

Expression of Peripheral Benzodiazepine Receptor (PBR) in Human Tumors: Relationship to Breast, Colorectal, and Prostate Tumor Progression[#]

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ABSTRACT

High levels of peripheral-type benzodiazepine receptor (PBR), the alternative-binding site for diazepam, are part of the aggressive human breast cancer cell phenotype in vitro. We examined PBR levels and distribution in normal tissue and tumors from multiple cancer types by immunohistochemistry. Among normal breast tissues, fibroadenomas, primary and metastatic adenocarcinomas, there is a progressive increase in PBR levels parallel to the invasive and metastatic ability of the tumor ($p < 0.0001$). In colorectal and prostate carcinomas, PBR levels were also higher in tumor than in the corresponding non-tumoral tissues and benign lesions ($p < 0.0001$). In contrast, PBR was highly concentrated in normal adrenal cortical cells and hepatocytes, whereas in adrenocortical tumors and hepatomas PBR levels were decreased. Moreover, malignant skin tumors showed decreased PBR expression compared with normal skin. These results indicate that elevated PBR expression is not a common feature of aggressive tumors, but rather

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may be limited to certain cancers, such as those of breast, colon-rectum and prostate tissues, where elevated PBR expression is associated with tumor progression. Thus, we propose that PBR overexpression could serve as a novel prognostic indicator of an aggressive phenotype in breast, colorectal and prostate cancers.

Key Words: Benzodiazepines; Cancer; Metastasis; Cholesterol.

Abbreviations: PBR, peripheral-type benzodiazepine receptor; GBM, glioblastoma multiforme; BPH, benign prostatic hyperplasia.

INTRODUCTION

Two classes of benzodiazepine receptors have been identified, the central-type benzodiazepine receptor located on the neuronal plasma membrane, part of the GABA_A/benzodiazepine receptor complex (1) and the peripheral-type benzodiazepine receptor (PBR)³ (2). Peripheral-type benzodiazepine receptor was identified in peripheral tissues because of its ability to bind the benzodiazepine diazepam (ValiumTM) (2). The pharmacological and molecular properties of these two receptors are distinct (2). PBR is an 18 kDa receptor protein which in steroid-synthesizing tissues, such as gonads, adrenal, placenta and brain, is extremely abundant. PBR primarily resides in the outer mitochondrial membrane where it regulates the transport of cholesterol to the mitochondrial inner membrane, the rate-determining step in steroidogenesis (2). Recent studies demonstrated that PBR is a high affinity cholesterol binding protein (3,4). PBR is also present in non-steroidogenic organs including kidney, lung, heart, liver, and skin (2). In addition, it has been shown that PBR is involved in mitochondrial respiration (5), regulation of cell proliferation (6), and apoptosis (7).

PBR ligand binding, protein and mRNA levels were found to increase in a manner parallel to the increased aggressive phenotype of a battery of human breast tumor cells (8,9). PBR in aggressive MDA-MB-231 cells and human breast metastatic tumor biopsies is localized primarily in and around the nucleus, in contrast to the largely cytoplasmic localization seen in less aggressive MCF7 cells and in normal breast tissue (8). Moreover, the ability of MDA-MB-231 cells to form tumors *in vivo* depends on the amount of PBR present in the cells (10). In addition, drug-induced reduction of PBR levels in MDA-MB-231 cells was accompanied by reduced expression of several genes with close ties to either cell proliferation, differentiation, or apoptosis and correlated with reduced cell proliferation *in vitro* and tumor growth in nude mice (11).

Although there is evidence that PBR ligand binding capacity is higher in human tumors, such as glioma, liver, colon, ovarian, and endometrial carcinoma, than in the corresponding normal tissue (6,8,12–15), there is no indication that this increased PBR expression correlates with metastasis and no data are available on the expression of PBR in human breast, prostate, lung, skin, adrenal, and testis tumors. In this report, we examined the expression of PBR in primary and metastatic human malignant tumor biopsies compared with corresponding normal tissue, benign lesions, and vicinal non-tumoral tissues. In addition, the correlation between PBR expression and tumor metastasis was evaluated. Our results show that elevated PBR expression is associated with breast, colorectal and prostate tumor progression.



MATERIALS AND METHODS

Human Biopsies

Human biopsies were obtained from Lombardi Cancer Center Tissue Resource at Georgetown University Medical Center, the Harvard Brain Tissue Resource Center (Belmont, MA) or ResGen (Huntsville, AL). Pathologists verified histological diagnosis and grading (Tables 1 and 2). Protocols for the use of human tissue were approved by the Georgetown University Internal Review Board. Samples for immunohistochemistry were fixed in 10% formalin and embedded with paraffin. Sections (5 μm) were cut and placed on glass slides. Frozen biopsy samples (-80°C) were used for total RNA extraction.

Immunohistochemistry

All sections were deparaffinized and immunostaining was performed using an affinity-purified anti-peptide rabbit polyclonal anti-PBR antiserum raised against the conserved amino acid sequence 9–27 at a concentration of 1 : 400 (2 $\mu\text{g}/\text{mL}$) as described (8). After overnight incubation at 4°C , the immunoreactivity was detected using horseradish peroxidase-conjugated anti-rabbit IgG (1 : 500) (Transduction Laboratories, San Diego, CA). Because PBR is highly expressed in adrenal tissue, immunoreactivity of rat and human adrenal tissue was used for positive control. For the negative control, the primary antibody was preabsorbed with 10 $\mu\text{g}/\text{mL}$ of the PBR peptide used to generate the antiserum. Counterstaining was carried out with Mayer's hematoxylin (Sigma Diagnostics, St. Louis, MO). Immunoreactivity was evaluated by two investigators (Han, Z and Papadopoulos, V) as described (16) with minor modifications. Cytoplasmic and nuclear labeling were evaluated using a semiquantitative method taking into account the staining intensity and the number of stained cells in different random fields. The immunostaining was scored by the percentage of positive cells vs. the total same type cells as –(no staining or $< 10\%$), +(mild, $10\%–30\%$), ++(moderate, $30\%–50\%$), +++(strong, $50\%–70\%$), and ++++(intense, $> 70\%$).

Quantitative Real-Time PCR

Frozen biopsy tissues and tumors were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's specifications, then total RNA was submitted to On-Column DNase I digestion with RNase-free DNase in order to remove genomic DNA contamination, and subsequently purified using the QIAGEN RNeasy Total RNA isolation kit (QIAGEN, Valencia, CA). Real-time quantity PCR was performed in ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) as previously described (17). Briefly, total RNA was reverse transcribed into cDNA. The resulting cDNAs were then processed for amplification of PBR using specific forward and reverse primers: 5'-TCTTCTTTGGTGCCCGACA-3' and 5'-CCAGCAGGAGATCCACCAAG-3'. Each sample was run in triplicate. Direct detection of the PCR products was achieved by measuring the increase in fluorescence caused by the binding of SYBR[®] Green I Dye to double-stranded (ds) DNA. The comparative C_T method was used to analyze the data. The amount of PBR mRNA expression was normalized to the endogenous reference (18S rRNA).



Table 1. Comparison of PBR expression in human breast, colon-rectum, and prostate biopsies.

Tissue	Histopathological type (case)	Mean age (y)	Gender (M/F)	Differentiation	PBR						<i>p</i> -value ^a
					-	+	++	+++	++++	N (%)	
Breast	Non-tumoral tissue (8)	39.9			—	3 (38)	5 (62)	—	—	—	<0.0001
	Fibroadenoma, adenosis (22)	40.0			—	9 (41)	13 (59)	—	—	—	
	W/o metastasis (9)	59.8		W(2), M(3), P(4)	—	3 (33)	4 (45)	2 (22)	—	—	
	infiltrating ductal carcinoma (8)				—	—	—	—	—	—	
	infiltrating lobular carcinoma (1)	54.4		W(2), M(4), P(12), U(5)	—	—	9 (39)	14 (61)	—	—	
Colon-rectum	With metastasis (23)				—	—	—	—	—	—	
	infiltrating ductal carcinoma (20)				—	—	—	—	—	—	
	infiltrating lobular carcinoma (3)				—	—	—	—	—	—	
	Non-tumoral tissue (6)	75.6	4/2		—	6 (100)	—	—	—	—	
	Adenoma (10)	64.6	8/2		1 (10)	2 (20)	6 (60)	1 (10)	—	—	
Prostate	Adenocarcinoma w/o metastasis (12)	64.4	5/7	W(3), M(8), P(1)	—	1 (8)	8 (67)	2 (17)	—	1 (8)	
	Adenocarcinoma with metastasis (21)	67.0	8/13	W(2), M(15), P(4)	—	2 (10)	5 (24)	4 (19)	10 (47)	—	
	Normal tissue (6)	60.8			—	2 (33)	4 (67)	—	—	—	<0.0001
	BPH (15)	71.2			—	10 (67)	5 (33)	—	—	—	
	Adenocarcinoma (16)	61.4		W(1), M(11), P(4)	—	—	2 (13)	8 (50)	6 (37)	—	

^aThe *p*-value was calculated using the Jonkheere-Terpstra test.

Notes: Immunohistochemistry was carried out by overnight incubation with rabbit anti-PBR antiserum (1 : 400) followed by HRP-conjugated anti-rabbit IgG (1 : 500) for 1 hour. The immunostaining was scored by the percentage of positive cells vs. the total same type cells as —(no staining or <10%), +(mild, 10–30%), ++(moderate, 30–50%), +++(strong, 50–70%), and ++++(intense, >70%). W, M, P, and U represent well, moderately, poorly and unknown differentiation.



Table 2. Comparison of PBR expression in human biopsies.

Tissue	Histopathological type (case)	Mean age (y)	Gender (M/F)	Differentiation	PBR					<i>p</i> -value ^a
					- N (%)	+ N (%)	++ N (%)	+++ N (%)	++++ N (%)	
Ovary	Normal tissue (7) Adenocarcinoma (20)	56, 2 unknown 55.2		W(3), M(1), P(8), U(8)	2 (28) 3 (15)	5 (72) 8 (40)	— 5 (25)	— 3 (15)	— 1 (5)	0.06
Lung	Non-tumoral tissue (10) Adenocarcinoma (8)	61.0 62.7	6/4 3/5	M(1), P(5), U(2)	— —	10 (100) 4 (50)	— 4 (50)	— —	— —	0.02
Brain	Non-tumoral tissue (3) GBM (11), anaplastic Astrocytoma (1)	70.6 60.5	2/1 7/5		— —	3 (100) 6 (50)	— 3 (25)	— 1 (8)	— 2 (17)	0.25
Skin	Normal tissue (6) Basal cell carcinoma (7) Squamous cell carcinoma (10) Melanoma (7)	49.6 60.2 64.6 53.0	2/4 4/3 6/4 3/4		— — — 1 (4)	4 (66) 1 (14) 9 (90) 6 (86)	1 (17) 6 (86) 1 (10) —	1 (17) — — —	— — — —	0.002
Adrenal	Normal tissue (5) Cortical adenoma (3), Cortical adenoma (1)	52.5, 1 unknown 52.5	2/2, 1 unknown 2/2		— — —	— — —	1 (20) 3 (100) 1 (100)	3 (60) — —	1 (20) — —	0.05
Liver	Normal tissue (12) Hepatocellular carcinoma (1)	60, 1 unknown 60.5	7/4, 1 unknown 9/4	low grade W(4), P(2), U(7)	— 3 (22)	1 (8) 1 (8)	3 (25) 4 (31)	7 (59) 1 (8)	1 (8) 4 (31)	0.44
Testis	Normal tissue (5) Seminoma (8)	33, 2 unknown 33.1			— 2 (25)	1 (20) 2 (25)	1 (20) 1 (13)	2 (40) 3 (37)	1 (20) —	0.25

^aThe *p*-value was calculated using the Jonkheere-Terpstra test.

Notes: Immunohistochemistry and scoring were carried out as described under *Materials and Methods* and in the *Notes of Table 1*. W, M, P, and U represent well, moderately, poorly, and unknown differentiation.



Statistical Analysis

A pattern of increasing or decreasing PBR expression over increasing severity of histopathological types was tested within each organ using the exact Jonckheere-Terpstra test (18) as implemented in StatXact (19). This analysis for ordered categorical data measures the evidence against a null hypothesis that all histopathological types within a single organ have the same staining intensity levels in the same proportions. The organs are breast, colon-rectum, prostate, brain, lung, ovary, skin, adrenal, liver, and testis cancers. The notation is as follows. τ_1 represents the normal samples, and τ_2 through τ_n represent each histopathological subtype by increasing severity where n is the number of histopathological subtypes. The two-sided alternative hypothesis is that either $\tau_1 \leq \tau_2 \leq \dots \leq \tau_n$ or $\tau_1 \geq \tau_2 \geq \dots \geq \tau_n$. A significant p -value indicates that there is a tendency for either increasing or decreasing PBR expression associated with increasing severity of histopathological types. With 10 organs being tested, a Bonferroni adjustment was used to control for multiple tests. PBR expression was considered to be significantly associated with histopathological severity if the p -value was less than 0.005 (0.05/10 organs).

Comparison of quantitative realtime PCR analysis of colon, breast carcinomas vs. their normal tissues was performed using unpaired t test (Prism, GraphPad, Inc., San Diego, CA).

RESULTS AND DISCUSSION

The immunoreactivity of purified anti-PBR antibody was verified by immunohistochemistry of rich in PBR rat and human adrenal tissues [Fig. 1(A and B)]. Preabsorption of the antibody with PBR peptide blocked this activity [Fig. 1(C and D)]. Immunoblot of rich in PBR mitochondria of MA-10 mouse Leydig cells and isolated recombinant PBR protein identified the corresponding 18-kDa protein (3,20). In addition, this antiserum recognized the 36-kDa PBR dimer present in human breast cancer cells (20).

We examined the expression of PBR in 10 types of human malignant tumor biopsies compared with corresponding non-tumoral tissues and benign lesions. PBR is constitutively present, at various expression levels, in all tissues examined (Fig. 2 and Tables 1 and 2). PBR was primarily seen in the epithelium of breast, colon, prostate, ovary, skin, and lung. Moreover, PBR was also present in adrenal cortical cells, hepatocytes, brain glial cells and Leydig and germ cells of testis. Both nuclear and cytoplasmic pattern of staining were observed, which is consistent with the pattern of PBR sublocalization in breast cancer cell lines and biopsies observed in our previous study (8). It is suggested that nuclear PBR is responsible for regulating movement of cholesterol into the nuclear membrane and that this regulation is related to its modulation of MDA-231 cell proliferation (8). However, it is still open to debate what role cholesterol may play in the nucleus and cell proliferation and how PBR regulates cell proliferation.

Breast PBR expression levels [Fig. 2(A1–A4); Table 1] increased noticeably with increasing severity of breast lesion histopathology ($p < 0.0001$). Non-tumoral, fibroadenoma, and adenosis cases were very similar with about 40% of cases with weak expression (+) and about 60% with moderate expression (++) . Primary adenocarcinoma samples contained 3 (33%) cases with weak (+), and 4 (45%) with moderate expression



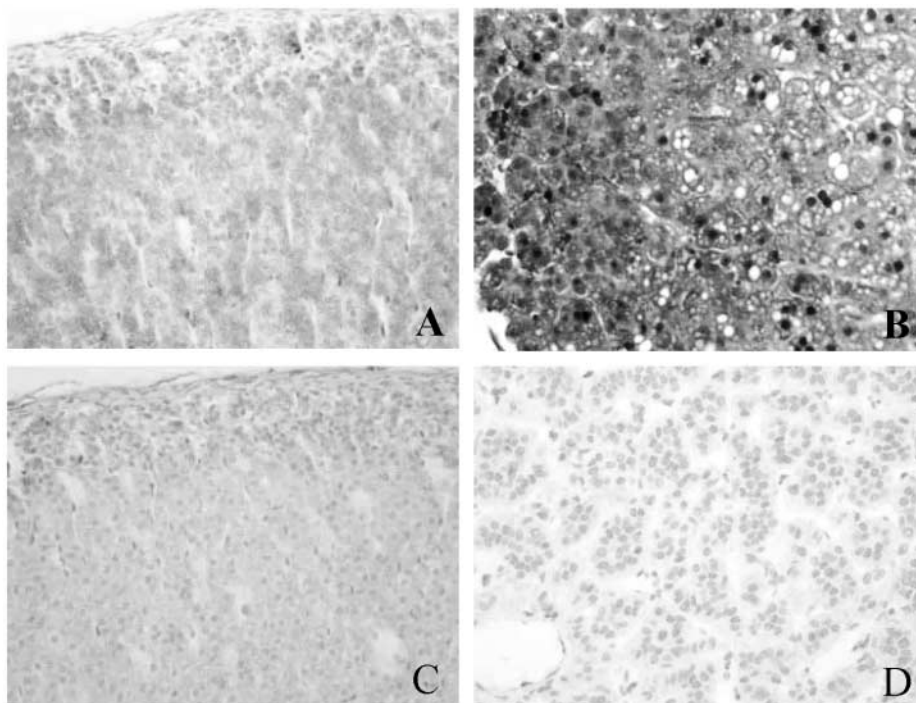


Figure 1. Immunohistochemistry controls. The immunoreactivity of the anti-PBR antiserum (2 $\mu\text{g}/\text{mL}$) used was verified by the positive staining of rich in PBR rat (A) and human (B) adrenal tissues. Antiserum preabsorbed with the PBR peptide (10 $\mu\text{g}/\text{mL}$) used to generate the antiserum, failed to recognize the antigen in these sections (C and D). (*Go to www.dekker.com to view this figure in color.*)

(++) and 2 (22%) with strong staining (+++). The metastatic cases had only 9 (39%) with moderate staining and 14 (61%) with strong staining. These data show that higher levels of PBR expression are present in aggressive breast tumor cells. Non-tumoral tissue, fibroadenoma and adenosis tissues gave nearly identical results, with increasing expression in primary adenocarcinoma and even more in metastatic adenocarcinoma. These data are in agreement with *in vitro* studies on human breast cancer cell lines showing that PBR protein and drug ligand binding capacity were increased in aggressive breast cancer cells relative to non-aggressive cells (8,9). Moreover, these results also agree with data showing that the ability of aggressive breast tumor cells to form tumors *in vivo* might depend on the amount of PBR present in the cells (10). Although in most cells PBR is primarily located in the mitochondria, a perinuclear/nuclear localization has been described in aggressive breast cancer and glioma cell lines (8,21). In the present study, both nuclear and cytoplasmic stainings were observed. In agreement with these protein data and the *in vitro* cell data (8), breast tumors showed increased PBR mRNA levels compared with normal breast tissue ($p < 0.05$) (Fig. 3).

PBR expression in aggressive colorectal carcinomas [Fig. 2(B1–B5); Table 1] is significantly different from non-tumoral tissue and benign lesions ($p < 0.0001$) and similar to that observed in breast cancer. All of the non-tumoral colorectal tissues presented only



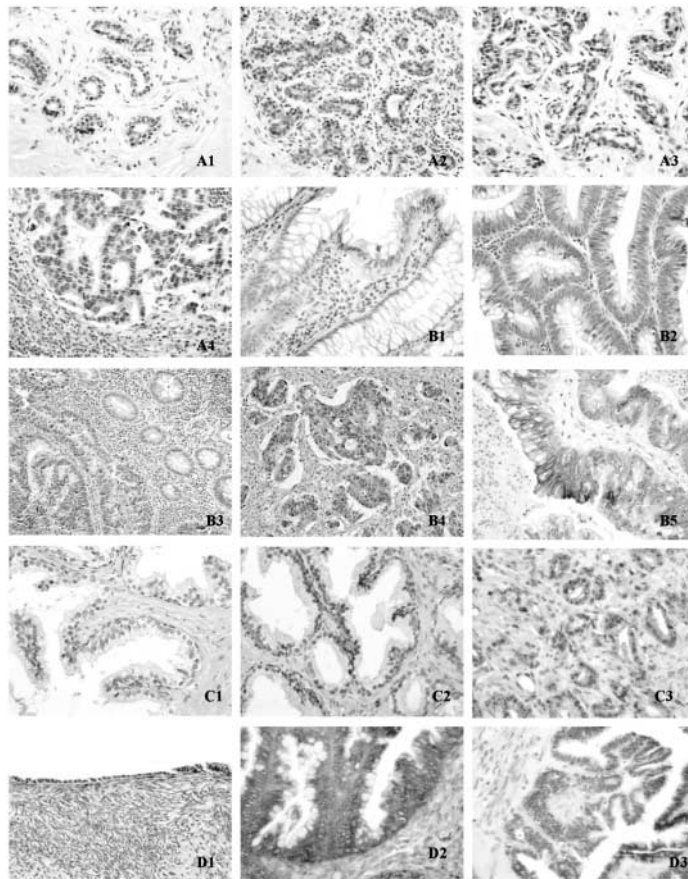


Figure 2. PBR expression in human biopsies. PBR immunohistochemistry was carried out as described under *Materials and Methods*. PBR was highly expressed in primary breast infiltrating intraductal carcinoma (A3) and the same type of carcinoma with lymph node metastasis (A4) compared with non-tumoral breast tissue (A1) and fibroadenoma (A2). The expression of PBR was increased in colorectal adenocarcinoma (B3, B4, and B5) relative to non-tumoral tissue (B1) and adenoma (B2). PBR expression was found increased in prostate adenocarcinoma (C3) relative to BPH (C2) and non-tumoral prostate tissue adjacent to BPH (C1). Normal ovary epithelium (D1) showed very weak staining but mucinous cystadenocarcinoma (D2) and serous adenocarcinoma of the ovary (D3) manifested increase of PBR expression. Lung adenocarcinoma cells (E2) expressed much more PBR than non-tumoral lung tissue (E1). PBR was highly expressed in the cytoplasm of normal adrenal cortical cells (F1) and the nuclei of low-grade adrenal cortical carcinoma tumor cells (F2). Hepatocellular carcinoma (G2) showed decreased PBR expression compared with its vicinal non-neoplastic hepatocytes and normal hepatocytes (G1). Normal epidermis (H1), basal cell carcinoma (H2), squamous cell carcinoma (H3) and melanoma (H4) all showed positive staining. PBR was seen in the germ cells and Leydig cells of normal testis (I1) and seminoma tumor cells (I2). PBR positive glial cells were rare in normal brain tissue (J1) whereas the population of positive tumor cells was markedly increased in anaplastic astrocytoma (J2) and GBM (J3). (*Go to www.dekker.com to view this figure in color.*)



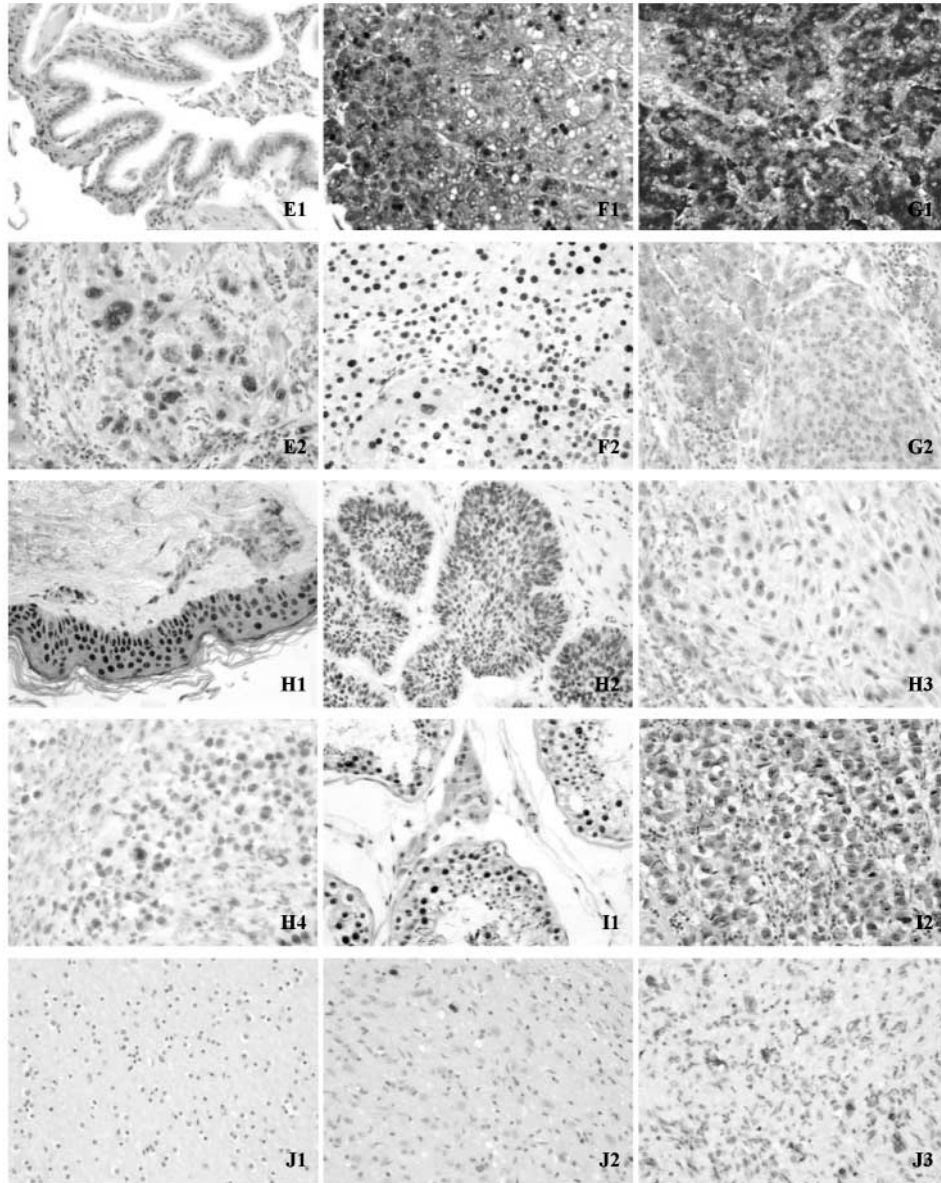


Figure 2. Continued.

weak (+) PBR staining. Adenoma and primary adenocarcinoma samples showed 60% and 67% moderate (++) expression, respectively. Ten (47%) of the metastatic adenocarcinoma samples were intensely stained (++++), with only 2 (10%) with weak expression. These data are in agreement with earlier studies reporting increased PBR drug ligand binding sites in colon adenocarcinomas as compared to normal human colon (22). At the mRNA level (Fig. 3), colon carcinoma showed significant increase of mRNA expression compared



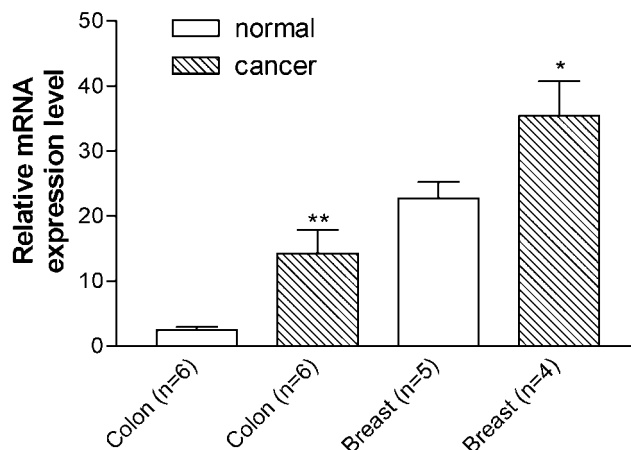


Figure 3. PBR mRNA levels in human breast and colon tumors. PBR mRNA levels were determined by quantitative real-time PCR carried out as described under *Materials and Methods*. The amount of PBR mRNA expression was normalized to the endogenous reference (18S rRNA). PBR mRNA levels are increased in both breast and colon tumors compared with their corresponding normal tissues. p values were determined using the unpaired t test. Results are means \pm SEM. Key: ** $p < 0.01$; * $p < 0.05$.

to normal colon tissue ($p < 0.01$). This result is consistent with the observation of prognostic significance of PBR overexpression in stage III colorectal cancer (23).

PBR immunoreactivity was seen in all the prostatic tissues [Fig. 2(C1–C3); Table 1] examined, but differed significantly between normal samples, BPH samples and adenocarcinomas ($p < 0.0001$). BPH cases [Fig. 2(C2)] exhibited mostly weak (+) staining (67%) with some moderate staining (++) . Adenocarcinoma cases [Fig. 2(C3)] had moderate (++) , 2 cases (13%), strong (+++) , 8 cases (50%), and intense (++++), 6 cases (37%), staining. These results indicate a strong correlation between PBR levels and prostate histopathology. Thus, PBR may be considered as one parameter along with prostatic specific antigen and prostate-specific acid phosphatase (24) in the diagnosis of prostatic adenocarcinoma. It has been reported that PBR drug ligand binding is present in both mitochondrial and microsomal fractions of Dunning G prostatic adenocarcinomas from orchietomized rats compared to tumors from sham operated controls. Treatment with testosterone repressed PBR drug ligand binding in both fractions to control values, suggesting a role of testosterone on the PBR density in these hormone-sensitive prostatic tumors (25).

To the best of our knowledge, there has been no report about the correlation between normal lung and lung tumor PBR levels. All 10 normal lung tissues [Fig. 2(E1); Table 2] showed weak expression (+), while half (4 cases) of the adenocarcinomas had weak (+) and half moderate staining (++) [Fig. 2(E2)]. This difference is significant ($p = 0.02$) and although it suggests that PBR might be involved in the progression of lung cancer, further investigation is required.

Brain tissue samples measured for PBR expression show a small trend indicating increased expression among GBM and anaplastic astrocytomas [Fig. 2(J1–J3); Table 2]. All



three of the non-tumoral tissue samples showed weak PBR expression, while 9 (75%) of the GBM and anaplastic astrocytoma samples had no staining (–), with 1 (8%) and 2 (17%) cases with weak (+) and moderate (++) expression, respectively. Immunoreactive PBR was found in the nuclei of the neurons and cytoplasm of glial cells with weak strength in normal brain, whereas its expression increases in the group of GBM and anaplastic astrocytoma. These results suggest that PBR is expressed constitutively at low level until the onset of the tumor. It has been suggested that PBR expression is associated with the malignancy grade of astrocytoma and affect the life expectancy of tumor-bearing patients (6).

There were notable differences between the normal ovarian tissue and ovarian carcinomas ($p = 0.06$), normal ovarian tissue had no staining (28%) and mild staining (72%) only [Fig. 2(D1); Table 2]. The majority of tumor samples express PBR with 40% weak staining, 25% moderate staining and 20% strong or intense staining, [Fig. 2(D2 and D3)]. As the fourth leading cause of death in women, epithelial ovarian carcinoma is usually diagnosed in advanced stages. However, it has been reported that a robust increase in the number of PBR binding sites in ovarian carcinoma occurs when compared with benign ovarian tumors and normal tissues (14). Thus, further investigation with more samples is needed to elicit the possible correlation of PBR expression with the progression of ovarian neoplasm.

Even though it is known that hormones regulate PBR levels, the molecular mechanisms involved in the regulation of PBR during tumor progression remain unknown. In contrast to the increased PBR levels found in epithelial tumors from breast, colon–rectum, prostate, lung, and ovary tumors, the opposite results were found in the tumors of skin, adrenal, liver, and testis.

Normal skin tissue and less aggressive tumors have significantly ($p = 0.002$) higher levels of staining compared to more advanced or malignant tissues [Fig. 2(H1–H4); Table 2]. Normal tissue showed PBR immunostaining ranging from weak (+, 66%), to moderate (++, 17%) and strong (+++, 17%) intensity, in agreement with a recent report (26), while basal cell carcinoma samples [Fig. 2(H2)] had mostly (86%) moderate (++) staining and the rest had weak (+) expression. Squamous cell carcinoma PBR immunostaining [Fig. 2(H3)] was mostly weak (+, 90%). Melanoma cases [Fig. 2(H4)] also presented mostly (86%) weak (+) staining. These data indicate that PBR is expressed at higher levels in the normal skin epidermal cells whereas its expression level decreased in the corresponding tumors. It has been suggested that PBR may participate in an antioxidant pathway in normal skin epidermal cells (26).

Table 2 presents information about the levels of PBR expression present in adrenal [Fig. 2(F1)], liver [Fig. 2(G1)] and testis [Fig. 2(I1)] tissues and their respective malignancies [Fig. 2(F2, G2 and I2)]. The trend observed suggests that PBR is expressed at high levels in normal adrenal cortical cells, hepatocytes and Leydig cells as well as germ cells (although in reduced levels) of testis, whereas its presence decreased in their corresponding tumors. However, there were not enough samples to evaluate convincingly the statistical significance of these data. The present findings with liver tumors do not support the observation showing upregulation of PBR expression in human hepatocellular carcinoma (12).

In conclusion, among breast, colorectal and prostate carcinomas, PBR expression dramatically increases compared to their non-malignant counterparts. Metastatic breast and colorectal adenocarcinomas manifest increased PBR expression relative to their primary malignancies. Brain, lung and ovarian tumors show a small trend in which



malignant tumors express more PBR than the corresponding non-tumoral tissues. We also observed that PBR is present in high levels in the cytoplasm of normal adrenocortical cells and hepatocytes, whereas PBR levels are lower in cortical adenoma, adenocarcinoma and hepatocellular carcinomas where it primarily localizes in the nuclei. PBR levels are higher in the epidermal cells of normal skin than in skin basal cell carcinomas, squamous cell carcinomas and melanomas. Testis seminomas show varied levels of PBR expression while the Leydig cells and germ cells contain PBR at very high and moderate levels, respectively. Despite the limited number of certain samples, these data provide a basis to study the predictive value of PBR expression in assessing the progression of the disease and the efficacy of various treatments. In addition, recent data showed the induction of apoptosis and cell death in various cancer cell lines by pharmacological concentrations of high affinity PBR drug ligands (8,27–29). Taken together, these results support further efforts on the evaluation of PBR as a predictive means of breast, colorectal, and prostatic tumor progression and the use of these drugs as anti-tumor agents in clinical trials.

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