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Original contribution

Clonality assessment of adenomatoid tumor supports its neoplastic nature ☆

Wei Wang MS[a,1], Huiting Zhu PhD[b,1], Jigang Wang PhD[a], Shuyang Wang PhD[a], Diyi Wang MS[c], Jingjing Zhao MS[b], Hongguang Zhu PhD[a,d,*]

[a]*Department of Pathology, Fudan University Shanghai Medical College, Shanghai 200030, China*
[b]*Department of Pathology, Shanghai First Maternity and Infant Hospital, Shanghai 200120, China*
[c]*Department of Pathology, The Affiliated Hospital of Taishan Medical College, Taian 271000, China*
[d]*Department of Pathology, Huashan Hospital, Fudan University, Shanghai 200040, China*

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Summary Adenomatoid tumor is a relatively rare disease that predominantly involves male and female internal genital tracts. Although its clinical and pathologic features are well characterized, there is still controversy regarding its nature as a true neoplasm or a variant of mesothelial hyperplasia of a reactive nature. We sought to resolve this debate by investigating the clonality of uterine adenomatoid tumor from 13 female cases. The mesothelial cells and surrounding normal myometrium were precisely harvested using laser capture microdissection, and genomic DNA was extracted for clonal analysis by assessing the patterns of X-chromosome inactivation. Fluorescent polymerase chain reaction amplification of a highly polymorphic short tandem repeat of the human androgen receptor (*HUMARA*) gene with and without methylation-sensitive restriction endonuclease HpaII digestion was performed on DNA extracted from mesothelial cells, using normal myometrium and male blood sample as controls. Of the 13 cases successfully amplified, all 10 informative cases showed concordant nonrandom X-chromosome inactivation pattern consistent with monoclonality. In comparison, surrounding normal myometrium showed a polyclonal pattern of X-chromosome inactivation, and male blood sample failed to be amplified after HpaII treatment. Our results demonstrate that adenomatoid tumor is a monoclonal disease favoring a neoplastic process. This neoplastic rather than reactive nature probably accounts for its frequently observed infiltrative growth pattern and the occurrence of diffuse adenomatoid tumor, especially when host immunity is compromised. Additional studies with larger sample sizes will be needed to conclusively prove our conclusion.

1. Introduction

Adenomatoid tumor is a relatively rare disease that is best known for its proclivity to involve both male and female internal genital tracts: epididymis, uterus, and fallopian tube [1]. It has also occasionally been reported to occur in other sites, such as the pancreas [2], the mediastinum [3], the heart

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* Corresponding author at: Department of Pathology, Fudan University Shanghai Medical College, Shanghai 200030, China.
E-mail address: hongguang_701@shmu.edu.cn (H. Zhu).

[1] Contributed equally to this work.

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[4], and the adrenal gland [5]. It is often incidentally found in women who are in middle age or older for unrelated medical conditions and in men manifesting as an intrascrotal mass without appreciable growth over years. This lesion is generally small, measuring a few centimeters, although larger or even giant ones have been described [6]. Microscopically, diverse morphologic patterns have been reported, and the characteristic one is of anastomosing cystic or slit-like structures lined by flattened or cuboidal cells intermixed with abundant hypertrophied smooth muscle fibers. Some adenomatoid tumors are predominantly composed of numerous cystic spaces, which entail differential diagnosis with lymphangioma. Adenomatoid tumor is considered to be a benign tumor of mesothelial origin [7] and harbor no potential for malignant transformation.

Although the clinical and pathologic features of adenomatoid tumor are thoroughly characterized [8,9], its nature and pathogenesis are by no means well understood. There is not even a uniform agreement on whether it is a true neoplasm or a pseudoneoplastic inflammatory process [1,10]. It was considered by some authors to be a variant of benign mesothelioma restricted to the genital tracts [11], and local invasion has also been noticed [12]. Nevertheless, no recurrent chromosomal aberrations have been detected so far, and histopathologically, it is also pretty common to observe chronic lymphocytic infiltration or even germinal centers in some adenomatoid tumors [8,9], highlighting the possibility that adenomatoid tumor may actually represent a variant of localized nodular mesothelial hyperplasia of a reactive nature [9,13].

The differentiation of a true mesothelial neoplasm from mesothelial hyperplasia can be confirmed by monoclonality, one of the distinguishing characteristics of neoplastic lesions [14,15]. It is accepted that tumor parenchymal cells are descended from a common progenitor and thus being monoclonal, while reactive lesions are often polyclonal [15]. An important and widely used indicator of clonality takes advantage of the inactivation pattern of the X chromosomes in females. Based on Lyon's hypothesis [16], 1 of the 2 X chromosomes in each cell is randomly inactivated in the form of hypermethylation at the late blastocyte stage of embryogenesis of females. Once this methylation pattern is established, it is conserved, reproducible, and transmitted permanently to all progeny cells during cell divisions. Hence, normal somatic tissues in females are composed of one half of cells with inactivated maternal X chromosomes and the other half with inactivated paternal X chromosomes. By comparison, neoplastic cells that are originated from the same progenitor show uniform X-chromosome inactivation pattern, and the same parental allele is inactivated in all the cells.

Techniques used to assess clonality follow the principle of Lyon's hypothesis. Polymerase chain reaction (PCR) amplification of certain allelic loci after methylation-sensitive restriction enzyme digestion is used extensively to analyze X-chromosome inactivation pattern. Of all the allelic loci candidates, the most widely used one is the human androgen receptor (*HUMARA*) gene on X chromosomes that contains a hypervariable trinucleotide-repeat (CAG) sequence in its first exon, and reported heterozygosity for this genetic locus can be as high as 90% in Caucasians [17]. Immediately upstream of the CAG repeats are invariable CpG sites on the inactivated X-chromosome that defy the digestion of methylation-restriction endonucleases HhaI and HpaII [17], which can only destroy corresponding sites on the unmethylated locus. Therefore, PCR amplification of the first exon of the *HUMARA* gene using primers flanking this locus will yield 2 products with different sizes in heterozygous females. Nonetheless, after methylation-restriction enzyme treatment, only one PCR product will be present in monoclonal tissues because all the cells have the same inactivated X chromosome that can be amplified. In polyclonal tissues, maternal or paternal X chromosomes undergo inactivation randomly, producing 2 PCR products after HpaII digestion. With this technique, it is possible to distinguish monoclonal disorders from polyclonal disorders.

In the present study, we aimed to apply the *HUMARA* test to elucidate the clonal status of adenomatoid tumor to explore whether it is a true mesothelial neoplasm or a pseudoneoplastic reactive entity.

2. Materials and methods

2.1. Specimen collection

Thirty-eight cases of formalin-fixed, paraffin-embedded adenomatoid tumor blocks were retrieved from the Department of Pathology, Shanghai First Maternity and Infant Hospital (Shanghai, China), and the Department of Pathology, the Affiliated Hospital of Qingdao University (Qingdao, China). We also took blood sample from a male volunteer to serve as a control. All the cases were initially diagnosed by an attending pathologist and reconfirmed by a consulting pathologist (H. G. Z.). Informed consent to use redundant tissue was obtained in all the cases.

2.2. Histologic evaluation

Histologic evaluation was performed on hematoxylin and eosin (HE)–stained slides. Because a great majority of adenomatoid tumors were cystic with flattened inconspicuous cell nuclei that were difficult to be precisely sampled without being contaminated by stromal cells, cases with cuboidal or prominent mesothelial cells were noticed during HE evaluation and selected for following microdissection.

2.3. Laser capture microdissection and genomic DNA extraction

Ten 6-μm-thick histologic sections were prepared from each selected block and adhered to a 1.4-μm membrane with