P = 0.0102). In IH and UT cases, when vimentin was not expressed, SMCs were organized in bundles. **Conclusion:** The SMCs on the PV in UT cases reach a more advanced stage of dedifferentiation that corresponds to a status more close to that of the natural obliteration, compared to IH cases, preventing herniation to occur.

Key words:
Apoptosis, inguinal hernia, processus vaginalis, programed cell death, undescended testis

INTRODUCTION

Inguinal hernia (IH) in children is inevitably related with the presence of a patent processus vaginalis (PV) peritonei but, even though 78% of cases with undescended testis (UT) are associated with a patent PV, herniation is very rare among them.[1] Hernia sacs of children have been shown to carry on their wall a considerable amount of smooth muscle cells (SMCs) organized in bundles[2] and with a mature phenotype, as demonstrated immunohistochemically.[3,4] This is considered as a result of a halted programmed cell death (PCD) process and a reason for nonobliteration of the PV, since SMCs are not observed on peritoneal tissue and on obliterated PV specimens used as controls in the abovementioned investigation.[4] The question remains on the configuration and developmental status of the SMCs on the PV of children with UT, which even though it remains unobliterated, it does not cause herniation.

MATERIALS AND METHODS

We conducted a case control study in order to determine differences in the PV configuration of the SMCs on the PV of cases with UT, which fail to develop herniation, and cases with IH. Twenty-six consecutive male patients were operated on for IH and 16 for unilateral UT participated

Address for correspondence:
Dr. Dimitrios Sfoungaris,
Second Department of Pediatric Surgery, Aristotle University of Thessaloniki, General Hospital Papageorgiou, Thessaloniki Ring Road, Nea Efkarpia, 56403, Thessaloniki, Greece.
E-mail: surgicalpediatrics@gmail.com

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in this study, after informed consent was received from their families. Surgical repair of their pathology was carried through inguinal approach and opening of the anterior wall of the inguinal canal. In two UT cases, no PV was found among the cord structures and these cases were excluded from the study. The hernia sac or the PV was dissected, ligated, divided to the level of the deep inguinal ring, as part of the procedure, and harvested as specimen, which was offered for further investigation in the pathology laboratory. The laboratory personnel were blinded for each patient's condition.

The hernia sacs of 26 boys with IH, aged from 2 days to 16 years (mean 44.31 months) and the PV of 14 children with UT, aged from 13 months to 13 years (mean 30.28 months) were included in this study. There were no significant differences in age between the two groups (*t*-statistics among unrelated groups, $P = 0.213$).

All tissue samples were fixed in 10% formalin buffer and sections from the specimens were embedded in paraffin blocks. Four micron thick paraffin sections were stained with hematoxylin and eosin. Unstained paraffin sections were used for immunohistochemical stains. Immunostains for vimentin (clone M0725, DakoCytomation), smooth muscle actin (SMA) (clone 1A4, DakoCytomation), desmin (clone D33, DakoCytomation), and caldesmon (h-CD, DakoCytomation) were performed. After deparaffinization in xylene and hydration in graded ethanol solutions, the sections were subjected to pretreatment in order to enhance antigen retrieval. A standard streptavidin-biotin method was used. As a final step, counterstaining with hematoxylin was performed.

Immunohistochemistry evaluation

Whole section surface was examined carefully for SMC identification by two experienced pathologists (TK and IK). The pathologists were blinded to clinical information of each specimen. The immunoreactivity of SMCs to all abovementioned immunostains was evaluated. Cells were considered as positive for vimentin, SMA, desmin, and caldesmon when specific intracytoplasmic and/or membranous staining was observed. Simultaneous staining of tissue sections from intestinal wall was used as positive control for the antibodies. Omission of primary antibody was used as negative control for each specimen to assess the specificity of staining.

The data were analyzed statistically using computer software. The *t*-statistics and χ^2 test were conducted with the help of IBM SPSS 18.0 package. The sensitivity and specificity tests were conducted with the help of MedCalc online statistical calculator. The probability level $P = 0.05$ or less was chosen to represent statistical significance.

RESULTS

Conventional histology showed fibrous connective tissue lined across the inner surface with mesothelial cells. Underneath the mesothelium, there was a layer of loose connective tissue and following that, a layer of dense fibrous connective tissue.

The expression of SMA, desmin, and h-caldesmon was distributed in the connective tissue of the specimens obtained from both IH and UT.

The findings which were different among the UT and IH groups [Table 1] were as follows:

- The SMCs from UT specimens were dispersed on the PV. Vimentin was expressed in 13 (93%) out of the total 14 cases [Figure 1]. Organized bundles of SMCs were found only in the case where vimentin was not expressed
- The SMCs from IH specimens were found to be organized in bundles in 14 (54%) out of 26 specimens of hernia sacs and these were negative to vimentin stain [Figure 2]. The SMCs were dispersed in the rest 12 (46.1%) specimens, where SMCs were positive to vimentin stain.

Among the UT and IH cases, the differences in incidence of SMC bundles and vimentin stain are of statistical importance (χ^2 test, $P = 0.0102$).

In IH and UT cases, whenever organized bundles of SMC were apparent, positivity to SMA was constantly evident, whereas vimentin was not expressed. In cases with expression of vimentin, there were no SMC bundles but rather diffusely distributed SMC, which were not organized in functional structures.

When a herniation was present, the probability of the PV to carry SMCs in bundles, staining negative to vimentin (i.e., a predominantly mature phenotype) is 53.85%, with a confidence interval (CI) from 33.37% to 73.41% (true positive rate).

When a herniation was not present, the probability of the PV to carry dispersed SMCs that stain positive to vimentin (i.e., a predominantly immature phenotype, more

Table 1: Vimentin expression in IH and UT cases

	Vimentin (%)		Total
	Not expressed	Expressed	
IH	14 (54)	12 (46)	26
UT	1 (7)	13 (93)	14

UT – Undescended testis; IH – Inguinal hernia

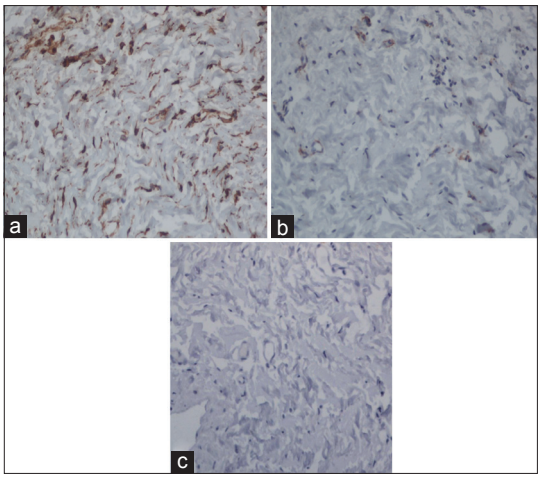

Figure 1: Smooth muscle cells on the processus vaginalis of a case with undescended testis. (a) Smooth muscle cells positive for vimentin (×200). (b) Negative for smooth muscle actin (×200). No bundles of smooth muscle cells are observed. (c) Negative control (×200)

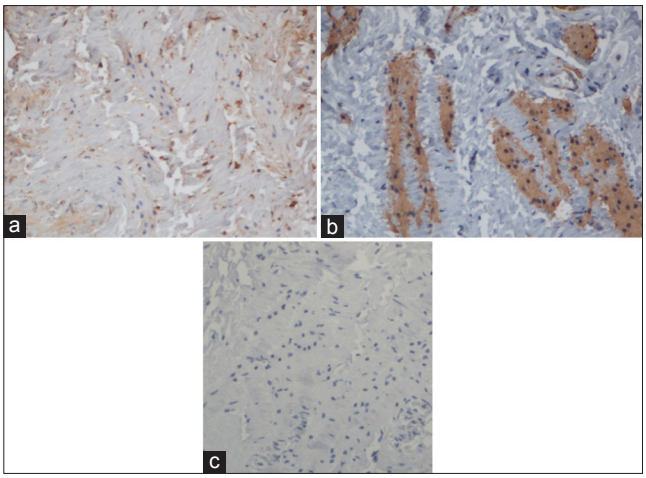

Figure 2: Smooth muscle cells on an inguinal hernia sac. (a) Smooth muscle cells in bundles negative for vimentin (×200). (b) Smooth muscle cells in bundles positive for smooth muscle actin (×200). (c) Negative control (×200)

advanced in the process of dedifferentiation and in a state more close to the natural outcome of apoptosis) is 92.86% with CI from 66.13–99.82% (true negative rate).

DISCUSSION

The PV develops as a diverticulum of the peritoneal membrane inside the gubernaculum, surrounded by mesenchyme. At 25–28 weeks of gestation, the testis descends rapidly through the inguinal canal, which has just formed, and then migrates down into the scrotum, arriving there at about 35–40 weeks of gestation.[5] The gubernaculular mesenchyme gives rise to muscle cells, which appear starting differentiation in 12–19 weeks fetuses, are still apparent in 20–25 weeks fetuses[6] and may reflect a further differentiation of the previously appearing myofibroblasts.[7]

The normally occurring obliteration of the PV before birth is probably a consequence of the elimination of the SMC from the PV through the process of PCD, also known as apoptosis, which starts by SMC dedifferentiation from the evolved contractile to the primitive synthetic phenotype.[3,7] This process has been shown to take place in cell culture, during atherogenesis, and after sympathectomy.[8-12] These two distinct phenotypes of SMC can be identified by their ultrastructural equipment and by the type of cytoskeletal proteins: In the process of differentiation/maturation of SMCs from the synthetic to the contractile phenotype, a progressively increased expression of desmin, smooth muscle a-actin and h-caldesmon, as well as a decreased expression of vimentin is identified. In the process of dedifferentiation and PCD, immature SMC of synthetic phenotype appear, characterized by well-developed Golgi apparatus and reduced contractile myofilaments, which express vimentin as an intermediate filament.[11,12]

The completion of the PCD process (apoptosis) is indicated by the elimination of SMCs on the PV which obliterates and a degenerated gumbernaculum.[3,4]

Several factors can intervene with the normal process of PCD and may lead to incomplete obliteration of the PV.[2] Hernia sacs of boys and girls contain SMCs, indicating that PCD was not completed.[3,4] In this study, SMCs were also found on the PV of UT, in accordance with other researchers that used conventional histology techniques.[2] In our study, through the use of immunohistochemical technique we are able to trace the more advanced dedifferentiated stage of the SMCs on the UT specimens by the increased incidence of vimentin expression (93%), in comparison to IH cases, where vimentin is expressed less often (46%).

The intimate anatomic and chronological relation of scrotal testicular descent and PV occlusion has led to several speculations, on how some of these factors affect PV development and testicular descent.[3,5] Androgens affect testicular descent by acting directly on the gumbernaculum and through their effects on the sensory nucleus of the genitofemoral nerve (GFN). Unilateral transection of the GFN in rodents causes ipsilateral cryptorchidism[13] affecting gubernacular migration and muscle cell differentiation, acting via the neurotransmitter calcitonin gene-related peptide (CGRP). CGRP elicits rhythmic contractions of rodent neonatal gubernacula in culture and stimulates the growth and differentiation of neonatal myogenic cells.[5,13] Several studies indicate that sympathetic innervation increases vascular smooth muscle contractile protein expression and thereby suggest that sympathetic nervous system promotes and/or maintains vascular smooth muscle differentiation.[14,15] On the other hand, sympathectomy influences the phenotypic modulations of SMCs towards a

dedifferentiated state, with higher expression of vimentin and lower expression of h-caldesmon.[8-10] PCD appears to be guided by a decrease in sympathetic and concomitant increase in parasympathetic tonuses of varying intensity, in alteration sequence and/or sustenance during a critical time period.[3] An upregulated sympathetic tonus prevents the natural process of dedifferentiation, PCD is not induced or is discontinued and mature SMC remain on the PV organized in bundles, preventing its obliteration and causing IH.[4] If a decrease in sympathetic tonus does takes place, but in a not so well-tuned sequence, it may cause partial dedifferentiation of the SMC of the PV. This has been shown to take place also in the partially closed sac in hydroceles where mature SMC are present alongside with high expression of vimentine.[4] In the present study, our findings are consistent with stimuli less efficient than normal for the completion of PCD, but more intense than those in IH cases.

CONCLUSION

In IH cases, the SMCs on the unobliterated PV are in a more differentiated state compared to those in UT cases where a step closer towards the normal evolutionary endpoint of PV obliteration has been reached. The clinical expression is analogous: In contrast to what happens in IH cases, the PV in UT does not permit visceral herniation, even though it remains open.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Donnell SC, Rickwood AM, Jee LD, Jackson M. Congenital testicular maldescent: Significance of the complete hernial sac. Br J Urol 1995;75:702-3.
2. Piçarro C, Tatsuo ES, Amaral VF, Gomez RS, Cruzeiro PC, Lanna JC. Morphological comparison of processus vaginalis from boys with undescended testis and hernia sacs from boys with inguinal hernia. Eur J Pediatr Surg 2009;19:145-7.
3. Tanyel FC. Obliteration of processus vaginalis: Aberrations in the regulatory mechanism result in an inguinal hernia, hydrocele or undescended testis. Turk J Pediatr 2004;46 Suppl: 18-27.
4. Mouravas VK, Koletsa T, Sfougaris DK, Philippopoulos A, Petropoulos AS, Zavitsanakis A, *et al.* Smooth muscle cell differentiation in the processus vaginalis of children with hernia or hydrocele. Hernia 2010;14:187-91.
5. Hutson JM, Nation T, Balic A, Southwell BR. The role of the gubernaculum in the descent and undescent of the testis. Ther Adv Urol 2009;1:115-21.
6. Youssef EH, Raslan NA. Study of the factors which affect the descent of the testicles in man. Acta Anat (Basel) 1971;79:422-44.
7. Tanyel FC, Talim B, Atilla P, Müftüoglu S, Kale G. Myogenesis within the human gubernaculum: Histological and immunohistochemical evaluation. Eur J Pediatr Surg 2005;15:175-9.
8. Fetalvero KM, Shyu M, Nomikos AP, Chiu YF, Wagner RJ, Powell RJ, *et al.* The prostacyclin receptor induces human vascular smooth muscle cell differentiation via the protein kinase A pathway. Am J Physiol Heart Circ Physiol 2006;290:H1337-46.
9. Puzdrova VA, Kudryashova TV, Gaynullina DK, Mochalov SV, Aalkjaer C, Nilsson H, *et al.* Trophic action of sympathetic nerves reduces arterial smooth muscle Ca(2) sensitivity during early post-natal development in rats. Acta Physiol (Oxf) 2014;212:128-41.
10. Kacem K, Sercombe C, Hammami M, Vicaut E, Sercombe R. Sympathectomy causes aggravated lesions and dedifferentiation in large rabbit atherosclerotic arteries without involving nitric oxide. J Vasc Res 2006;43:289-305.
11. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004;84:767-801.
12. Schwartz SM, Campbell GR, Campbell JH. Replication of smooth muscle cells in vascular disease. Circ Res 1986;58:427-44.
13. Goh DW, Momose Y, Middlesworth W, Hutson JM. The relationship among calcitonin gene-related peptide, androgens and gubernacular development in 3 animal models of cryptorchidism. J Urol 1993;150 (2 Pt 2):574-6.
14. Damon DH. Sympathetic innervation promotes vascular smooth muscle differentiation. Am J Physiol Heart Circ Physiol 2005;288:H2785-91.
15. Sasaki Y, Hutson JM, Clarke MC. The role of sympathetic innervation in the developing rat gubernaculum. J Pediatr Surg 2007;42:350-4.