Molecular Evidence Supporting the Neoplastic Nature of Some Epidermoid Cysts of the Testis

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Context.—Loss of heterozygosity (LOH) on chromosomes 9p and 12q is common in germ cell tumors of the testis. Loss of heterozygosity of 17p13 has also been demonstrated in germ cell tumors. The incidence of LOH in epidermoid cysts, a possible special form of teratoma, has not been previously determined.

Objective.—To determine the frequency of LOH in epidermoid cysts.

Design.—Eight testicular epidermoid cysts and surrounding parenchyma were microdissected from formalin-fixed, paraffin-embedded tissue, and the genomic DNA was extracted using proteinase K. Polymerase chain reaction analysis targeted regions on chromosome 9p21 (D9S177 and D9S161 loci), chromosome 12q22 (D12S1051 locus), and chromosome 17p13 (TP53 locus). Gel electrophoresis followed by autoradiography was used to detect LOH.

Results.—All 8 of the epidermoid cysts were informative at a minimum of 1 of 4 loci. Three demonstrated LOH. In 2 epidermoid cysts LOH occurred on chromosome 12. Loss of heterozygosity on chromosome 17p13 was not present in any of the tumors.

Conclusions.—Epidermoid cysts harbor allelic loss at some of the same loci identified in malignant testicular germ cell tumors. Our findings support that some examples of epidermoid cysts are neoplastic, although their low frequency of LOH also supports that they are genetically different from malignant germ cell tumors.


Epidermoid cysts are benign lesions accounting for less than 1% of all testicular tumors. Grossly, the cysts are located within the testicular parenchyma and are filled with keratinous debris. Microscopically, well-differentiated stratified squamous epithelium lines the cysts. Epidermoid cysts lack the adnexal structures found in dermoid cysts and also lack other tissue types found in mature teratomas. Unlike germ cell tumors, including mature teratomas, epidermoid cysts are not associated with intra-tubular germ cell neoplasia, and their behavior is uniformly benign. Little is known about the histogenesis and possible genetic alterations in epidermoid cysts of the testis.

Several authors have proposed possible origins for epidermoid cysts. Some authors have suggested that epidermoid cysts may originate from squamous metaplasia of the mesothelium, rete testis, or seminiferous tubular epithelium. Others indicate that epidermoid cysts arise from germ cells and represent monodermal teratomas.

Loss of heterozygosity (LOH) studies may help elucidate the histogenesis of epidermoid cysts. Loss of heterozygosity on the short arm of chromosome 9 and the long arm of chromosome 12 is well documented in testicular germ cell tumors, including mature teratomas. Loss of heterozygosity at 17p13 has been documented in 33% of teratomas and 22% of nonseminomatous germ cell tumors. The incidence of LOH in epidermoid cysts of the testis has not been previously determined. In this study, we examined the prevalence of LOH at 4 microsatellite markers on chromosomes 9p21 (D9S177 and D9S161), 12q22 (D12S1051), and 17p13 (TP53) in 8 epidermoid cysts of the testis.

Materials and Methods

Epidermoid cysts were diagnosed based on the characteristic squamous epithelial lining surrounding a cystic space filled with keratin. Additionally, all of the lesions lacked evidence of intra-tubular germ cell neoplasia or teratomatous-type elements other than squamous epithelium. Formalin-fixed tissue from 8 testicular epidermoid cysts was microdissected from paraffin-embedded, hematoxylin-eosin-stained tissue using a 28-gauge needle and direct visualization with a light microscope, as previously described. Normal testicular parenchyma adjacent to the epidermoid cysts served as the DNA control. The Figure illustrates one of the epidermoid cysts prior to (Figure, A) and after (Figure, B) microdissection. Genomic DNA was obtained by placing microdissected tissue in a 15-μL aqueous solution of 10mM Tris, 1mM EDTA, 1% Tween 20, and 0.2 g/mL proteinase K (pH 8.3) and incubating overnight at 37°C.

Polymerase chain reaction (PCR) analysis targeted regions in chromosomes 9p21 (D9S177 and D9S161 loci), 12q22 (D12S1051 locus), and 17p13 (TP53). Polymerase chain reaction amplification was performed according to previously reported methods. Gel electrophoresis was performed using 3 μL of the mixture loaded onto 6.5% polyacrylamide denaturing gels without formamide. Electrophoresis followed by autoradiography with Kodak X-OMAT AR film for 8 to 16 hours was used to detect LOH by

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Precise microdissection of an epidermoid cyst of the testis (case 3) and representative results of loss of heterozygosity (LOH) analysis. A, Tumor before microdissection (hematoxylin-eosin, original magnification ×200). B, Tumor after microdissection (hematoxylin-eosin, original magnification ×200). C, Loss of heterozygosity results. DNA was prepared from the epidermoid cyst (T) and normal testicular parenchyma (N) and amplified by polymerase chain reaction using polymorphic marker D9S177. Arrow points to the allelic loss in the epidermoid cyst.

**Summary of Loss of Heterozygosity Analysis in Epidermoid Cysts of the Testis from 8 Patients**

<table>
<thead>
<tr>
<th>Case No.</th>
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<td>D12S1051</td>
<td>TP53</td>
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<tr>
<td>3</td>
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<td>NL</td>
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</table>

* NI indicates noninformative; NL, no loss of alleles; and LOH, loss of heterozygosity.

**RESULTS**

The Table summarizes the LOH results at all 4 loci. Loss of heterozygosity was detected at both of the 9p21 loci and at the 12p22 locus. Seven tumors were informative at 9p21 (D9S177) and 9p21 (D9S161). Case 6 demonstrated LOH at 9p21 (D9S161). Allelic loss at 9p21 (D9S177) was demonstrated in case 3. One of 5 informative tumors at locus 12p22 (D12S1051) had LOH. All cases were informative at 17p13 (TP53). Loss of heterozygosity on chromosome 17p13 (TP53) was not detected in any of the epidermoid cysts. The Figure, C, illustrates an example of LOH at locus D9S177 (case 3).

**COMMENT**

Epidermoid cysts are considered benign testicular lesions, usually located adjacent to the tunica albuginea. Unlike germ cell tumors, including mature teratomas, epidermoid cysts are not associated with intratubular germ cell neoplasia. Therefore, whether epidermoid cysts are neoplastic is unclear, as is their relationship to mature teratoma.

Molecular evidence shows that multiple genetic alterations occur during the development of testicular germ cell tumors. Numerous studies indicate that seminomatous and nonseminomatous germ cell tumors harbor LOH at multiple loci. Murty et al demonstrated LOH in greater than 40% of germ cell tumors on the long arm of chromosome 12. Loss of heterozygosity in germ cell tumors has also been identified on chromosomes 9p and 17p. Peng et al demonstrated a 22% LOH in the tumor suppressor gene p53 locus (TP53) in germ cell tumors, but none had p53 mutations. The authors suggested that the LOH on 17p may represent the baseline level in this patient population in a cytogenetically abnormal chromosome.

We investigated the incidence of LOH at 4 microsatellite markers on chromosomes 9p21 (2 loci), 12q22, and 17p13. Previous studies demonstrated that LOH at these loci were frequently found in testicular germ cell tumors, particularly in teratomas. We found LOH in 3 of 8 epidermoid cysts of the testis. In these 3 cases, there was partial overlap with genetic changes commonly seen in nonseminomatous germ cell tumors, although, interestingly, LOH was not detected at the 17p13 locus.

Our findings indicate that epidermoid cysts harbor allelic loss at some of the same loci identified in malignant germ cell tumors, thus providing evidence of a partial genetic link between epidermoid cysts and mature teratomas, but simultaneously emphasizing that there are fundamental differences between these 2 lesions, given the low frequency of these changes. The significance of the LOH in epidermoid cysts is not entirely understood; however, we believe these results support the concept that epidermoid cysts are neoplasms of germ cell derivation rather than metaplastic in origin. A prior study suggested that there are at least 2 pathways to a mature teratoma in the testis, one from a malignant intratubular germ cell and the second from a germ cell that has not undergone malignant transformation. Perhaps the LOH seen in epidermoid cysts reflects the neoplastic change in a nonmalig-
nant germ cell that undergoes teratomatous differentiation.

The neoplastic origin of epidermoid cysts is further supported by the similarity of several clinical features between epidermoid cysts and germ cell tumors, including peak age incidence, race predilection (a higher frequency in whites than in blacks), location (a higher frequency of epidermoid cysts in the right testis than in the left testis), and the occurrence of epidermoid cysts in cryptorchid testes.4

Additional genetic studies should be performed to further characterize the relationship of epidermoid cysts to malignant germ cell tumors, including mature teratomas. The most important of these studies is analysis of genetic alterations involving chromosome 12.6 Testicular germ cell tumors usually have an overrepresentation of chromosome 12p.17,18 Most commonly, the change occurs by the formation of isochromosome 12p. A lack of commonly detected genetic alterations in chromosome 12p of epidermoid cysts would provide data that these neoplasms arise from a different pathway than malignant germ cell tumors.

Despite partial genetic similarity, the natural clinical history of epidermoid cysts supports their benign nature, and the aggressive therapy used for nonseminomatous germ cell tumors should not be undertaken.

References