*GENE LOSS IN HUMAN TERATOMAS**

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Abstract.—If benign cystic teratomas (dermoid cysts) of the ovary arise from a germ cell that has undergone meiosis, they should be missing genes which are present in the person. Three independently segregating allelic isozymes in 11 benign cystic teratomas of the human female ovary were compared with normal tissue of the same case. Dermoid cysts from persons heterozygous for these isozymes are frequently homozygous for that particular gene product. One of two dermoid cysts is homozygous for glucose-6-phosphate dehydrogenase, two of four tumors are homozygous for phosphoglucomutase at the PGM_1 locus, and two (or more) of eight tumors are homozygous for phosphoglucomutase at the PGM_3 locus in women heterozygous for these allelic isozymes. These findings are consistent with the hypothesis that these tumors arise from a germ cell which has undergone meiosis with varying degrees of crossing-over.

Introduction.—Benign cystic teratomas (dermoid cysts) are found relatively frequently in human females. Most of them arise in the gonads; less frequent sites are the mediastinum, the retroperitoneum, and the presacral and coccygeal regions.[1] The different theories of their origin have arisen from studies of their location, of the degree and kind of organization of the tissue types within them,[1] and of their possible relationship with other gonadal tumors.[2, 3] They have a normal diploid karyotype.[4]

This study was undertaken in an attempt to test the theory that dermoid cysts in human females arise from a germ cell after, or at the time of, meiotic division. Mammalian eggs begin meiosis *in utero*, and by the time of birth they are at diplotene.[5] A tumor formed after meiotic division is completed should be missing half the chromosomes of the individual. One would then expect that some genes which are heterozygous in the patient would be homozygous in the tumor. This study uses two common electrophoretic variants (allelic isozymes) of two autosomal, independently segregating genes for phosphoglucomutase (PGM)[6, 7] and the X-linked gene, glucose-6-phosphate dehydrogenase (G6PD)[8] as genetic markers to compare the phenotype of the host with that of the tumor.

Methods.—Fresh dermoid cysts and other tumors were obtained incidental to surgery, autopsies, and therapeutic abortions at Children's Hospital, Mount Zion Hospital, Kaiser Foundation Hospital, University of California Medical Center, and the Presbyterian Medical Center, all in San Francisco. On gross examination the tumors were diagnosed by their characteristic appearance; this impression was later confirmed histologically. Samples from the growth nidus of the tumor were taken for enzyme analysis and stored in a CO_2 freezer. Additional segments from the same area were used for cell culture. Cell cultures were obtained by mincing samples into small fragments and growing them in Eagle's minimal essential medium in Earle's balanced salt solution (GIBCO) with fetal calf serum 12% (GIBCO), penicillin, and streptomycin. Some cell lines consisted of spindle cells only, and others had an admixture of spindle cells and epithelial cells.

The allelic isozymes Gd^A and Gd^B of G6PD were studied. There are two genotypes Gd^A and Gd^{A-} that have the same electrophoretic mobility. These two were not differentiated and are grouped together by their common electrophoretic phenotype and designated GdA. G6PD was not run on non-Negro tissues since Gd^A and Gd^{A-} genotypes are seen only in Negroes. The isozymes were analyzed by vertical starch gel electrophoresis according to the method of Bowman[9] and were stained as previously described.[10]

There are three loci for PGM, designated PGM_1, PGM_2, and PGM_3. They show no demonstrable linkage.[11] PGM_2 variants are rare and none were found in this series, but there are two common alleles at both the PGM_1 and PGM_3 loci. At each locus the patient can be homozygous (PGM 1 or PGM 2) or heterozygous (PGM 2-1). Samples used for PGM isozyme analysis were run on vertical starch gel electrophoresis and stained according to the method of Spencer, Hopkinson, and Harris,[6] with the addition of 1% agar to the staining mixture.

Results.—Eleven fresh ovarian dermoid cysts were obtained from ten females. Their isozyme phenotypes are shown in Table 1. The three tumors from Negro females were analyzed for their electrophoretic mobility of G6PD. Two of the women are heterozygous (GdAB) (Fig. 1), and one is homozygous (GdA). The dermoid cyst from one of the GdAB women is GdAB. The dermoid cyst from the other GdAB woman is GdB, there being no GdA activity in multiple analyses from the growth nidus. The dermoid cyst from the homozygous GdA individual is GdA. A sex-chromatin (Barr) body is present in the cell nuclei of all three tumors.

There are four women who have the PGM_1 2-1 phenotype, indicative of heterozygosity at the PGM_1 locus. In this group two cases (nos. 238 and 271) have dermoid cysts which are PGM_1 2-1; one case (254) has a dermoid cyst which is PGM_1 1, and the fourth dermoid (case 291) is PGM_1 2 (Fig. 1). With one exception (case 254), multiple samples of the tumor gave similar results. This exceptional tumor is PGM_1 1 in some areas; in other areas there is, in addition, a faint PGM_1 2 band. Sections from the latter areas reveal active inflammation with inflammatory cells interpreted as being of host origin. In all PGM_1 1 and PGM_1 2 cases, the tumor phenotype is the same as that of the patient's normal tissues.

Although it was possible to separate the PGM_3 1 phenotype from the PGM_3 2-1 and the PGM_3 2 phenotypes, it was not possible in all cases to separate the PGM_3 2-1 from the PGM_3 2 phenotype. Eight cases are PGM_3 2-1 or PGM_3 2. Two of them (cases 238 and 258) have dermoid cysts which are all PGM_3 1. It is assumed that these two tumors show absence of gene effect at the PGM_3 2 locus in PGM_3 2-1 patients. Tumors in the other four cases have the same PGM_3 phenotype as that of normal tissues from the same case.

Monolayer cell cultures were obtained from normal tissue and dermoid cysts of five cases. All tumors karyotyped are 46,XX. In all cases the G6PD and PGM phenotype is the same as in the *in vivo* analyses, including those dermoids that show absence of gene effect at alternate alleles. The tumor phenotype remained constant throughout repeated transfers, the maximum being 12.

Other tissues were analyzed for PGM and G6PD during the course of the study. Liver, kidney, adrenal gland, thyroid gland, uterus, and skeletal muscle obtained from autopsies all have the same PGM_1 phenotype in the same case, confirming the finding of Hopkinson and Harris.[7] Random samples of 11- to 13-week

TABLE 1. *Comparison of electrophoretic phenotype of normal tissues with that of dermoid cysts.*

Case	Tissue	PGM$_1$ *In vivo*	PGM$_1$ Cell culture	PGM$_2$ *In vivo*	PGM$_2$ Cell culture	G6PD *In vivo*	G6PD Cell culture
233	Ovary	1(2)*	...	2-1?†	...	AB(3)	...
	Appendix	1(1)	...	2-1?	...	AB(3)	...
	Blood	1(1)	...	...	...	AB(1)	...
	Dermoid cyst	1(2)	...	2-1?	...	B(6)	...
238	Ovary	2-1(1)	2-1	2-1	2-1	AB(2)	AB
	Appendix	2-1(1)	...	...	...	AB(2)	...
	Blood	2-1(1)	...	...	...	AB(1)	...
	Dermoid cyst‡	2-1(3)	2-1	1	1	AB(4)	AB
246	Ovary	1(2)	...	2-1?	...	...	...
	Blood	1(1)	...	...	...	A(1)	...
	Dermoid cyst	1(4)	...	2-1?	...	A(4)	...
254	Ovary	2-1(5)	2-1	2-1	2-1	...	...
	Appendix	2-1(3)	...	2-1	...	...	...
	Dermoid cyst‡	1(25)	1	2-1	2-1	...	...
255	Fallopian tube	1(1)	...	1	...	...	...
	Leukocytes	1(1)	...	...	...	...	...
	Blood	1(1)	...	...	...	...	...
	Dermoid cyst A	1(2)	...	1	...	...	...
	Dermoid cyst B	1(3)	...	1	...	...	...
258	Myometrium	1(4)	...	2-1	2-1	...	...
	Blood	1(1)	...	...	...	...	...
	Dermoid cyst‡	1(3)	1	1	1	...	...
265	Ovary	2(4)	2	...	2-1	...	...
	Ovarian capsule	2(1)	2	2-1	2-1	...	...
	Fallopian tube	2(2)	...	2-1	...	...	...
	Blood	2(1)	...	...	...	...	...
	Dermoid cyst	2(4)	2	2-1	2-1	...	...
271	Ovarian capsule	2-1(1)	...	...	...	...	...
	Ovary (opposite)	2-1(3)	...	1	...	...	...
	Blood	2-1(1)	...	...	...	...	...
	Dermoid cyst‡	2-1(5)	2-1	1	1	...	...
289	Ovary	1(3)	...	2-1?	2	...	...
	Skin	1(1)	...	2-1?	2	...	...
	Blood	1(1)	...	...	...	...	...
	Dermoid cyst‡	1(8)	...	2-1?	2	...	...
291	Fallopian tube	2-1(4)	...	2-1?	...	...	...
	Dermoid cyst	2(8)	...	2-1?	...	...	...

* Number in parentheses indicates number of separate samples analyzed.
† Question mark used to indicate those analyses in which one cannot differentiate the PGM$_2$ 2 from the PGM$_2$ 2-1 phenotype.
‡ Karyotype analysis: 46, XX.

human embryos obtained incidental to therapeutic abortions were also analyzed, and these too have the same PGM$_1$ phenotype throughout.

PGM isozymes were studied in other tumors. In those that came from Negro females, G6PD was also analyzed. The results of the tumor analyses are shown in Table 2. There are 21 leiomyomas from GdAB, PGM$_1$ 2-1 women. The myomas are either all GdA or GdB, and each myoma has a PGM$_1$ 2-1 phenotype. Gene inactivation in these tumors at the G6PD, but not PGM, locus has been

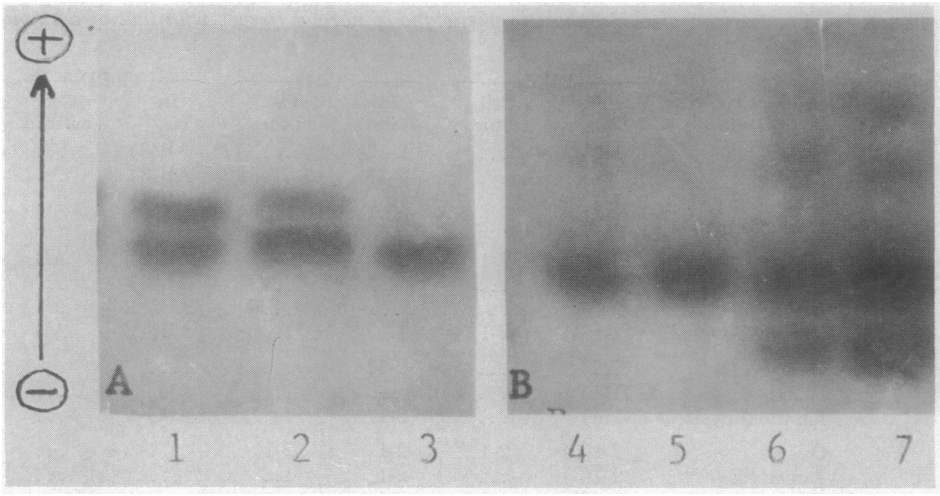

FIG. 1.—(*A*) G6PD electrophoretic patterns of dermoid cysts from heterozygous cases *1*, Case 238, dermoid cyst; *2*, normal GdAB control; *3*, case 233, dermoid cyst.
(*B*) *4* and *5*, PGM_1 2 phenotype in a dermoid cyst, case 291, compared with *6* and *7*, showing PGM_1 2-1 phenotype of ovary and fallopian tube from the same case.

previously noted.[12] Cases similar to 252, where there is an excessive number of tumors of a single phenotype, have also been reported.[13] The three cases of nodular goiter have the same pattern as uterine leiomyomas. Here one also sees gene inactivation of the G6PD locus, but not the PGM_1 locus. Isozyme bands at the PGM_3 locus were not sharp enough to resolve. Five PGM_1 2-1 cases have a malignant tumor. One tumor (case 288) is PGM_1 2 in three of four metastatic sites, and the tumors in the other cases have the same phenotype as that of the host. Of the three cases of malignant tumors from GdAB women, one is homozygous (GdB) and two are heterozygous (GdAB) in some areas and homozygous (GdB) in others.

Discussion.—This report reveals a frequent absence of gene effect at alternate alleles for two autosomal loci, PGM_1 and PGM_3, and the X-linked locus G6PD in ovarian dermoid cysts. The gene locus showing loss of gene effect varies from tumor to tumor and, at least at the PGM_1 locus, is not limited to any one allele. In case 238, there is loss of gene effect at the PGM_3 but not G6PD locus; in case 254, there is loss of gene effect at the PGM_1 but not at the PGM_3 locus. The phenotype of the tumor is not dependent upon the histological types within it. With the exception of case 254, where there are inflammatory cells of host origin, there is a similar admixture of histological cell types in each tumor, and multiple samples of the same tumor have the same phenotype. A reasonable explanation for these findings is that there is a very early origin of the gene differences between the host and the tumor; this suggests that there is a single cell, different from the other cells of the host, which gives rise to the tumor.

Absent gene effect at one of two alternate alleles could be due to gene inactivation or to gene loss. Since absent gene effect is maintained in cell culture, inactivation by a labile type of regulatory mechanism seems unlikely. While one cannot be sure that there are no random point mutations leading to absent gene

TABLE 2. *Electrophoretic phenotype of normal tissues and various tumors from patients heterozygous for phosphoglucomutase (PGM_1) and/or glucose-6-phosphate dehydrogenase (G6PD).*

Case	Tumor diagnosis	PGM_1		G6PD	
		Normal tissue	Tumor	Normal tissue	Tumor
198	Nodular goiter	2-1	2-1	AB	A
211	Nodular goiter	1	1	AB	A
223	Nodular goiter	2-1	2-1	AB	B
	2 Leiomyomas, uterus	2-1	2-1	AB	Both A
239	Granular cell myoblastoma, tongue	1	...	AB	AB
241	8 Leiomyomas, uterus	2-1	All 2-1	AB	(6 A) (2 B)
252	13 Leiomyomas, uterus	2-1	All 2-1	AB	(1 A) (12 B)
235	Metastatic leiomyosarcoma, uterine primary	1	...	AB	(B > AB) (AB) (AB) (AB) (B)*
249	Giant cell CA, thyroid, or rhabdomyosarcoma	2-1	2-1	AB	AB and B areas
	5 Metastatic tumors	2-1	2-1	AB	AB
259	Metastatic adenocarcinoma, lung	2	...	AB	B
284	Metastatic squamous cell carcinoma	2-1	2-1	...	...
288	Metastatic leiomyosarcoma, uterine primary	2-1	(2) (2) (2 ≫ 2-1)*	...	...
293	Malignant mixed tumor, parotid	2-1	2-1	...	...
297	Adenocarcinoma, lung	2-1	2-1	...	...
300	Adenocarcinoma, ovary	2-1	2-1	...	...

* Separate metastatic sites. (>) indicates much greater amounts of one phenotype than of the other.

activity, the implied frequency of these point mutations would make the mutation load excessive. Karyotype analysis of these tumors reveals no loss of chromosomal material to account for gene loss. The most likely possibility is that there is meiotic division leading to gene loss; it is less likely that there is somatic cell crossing-over. In both cases, the karyotype analysis could show normality.

In somatic cells of mammalian females, there is presumably random and fixed inactivation of alternate alleles at the G6PD locus and of the heterochromatic X-chromosome containing this gene.[14–18] A tumor arising from a single somatic cell in a woman heterozygous for G6PD should be functionally hemizygous; this was found in uterine leiomyomas,[19] multiple myeloma,[20] chronic myelogenous leukemia,[21] and in the two cases of nodular goiter in GdAB women in this series.

There are two dermoid cysts from GdAB women. One tumor is all GdB in multiple samples and the other is GdAB. The GdB tumor could arise from a single somatic cell or several cells which, by chance, have a GdB phenotype. Since there is fixed inactivation of alternate alleles at this locus, it is unlikely that the GdAB tumor has a single somatic cell origin. A single germ cell, however, could give rise to both types of tumors, if it undergoes first or second meiotic division prior to, or at the time of, tumor formation and if crossing-over occurs between the centromere and the gene locus. The final tumor phenotype will depend upon the kind of meiosis and upon whether or not crossing-over has occurred.

The G6PD findings are compared with autosomal genes for PGM which do not show gene inactivation of alternate alleles.[12, 22] At both the PGM_1 and PGM_3 loci, there is frequent absence of gene effect in the different tumors. It is found in two of the four PGM_1 2-1 cases and in at least two of eight possible PGM_3 2-1 cases. Gene loss incidental to meiotic division could account for this fact.

The findings of this report are consistent with tumor formation following the first meiotic division or with tumor formation following the failure of the first meiotic division and the occurrence of the second meiotic division. Other mechanisms, such as chiasma interference affecting the orientation of diads, and the distance between the centromere and the different gene loci could also modify gene segregation. It is obvious that more cases and more genetic markers are necessary to determine which mechanisms are operating.

There is genetic evidence for somatic crossing-over in mammalian cells at the histocompatibility locus in several types of mouse tumors.[23] It is unlikely that this mechanism accounts for the findings in this series of dermoid cysts. In contrast to these tumors, dermoid cysts are benign tumors, have a normal female karyotype, and form varied but normal histologic cell types. They arise in areas normally seeded by germ cells,[24] and a germ cell origin could account for the gene loss of the tumors.

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