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Genetics

PAGE-1, an X chromosome-linked *GAGE*-like gene that is expressed in normal and neoplastic prostate, testis, and uterus

(cancer/expressed sequence tags/cDNA/libraries)

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ABSTRACT We have used a combination of computerized database mining and experimental expression analyses to identify a gene that is preferentially expressed in normal male and female reproductive tissues, prostate, testis, fallopian tube, uterus, and placenta, as well as in prostate cancer, testicular cancer, and uterine cancer. This gene is located on the human X chromosome, and it is homologous to a family of genes encoding GAGE-like proteins. GAGE proteins are expressed in a variety of tumors and in testis. We designate the novel gene *PAGE-1* because the expression pattern in the Cancer Genome Anatomy Project libraries indicates that it is predominantly expressed in normal and neoplastic prostate. Further database analysis indicates the presence of other genes with high homology to *PAGE-1*, which were found in cDNA libraries derived from testis, pooled libraries (with testis), and in a germ cell tumor library. The expression of *PAGE-1* in normal and malignant prostate, testicular, and uterine tissues makes it a possible target for the diagnosis and possibly for the vaccine-based therapy of neoplasms of prostate, testis, and uterus.

Expressed sequence tags (ESTs) are sequences derived from randomly selected clones from various cDNA libraries (1–6). Each cDNA clone is generated from a transcript, and the frequency and distribution of the many different transcripts in any given tissue depends on the tissue specific activity of the genes. The translation of transcript frequency and distribution into frequency and distribution of EST sequences depends not only on the specificity and magnitude of mRNA expression but also on other factors such as mRNA stability and clonability of these EST sequences. Therefore, a specificity or frequency analysis of ESTs only provides a guide for the prediction of expression patterns. Nevertheless, ESTs provide a valuable source of information that may be utilized to predict the expression patterns of specific genes in different tissues.

The recently developed Cancer Genome Anatomy Project (CGAP) of the National Cancer Institute uses microdissection and laser-capture techniques to generate defined and tissue/tumor-specific EST libraries (http://www.ncbi.nlm.nih.gov/ncicgap; refs. 4–6). CGAP has already accumulated a vast number of tissue-specific sequences, and the CGAP sequence database is rapidly growing with the continuous addition of sequences from different tissues and tumor types. There are many ways by which the EST sequence data can be processed to cluster, sort, and filter the cDNA sequences, to identify genes that are specifically expressed in certain tissues. Database "mining" for cDNAs that are preferentially or exclusively expressed in defined tissues, or in malignant/neoplastic tissues, provides lists of potential target genes for cancer therapy (4–7). Although in many cases these "candidate genes," which appear tissue specific in database analyses, cannot be confirmed in their specificity by experimental techniques (e.g., Northern blots or PCR), a reasonable number of candidate genes remain for which the predicted and desired expression pattern can be experimentally confirmed (7, 8). These specifically expressed genes are of interest because of their functions in cell or tumor biology and may also be directly used as markers for cancer diagnosis and targets for cancer therapy.

We have established a computer screening strategy to identify genes that are preferentially expressed in normal prostate and in prostate cancer (7). Using this approach in combination with experimental verification, we have found several candidate genes that are preferentially expressed in the prostate and are evaluating whether these genes can be used as targets for the diagnosis or therapy of prostate cancer. Here we describe the identification of another gene that was found by relaxing the specificity requirements for candidate ESTs in our screening procedure. Instead of removing or giving low ranking to EST clusters that are expressed in nonprostate tissues, we have allowed ESTs that occur in tumors and in a limited number of nonessential normal tissues. Our rationale for this approach is that the expression of a gene in a nonessential tissue and in more than one type of tumor does not exclude it as a target for therapy. In fact, expression in several types of tumors is desirable because this broadens the application of reagents that are developed on the basis of such targets.

Here we describe the identification of an X chromosome-linked gene that is expressed in normal and malignant male and female reproductive tissues. This gene, *PAGE-1*, is homologous to a family of MAGE/GAGE-like proteins and is expressed in normal prostate, testis, uterus, fallopian tube, and placenta, as well as in prostate, testicular, and uterine cancers.

MATERIALS AND METHODS

Computer Analysis of EST Sequences. The National Center for Biotechnology Information (NCBI) dbEST/CGAP database (http://www.ncbi.nlm.nih.gov/ncicgap; refs. 4–6) was used as a source for cDNA sequences. The ESTs from human tissues and tumors were downloaded from ftp://ncbi.nlm.nih.gov/repository/dbEST. The cDNA libraries that we processed are listed in http://www.nci.nlm.nih.gov/UniGene/Hs.Home.html; http://www-bio.llnl.gov/bbrp/image/humlib_info.html; http://genome. wustl.edu/est/est_protocols/libraries.html; http://inhouse.ncbi.nlm.nih.gov/cgi-bin/

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Abbreviations: EST, expressed sequence tag; CGAP, Cancer Genome Anatomy Project; NCBI, National Center for Biotechnology Information; RT-PCR, reverse transcription–PCR.

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UniGene/lbrowse?org=Hs&OTP=cgap. The EST sequences were clustered and sorted as described before (7). However, the candidate gene list was updated by using the EST dataset of April 25, 1998. This dataset contains 1,001,294 human EST sequences from 656 libraries. Two updated candidate lists were prepared, one with the specificity cutoff for prostate of three as before and another with the cutoff value of six. The top portions of these tables are available at our web site, http://rex.nci.nih.gov/RESEARCH/basic/lmb/mms/biblio.htm.

Molecular Biology Techniques. EST-plasmids were obtained from the IMAGE Consortium (Genome Systems). The identities of the sequences were confirmed and extended by automated fluorescent DNA sequencing using an Applied Biosystems rhodamine-terminator cycle sequencing kit. PCR was performed on a Biometra Thermocycler using Boehringer Mannheim high-fidelity reagent kits and the Hot-Start technique. Northern blots containing 2 μg of poly(A)$^+$ mRNA from various tissues and cancer cell lines (CLONTECH), blots with 20 μg per lane total tumor RNA (Invitrogen), mRNA dot blots (CLONTECH), and a Somatic Cell Hybrid Southern Blot (Oncor) were hybridized with random-primed ^{32}P-labeled DNA fragments. The specific activity of the labeled probe was 1 mCi/μg (1 mCi = 37 MBq). The membranes were blocked for 4 hr in hybridization solution (50% formamide, without probe), hybridized for 15 hr with the probe at 55°C, rinsed in 2× SSC/0.1% SDS, and washed once with 2× SSC/0.1% SDS and twice with 0.2× SSC/0.1%SDS at 55°C.

RESULTS

Database Mining of Genes That Are Preferentially Expressed in Prostate, Prostate Cancer, and Other Tumors. The database analysis was performed on the complete human EST sequence set in the dbEST database (NCBI dbEST/CGAP; refs. 4–6) as of April 25, 1998, which included 1,001,294 ESTs in 656 different libraries. The majority of the ESTs (>650,000 ESTs, >64% of the total ESTs) came from Soares libraries and/or the National Cancer Institute CGAP.

Our EST database clustering and filtering program, originally designed to identify genes that are very specifically expressed in prostate and prostate cancer (7), was updated with the additional EST data. We "relaxed" the specificity requirement for the selection of potentially useful EST clusters because we observed candidate genes on our search list that were not entirely prostate specific but might still be acceptable and useful as targets for the diagnosis or therapy of prostate cancer. For example, EST clusters that show several "expression-hits" in nonprostate/cancer tissues are still interesting if the nonprostate expression specificity is found in libraries other than prostate that come from tumors or nonessential tissues. Therefore, in selecting candidates for further, experimental processing, we "tolerated" the occurrence of ESTs from candidate clusters in a limited number of normal tissues. These were placenta, other gender-specific tissues, and fetal tissues. In identifying target antigens for tumor therapy, the expression of a gene in more than one type of tumor is not an impediment to the applicability of such targets; in fact, it may be desirable because expression of a given protein in multiple tumors will broaden the application of reagents that are developed on the basis of such targets. The expression of "tumor" proteins in certain normal tissues may be neglected if the expression is in reproductive tissues. Expression in uterus, ovary, or placenta is not relevant for males, and prostate or testis expression is not relevant for females.

The output of our recent database analysis is a list of clones that occur frequently in prostate and prostate cancer, as well as in other tumors, and that may also be present in some normal tissues. We sorted this list according to EST frequency in prostate and prostate tumors. Since the EST frequency in libraries of defined tissues approximately correlates with the level of tissue-specific expression of the corresponding gene,

Table 1. Comparison of the distribution of *PAGE-1* sequences in EST libraries with *PAGE-1* Northern hybridization signals

	Computational analysis		mRNA analysis		
Tissue	No. of *PAGE-1* ESTs	*% PAGE-1*/ tissue ESTs	Dot blot	Northern	RT-PCR
Prostate	5	5/22,334 (0.022%)	++	++	+
Prostate ca.	7	7/20,871 (0.031%)			+
Testis	0	0/31,263	+	+	
Testis ca.	0	0/1,123			+
Uterus	3*	0/22,333 (0.013%)	+	+	
Uterus ca.		0/1,112		++	
Ovary	0	0/5,573	(+)	−	
Ovary ca.	0	0/21,989		−	
Fallopian tube	0	0/0		++	
Placenta	8	8/49,467 (0.016%)	+++	+++	+
Other tissues/ca.†	0	0/823,754	−	−	−

The human cDNA sequence libraries (dataset of April 25, 1998) were processed by computer analyses as previously described (7). Individual *PAGE-1* ESTs are nh24e10.s1, nc27g01.r1, nh24a11.s1, nf19h11.s1, and nr35f03.s1 (prostate); nh32c06.s1, nt72b09.s1, nc33g02.s1/r1, nc79f08.s1/r1, and nt78f01.s1 (prostate cancer); EST81031, EST80996, C18969, C18137, yi82c07.s1/r1, and yw73c12.s1 (placenta); and zr65g11.s1/r1 and aa07e08.s1 (uterus; see * below). *% PAGE-1*/tissue: the number of *PAGE-1* ESTs was divided by the total number of tissue-specific ESTs. Original dot-blot and Northern results are shown in Figure 2. +, ++, and +++ indicate the signal intensity on the dot blots or Northern blots: signal, strong signal, and very strong signal, respectively. Ovary gave a very weak signal after prolonged exposure of the autoradiograph.

*The ESTs with uterus specificity were part of pooled uterus-containing libraries; the other tissues of this library did not show and *PAGE-1* expression in hybridization analyses.

†Other tissues in the database analysis are those that were represented by dbEST files in May 1998. Other tissues that were tested by hybridization analyses were brain, spinal cord, heart, aorta, skeletal muscle, colon, bladder, stomach, pancreas, pituitary, adrenal, thyroid, salivary, mammary, kidney, liver, small intestine, spleen, thymus, peripheral leukocyte, lymph node, bone marrow, appendix, lung, trachea, fetal brain, fetal heart, fetal kidney, fetal liver, fetal spleen, fetal thymus, fetal lung, and cancer cell lines HL60, HeLa, K562, Molt4, Raji, SW480, A549, and G361.

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this tissue-specific ranking may identify genes that are preferentially expressed in prostate and prostate cancer. The top portion of the output list of our database analysis, and the "ranking" according to prostate/tumor specificity is available at http://rex.nci.nih.gov/RESEARCH/basic/lmb/mms/biblio.htm.

A cDNA Cluster That Is Predominantly Represented in Prostate and Prostate Cancer Libraries Encodes an X-Linked GAGE-Like Protein. One of the cDNA clusters present on the database search list was observed to be preferentially present in prostate and prostate tumor libraries, and additionally, in placenta and in a mixed pooled library that contained mRNA from uterus. This cluster was ranked 20th in our original list (7) and 8th in the new table. The computational analysis portion of Table 1 lists the distribution of individual EST sequences that correspond to this cDNA cluster in several different EST libraries. ESTs from this cluster are most abundantly found in libraries from prostate and prostate cancer, where they represent 0.022% (prostate) and 0.031% (prostate cancer) of the total cDNA sequence population. They were also represented in placenta libraries (0.016%) and in a library pool that contained cDNA from uterus (0.013%). Homology analyses showed that the sequence of this cDNA cluster is similar to a family of GAGE-like proteins (9–11). An alignment of the protein sequence that is predicted from its reading frame with the sequences of members of the GAGE family is shown in Fig. 1*A*. The homology to GAGE is highly significant, but it is not as pronounced as that of the other GAGE proteins to each other, and we observed some weaker similarity to MAGE proteins (11–16). Because this novel member of the MAGE/GAGE protein family appears to be strongly expressed in prostate and placenta we named it PAGE-1. Further database searches identified additional EST clusters with significant similarity to PAGE-1 and less similarity to GAGE and MAGE. This suggests that PAGE-1 is a member of a family of related proteins like MAGE and GAGE. An alignment of PAGE-1 with sequences of PAGE-2 (predominantly in testis) and PAGE-3 (one EST from a pooled, testis-containing library) is shown in Fig. 1. In addition, we identified several other EST clusters with homology to PAGE as well as to GAGE, but which do not have the striking similarities that the other GAGE family members have to each other (Table 2). Representatives of some of these cDNA clusters are the ESTs yd88e11 (fetal liver/spleen), yw86a06 (placenta), and yi21h01 (placenta). The relation of the sequences of GAGE and PAGE is shown in a graph form (dendrogram) in Fig. 2. There are two sequence stretches in PAGE-1 that contain Arg-Gly-Asp (RGD) motifs, and the surrounding sequence is similar to an RGD-containing sequence present in the metabotropic glutamate receptor 6 (17). RGD motifs are frequently found in cell adhesion proteins, and it has been suggested that RGD sequences in several receptor molecules are involved in cell–cell interactions (18).

Several GAGE/MAGE-like proteins have recently been described as CT antigens—i.e., proteins that are expressed preferentially in cancers and testis (11–16). It has been shown that many MAGE genes are positioned in at least two clusters on the human X chromosome (10, 11, 19, 20). We have found, by radioactive hybridization of a somatic cell hybrid Southern blot, that the PAGE-1 gene is also located on the X chromosome (data not show). A recent database deposit of mapped X chromosomal transcript sequences confirms the X chromosome mapping of PAGE-1 and places PAGE-1 at position Xp11.23 (21).

PAGE-1 Is Expressed in Normal Prostate, Testis, Uterus, Fallopian Tube, and Placenta, and in Prostate and Uterine Cancers. To evaluate experimentally the specificity of expression of PAGE-1, which was suggested by the database analysis to be expressed preferentially in prostate and prostate tumors, we hybridized dot blots and Northern blots of mRNAs from different tissues with a radioactive labeled *PAGE-1* probe. The results of these experiments, which were done with a 140-bp

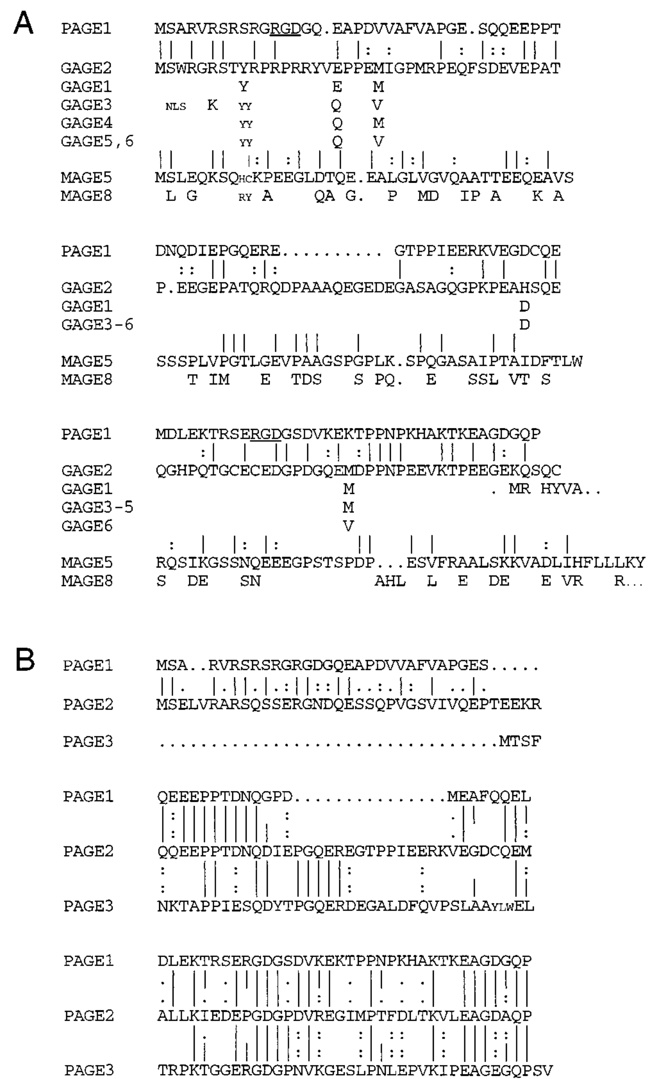

FIG. 1. Similarity of PAGE-1, GAGE, and MAGE. (*A*) The predicted PAGE-1 reading frame is derived from the full-length PAGE-1 EST clone nh32c06. The GAGE and MAGE sequences are from SwissProt: GGE1, GGE2, GGE3, GGE4, GGE5, GGE6, MAG5, and MAG8_HUMAN. Note that the "MAGE alignment" matches amino acids that occur in MAGE5 and/or MAGE8, which are similar to PAGE-1 and/or GAGE1–6; the homologies between single members of the MAGE and PAGE and GAGE protein families are weaker. (*B*) Alignment of PAGE-1 with other PAGEs. PAGE-2 was translated from the EST ai61a04 EST cluster and PAGE-3 from om29f08. PAGE-3 was translated from one single EST and it is possible that the truncated N terminus results from a sequence artifact (the homology extends further to the N terminus in another reading frame). Several other so-far-undefined EST clusters were found that have homology to PAGE as well as to GAGE. These clusters do not have the striking similarities that the other GAGE family members have to each other, but they are also not significantly more similar to PAGE than to GAGE. Representatives of some of these cDNA clusters are the ESTs yd88e11 (fetal liver/spleen), yw86a06 (placenta), and yi21h01 (placenta).

probe under very stringent hybridization conditions, are shown in Fig. 3 and summarized in Table 1. Dot-blot hybridizations (Fig. 3*A*) show a significant level of *PAGE-1* expression in normal prostate. We also found weaker expression in testis, and very strong expression in normal placenta. Additional signals were observed in uterus and a very weak signal in ovary. The very strong placental expression, which was stronger than prostate and the expression in ovary and testis, was not predicted from the results of the database analysis. The

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Table 2. Distribution of other members of *PAGE-1*-like ESTs in the database

EST cluster	Tissue distribution					
	Prostate/Ca.	Testis	Placenta	Germ cell/Ca.	Pool (with uterus)	Pool (with testis)
PAGE-1	nh24e10		yw73c12			aa07e08
	nc24a11		yi82c07			zr65g11
	nh27g01		C18137			
	nf19h11		C18969			
	nr35f03		EST80996			
	nh32c06/Ca.	EST81031				
	nc33g02/Ca.					
	nt72b09/Ca.					
	nt78f01/Ca.					
	nc79f08/Ca.					
PAGE-2		ai61a04		0m68f10/Ca.		0m13c03
		zv62h08				0j89d1
		aj29d06				
		az58h12				
PAGE-3						om29f08

ESTs with homology to *PAGE-1* were identified by BLAST and FASTA (27, 28). "/Ca." indicates ESTs from tumor libraries. *PAGE-2* and *PAGE-3* are sequences that are more homologous to *PAGE-1* than to any member of the GAGE protein family (see Figs. 1 and 2).

expression in uterus is congruent with the appearance of *PAGE-1* ESTs in a library that was derived from pooled mRNAs, including uterus. Among the 58 normal tissues and cancer cell lines that we tested, only prostate, testis, placenta, uterus, and ovary showed *PAGE-1* hybridization signals in dot blots. The signal with ovary was very weak in dot blots. In Northern blots, prostate, testis, placenta, and uterus, but none of the other tissues, displayed a clear ≈500-nt band that hybridized with the *PAGE-1* probe (Fig. 3*A*). Further analyses of Northern blots with mRNA preparations from different uterine cancer samples showed that *PAGE-1* is in uterine cancers. Fig. 3*B Left* is a Northern blot containing total RNA from different uterine tumors (lanes 1, 3, 5, and 7) and corresponding normal uterus (lanes 2, 4, 6, and 8). The *PAGE-1* signal is apparent in all normal uterus and uterine cancer samples, and in some instances the *PAGE-1* signal in mRNA from uterine cancer is stronger than in the adjacent normal tissue (Fig. 3*B*). On the other hand, expression of *PAGE-1* in ovary, which we tested because of the weak dot-blot hybridization signal, could not be confirmed. Fig. 3*B Right* shows that Northern blots containing mRNA from ovarian cancer and adjacent normal ovary showed no evidence of *PAGE-1* expression; however we did find *PAGE-1* in mRNA from normal fallopian tube (Fig. 3*B Center*). To confirm the presence of *PAGE-1* transcripts experimentally in malignant prostate we analyzed a prostate cancer cDNA preparation (from Invitrogen) by RT-PCR with primers that specifically amplify a full-length *PAGE-1* cDNA fragment. Fig. 4 shows that *PAGE-1* mRNA can be detected by PCR in cDNA samples from malignant prostate, as well as in normal prostate and in a testicular tumor. The PAGE-1 fragment was also obtained by PCR from a placental cDNA library, but not from libraries from human muscle or liver. In the prostate tumor cell lines LnCAP and PC3, *PAGE-1* expression could be detected by hybridization analyses of RT-PCR products (data not shown) but not by Northern blots or RT-PCR using ethidium-bromide-stained agarose gels. These experimental observations, combined with the fact that *PAGE-1* is present in CGAP cDNA libraries derived from different prostate cancers (Table 1), indicates that *PAGE-1* is predominantly expressed in normal and neoplastic male and female reproductive tissues and particularly in prostate, testis, and uterus.

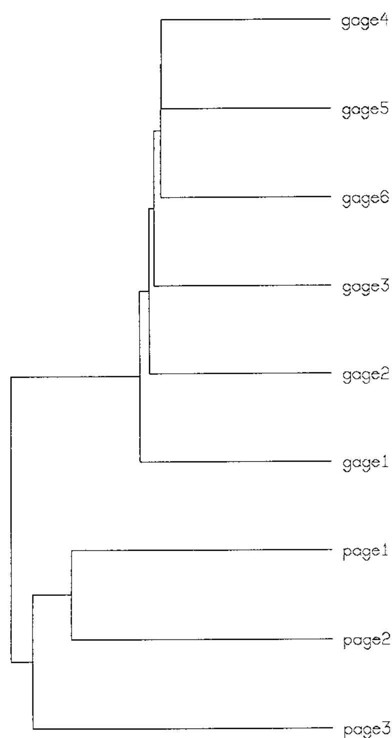

FIG. 2. Relation of the sequences of GAGE, PAGE, and other so-far-uncharacterized EST clusters. The GCG program PILEUP was used to compare the multiple protein sequences of the GAGE and PAGE protein family. The dendrogram shows that PAGE proteins are a separate group of proteins that are less related to GAGE proteins.

DISCUSSION

PAGE-1 is a human X-linked gene that is strongly expressed in prostate and prostate cancer, but is also expressed in other male and female reproductive tissues: testis, fallopian tube, placenta, uterus, and uterine cancer. PAGE-1 shows similarity with the GAGE protein family, but it diverges significantly from members of the family so that it appears to belong to a separate family. This, and the existence of other genes, *PAGE-2* and *PAGE-3*, that share more homology with *PAGE-1* than with members of the GAGE family, indicates that the PAGE proteins constitute a separate protein family.

The specificity of *PAGE-1* expression in normal and malignant tissues that are associated with male and female reproductive function coincides with the localization of this gene on the X chromosome. This observation provides a link between sex-chromosomal genes and reproductive functions. For example, it

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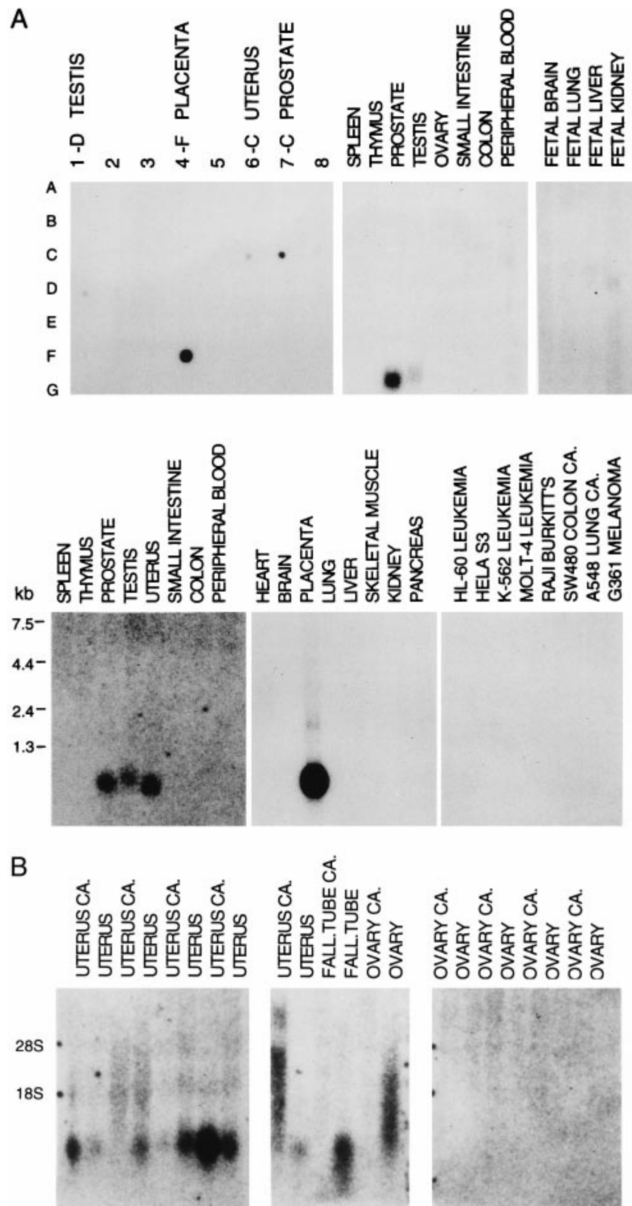

FIG. 3. Hybridization analysis of PAGE-1 expression. (*A*) A multiple tissue dot blot (*Left*) and Northern blots (*Center* and *Right*) were probed with a 140-bp ^{32}P-labeled *PAGE-1* probe under very stringent hybridization conditions (50% formamide, 55°C). Specific *PAGE-1* signals were observed in prostate, testis, placenta, and uterus, but not in other tissues (Table 1 legend lists the analyzed tissues). Because the hybridization probe had some similarity with another *PAGE-1*-like EST cluster that is expressed in testis (*PAGE* represented by the EST zv62h08, Table 2), we additionally used a probe with minimal homology to zv62h08 to confirm that the signal in testis corresponds to the expression of the authentic *PAGE-1*. (*B*) Blots containing 20 μg per lane total RNA from normal or malignant ovary (*Right*), fallopian tube (*Center*), and uterus (*Left*) were hybridized under stringent conditions. *PAGE-1* is expressed in fallopian tube, uterus, and uterine cancer, but not in ovary and ovarian cancer.

is known that a testis-defining gene (*SRY*) is located on the Y chromosome (22), and other sex-determining genes are predicted to be positioned on the X chromosome (23, 24). The genes encoding the family of MAGE proteins are located on Xp21 and Xp28 and are expressed in testis and tumors. *PAGE-1* also is located on the X chromosome and is expressed in male- as well as female-specific tissues. It is interesting to speculate that MAGE and/or PAGE proteins are involved in sex determination. One important question in this context is in which cells of the

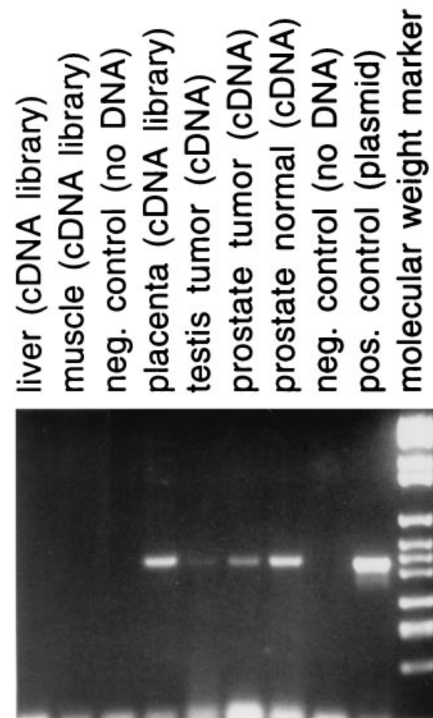

FIG. 4. RT-PCR analysis of *PAGE-1* expression. Ethidium bromide-stained 2.5% agarose gel; *PAGE-1* cDNA was amplified with 5′- and 3′-end-specific PAGE primers (40 cycles 94°C, 58°C, 72°C, 1 min each).

reproductive tissues *PAGE-1* is expressed. Because of the high homology of the various members of the GAGE family, this question can probably not be solved by *in situ* hybridizations, but instead specific antibodies will be required. The presence of *PAGE-1* ESTs in microdissected CGAP tumor libraries suggests that *PAGE-1* is probably expressed in the epithelial cells from which most tumors originate.

In addition to the interesting basic questions about the molecular function of PAGE such as its cellular localization and the function of the RGD sequences, the expression pattern of *PAGE-1* opens the possibility of its usefulness in tumor diagnosis and in therapy. In males, *PAGE-1* is found in prostate and testis, as well as in prostate and testicular cancer. Obviously, expression in placenta, fallopian tube, or uterus is irrelevant for therapy of males. Conversely, testicular and prostate expression can be neglected in females, as well as the high expression in normal placenta.

The specific detection of PAGE-1 might be valuable for the diagnosis of prostate and testicular tumors, as well as uterine tumors. There are sufficient differences between PAGE-1 and other members of the PAGE and MAGE protein families to produce specific antibodies. Analyses with such antibodies are needed to confirm by immunohistology the expression specificity that is seen in database and mRNA analyses, and to evaluate whether anti-PAGE-1 antibodies may be a useful tool for tumor diagnosis.

Since removal of normal prostate, testis, or uterine tissue together with the cancerous lesions is part of standard cancer therapy, specific targeting and elimination of PAGE-1-positive normal and malignant tissue could be a promising therapeutic approach. One possibility of eliminating PAGE-1-expressing cells could be to use it as a cancer vaccine (11–16). Although we do not know if PAGE-1 is processed or presented by cells, and thus is suitable as a vaccine, the relation to MAGE and GAGE proteins strongly suggests that this could be the case (11–16). Among the many possible approaches to vaccination, one method is direct vaccination with plasmid DNA (25). We are able to obtain good expression of PAGE-1 protein with

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mammalian expression plasmids (data not shown), and it has been demonstrated that DNA immunization with such expression constructs leads to good immune responses (25, 26). Therefore, this method may generate anti-PAGE-1 responses and allow us to analyze if "PAGE-1-vaccination" can eliminate PAGE-1-expressing cells, as a therapeutic approach toward neoplasms of the prostate, testis, and uterus.

Note Added in Proof. While this paper was in press, we noticed that the gene name PAGE-1 had been assigned to a relative of the GAGE/PAGE protein family that is different from the PAGE-1 and other PAGE proteins that we describe in this publication. The PAGE-1 described in our publication is expressed in malignant as well as normal prostate. In contrast, the gene that is described in ref. 29 was isolated from LnCAP prostate cancer cells, but it is not expressed in normal prostate. Although related to GAGEs as well as the PAGEs described in this paper, PAGE-1 in ref. 29 differs significantly from both protein groups. To avoid confusion, renaming of PAGE-1 is necessary. Because our database analyses indicate the existance of other uncharacterized GAGE/PAGE-like genes, we will postpone the final renaming of PAGE-1 until a consensus nomenclature of (X)AGE gene is available. We suggest PAGE-4 as an interim solution for renaming the PAGE-1 described in this paper.

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