The P2X7 Receptor: A Novel Biomarker of Uterine Epithelial Cancers

Xin Li, Lingying Zhou, Ying-Hong Feng, Fadi W. Abdul-Karim, and George I. Gorodeski

Abstract

Objective: To determine expression of the P2X7 receptor in normal and in cancer uterine tissues. The rationale was that the receptor P2X7 regulates constitutive apoptosis in uterine epithelial cells, and previous studies showed diminished P2X7-mediated apoptosis in cancer uterine cells compared with normal cells.

Methods: A clinical, experimental feasibility study. Normal (n = 42) and cancer uterine tissues (n = 47) were obtained from a total of 72 women ages 25 to 75. End points for P2X7 mRNA were quantitative PCR and in situ hybridization, and end points for P2X7 protein were Western blots and immunostaining using anti-P2X7 antibody.

Results: (a) In normal uteri, P2X7 mRNA and protein were expressed predominantly in the epithelial (endometrial, endocervical, and ectocervical) cells. (b) Expression of the P2X7 mRNA and protein was absent from endometrials and endocervical adenocarcinoma tissues and from cervical squamous cell carcinoma tissues. (c) In cervical dysplasia, P2X7 protein was absent in the dysplastic lesions. (d) Semiquantitative analysis using P2X7 mRNA (normalized in each tissue to the constitutive glyceraldehyde-3-phosphate dehydrogenase) and P2X7 protein levels (normalized in each tissue to the constitutive tubulin) revealed that P2X7 mRNA and/or protein levels can distinguish uterine normal from cancer tissues at high degrees of sensitivity (92%, 100%) and specificity (100%, 90%).

Summary and Conclusions: (a) Levels of the P2X7 are lower in uterine epithelial cancer tissues than in the corresponding normal tissues. (b) The data suggest that tissue P2X7 mRNA and protein levels could be used as a novel biomarker to differentiate normal and cancer uterine epithelial tissues.

Introduction

The receptor P2X7 belongs to the P2X subfamily of P2 nucleotide receptors (1, 2), which are membrane-bound, ligand-operated channels (3-5). ATP is the naturally occurring ligand for the P2X7, and activation of the receptor by extracellular ATP stimulates several responses, including formation of pores in the plasma membrane that allow passage of cations up to 900 Da through the pores (6). In normal human cervical epithelial cells, Ca2+ influx via P2X7 pores mediates apoptosis constitutively by an autocrine-paracrine mechanism (7). Thus, ATP secreted by cells into the extracellular space reaches high nanomolar to low micromolar concentrations, which suffice to activate the P2X7 receptor (7, 8), and cells expressing the functional receptor are targeted to the proapoptotic effect of ATP (9).

Human uterine epithelial cells express two P2X7 isoforms: the full-length 85-65 kDa P2X7 receptor (P2X7; ref. 10) and an inactive 42 to 45-kDa truncated variant (P2X7-j) that lacks the entire intracellular COOH terminus, the second transmembrane domain, and the distal third of the extracellular loop of the P2X7 (9). The P2X7-j variant is deficient in ligand binding, pore formation, and mediation of apoptosis; however, it can interact and hetero-oligomerize with the full-length P2X7 (9). Because P2X7 receptor functions depend on oligomerization of P2X7 molecules into homotrimers (11), any interaction between the P2X7-j and the full-length P2X7 can block P2X7-mediated channel activity, pore formation, and induction of apoptosis (9).

Earlier studies showed that baseline and ligand-induced P2X7-mediated apoptosis is greater in normal human ectocervical epithelial cells than in cancer human cervical cells (7). More recent results showed that normal and cancer human cervical cells express the variant P2X7-j but normal cells express higher levels of the full-length P2X7 than cancer cells (9). These data provide a possible mechanistic explanation for the finding of lesser degree of P2X7-mediated apoptosis in cancer cervical cells. Accordingly, normal cells expressing predominantly the P2X7 isoform are more likely to form active [(P2X7)] homotrimers, whereas cancer cells expressing low levels of the P2X7 isoform are more likely to form inactive heterotrimers, such as the [(P2X7)2]/[(P2X7-j)] or the [(P2X7)]/[(P2X7-j)2] or the inactive [(P2X7-j)3] homotrimers (9).

The objective of the present study was to better understand the clinical significance of P2X7 receptor expression in vivo in normal and cancer human uterine tissues. Using semiquantitative analyses, we show that levels of P2X7 receptor mRNA and protein in tissues obtained from women with ectocervical, endocervical, and endometrial cancers are significantly lower than from the corresponding normal tissues. The results suggest that tissue levels of the P2X7 receptor can serve as a novel biomarker to detect uterine cancers in women.

Materials and Methods

Human Tissues and Cell Cultures. Discarded human uterine tissues from women undergoing hysterectomy for indications unrelated to the present study were obtained according to Institutional Review Board protocols 12-03-50 and 03-90-300 from the Human Tissue Procurement Facility of Uniformed Services University of the Health Sciences, Bethesda, Maryland.
University Hospitals of Cleveland and the Comprehensive Cancer Center Tissue Procurement Core Facility (Case Western Reserve University, Cleveland, OH) and from the Cooperative Human Tissue Network (National Cancer Institute, Bethesda, MD) through the Human Tissue Resource Network (Department of Pathology, Ohio State University, Columbus, OH). Tissues were collected over a period of 6 months based on availability. Some of the tissues were used for assay development and pilot experiments. The secondary intention of the study was to determine the size effect if differences among groups (normal versus cancers) were found and to conduct a feasibility comparative study. The data presented in this article are considered preliminary, and no formal power analysis was done. A minimum number of tissues to be included is the comparative part of the study.

On removal, uterine specimens were delivered to the Department of Pathology at University Hospitals of Cleveland or at the Ohio State University where the bulk of the tissue was used to establish the histologic diagnosis. Tissues selected by the pathologists for the purpose of the present study were snap frozen in liquid nitrogen, shipped on dry ice, and stored at −80°C until assayed. Some tissues were fixed and embedded in paraffin according to standard procedures. Tissue processing for RNA and protein assays was described (9).

Tissues were obtained from a total of 72 women ages 25 to 75. The study used histologically normal 15 endometrial, 3 endocervical, and 24 ectocervical tissues and 29 endometrial cancers (28 endometrioid adenocarcinomas and 1 mixed adenomatous-mullerian cancer), 6 endocervical cancers (all adenocarcinomas), and 10 ectocervical cancers (all squamous cell carcinomas). Normal plus cancer tissues were retrieved from uterine specimens of 15 women: 10 endometrial, 3 endocervical, and 2 ectocervical. The histologic diagnoses presented below were assigned by the Departments of Pathology at University Hospitals of Cleveland or at the Ohio State University.

Cell culture techniques of normal human keratinocytes and the transformed cervical cancer cell line CaSkii (12, 13) and the method of doxycycline-inducible expression of P2X7 or P2X7-j in Madin-Darby canine kidney cells were described (9).

**Quantitative Real-time PCR.** Quantitative PCR assays, including specific primers and conditions for amplification of the human full-length P2X7 gene (Genbank Y09561), the truncated variant P2X7-j (Genbank DQ399293), as well as the constitutive glyceraldehyde-3-phosphate dehydrogenase, were described (9). Quantitative PCR results were calculated using the comparative threshold cycle (Ct) method of relative quantitation. Verification of reverse transcription was done by sequencing the PCR products using the above antisense probe of the full-length P2X7 cDNA template (synthesized by using 100 ng/L DIG labeling mix (Roche, Indianapolis, IN), 2 µL transcription buffer, 40 units of 17 polymerase (Roche), and H2O in a total volume of 20 µL. The mixture was incubated at 37°C overnight, and the reaction was stopped by adding 0.8 µL of 0.5 mol/L EDTA. For ethanol precipitation, 2.5 µL of 4 mol/L lithium chloride plus 75 µL prechilled (−20°C) ethanol were added to the mixture followed by incubation at −80°C for 2 hours and centrifugation at 13,000 × g for 5 minutes at 4°C. The pellet was dried, dissolved in 50 µL diethyl pyrocarbonate water, and stored at −80°C.

Tissue slices on slides were deparaffinized, rehydrated, and postfixed with 4% paraformaldehyde. After treatment with 0.04 N HCl for 20 minutes at room temperature, tissues were digested with 20 µg/mL proteinase K for 20 minutes at 37°C. Cultured cells were plated on polylysine-coated coverslips and grown in culture medium to subconfluence. After fixation with 4% paraformaldehyde/PBS for 15 minutes at room temperature, cells were washed with PBS containing 0.1% active diethyl pyrocarbonate. The cells were permeabilized with 0.1% Triton X-100/PBS/diethyl pyrocarbonate for 5 minutes and equilibrate with 5× SSC/diethyl pyrocarbonate for 15 minutes. Specimens (tissues or cells) were prehybridized for 2 hours at 58°C in hybridization mix containing 5× SSC/50% formamide/40 µg/mL salmon sperm DNA and hybridized at 58°C overnight with 150 ng/µL sense or antisense probes for the full-length P2X7 in hybridization mix solution. Following the incubation, specimens were washed in 2× SSC at 15 min intervals, then in the same buffer for 30 minutes at 65°C, and in 0.1× SSC for 1 hour at 65°C and equilibrated with TN buffer containing 100 mmol/L Tris and 150 mmol/L NaCl (pH 7.5). Specimens were incubated with alkaline phosphatase–conjugated anti-digoxigenin antibody (1:1,000; Roche) in blocking solution for 2 hours at room temperature. Excess antibody was removed by two 15-minute washes in washing buffer and once by washing with detection buffer (Roche). Color development was done at room temperature in BM Purple alkaline phosphatase substrate (Roche) for 20 minutes in the dark, and staining was stopped with detection buffer. For cell cultures, nuclei were counterstained with methyl green or fast red and slides were dried with ethanol and mounted with VectaMount (Vector Laboratories, Burlingame, CA). Staining was evaluated using Nikon Eclipse 80i microscope (Nikon, Melville, NY).

**Protein Methods.** Western blots were done as described (14). Aliquots of postnuclear supernatants of cell lysates were normalized to 15 µg protein, separated in SDS-PAGE, and blotted by Western analysis. The methods of immunostaining and densitometry were described (14). P2X7 receptor poly-peptides (P2X7 and P2X7-j) were visualized using rabbit polyclonal anti-P2X7 receptor (in the absence or presence of its antigen peptide; Alomone Laboratories, Jerusalem, Israel; refs. 10, 14). The anti-tubulin and anti-E-cadherin antibodies as well as secondary antibodies were described (10, 14-16).

All chemicals, unless specified otherwise, were obtained from Sigma Chemical (St. Louis, MO). Data were tabulated in contingency tables, and significance of differences between groups was estimated by χ2 test.

**Results**

**P2X7 and P2X7-j Expression.** P2X7 expression in normal and cancer endometrial and cervical tissues was determined in terms of immunoreactivities with the anti-P2X7 antibody that could be blocked by coincubation with the P2X7 antigen. Results of Western blots are shown in Fig. 1A (lanes 1 and 3 (CaSkii) and lanes 2 and 4 (patient 120)) and confirm (9, 10, 14) the expression of specific P2X7 immunoreactivities of the full-length P2X7 (65-85 kDa) and of the truncated P2X7-j isoform (42-45 kDa; Fig. 1A). Results of immunostaining are shown in Fig. 1B and show loss of immunoreactivity with the anti-P2X7 antibody in samples coincubated with the P2X7 antigen.

Protein and mRNA levels of the truncated P2X7-j isoform were similar in lysates of histologically normal and cancer
endometrial and ectocervical tissues (Fig. 1A and C). However, the protein and mRNA levels of the full-length P2X7 were significantly higher in the normal than in the cancer tissues (Fig. 1A and C).

**P2X7 Protein Expression**

**Normal Tissues.** In normal uteri, P2X7 immunoreactivity was localized predominantly in the endometrial, endocervical, and ectocervical epithelium, as correlated and confirmed by costaining with the epithelial marker E-cadherin (Fig. 2, a-f, o-r, and w-zh). In all three types of epithelia, the P2X7 staining was observed both in the membrane and in the cytoplasm of the epithelial cells (Fig. 2, c, q, and zg). In the endometrium and endocervix, the P2X7 antibody stained the endometrial glands (Fig. 2, a-f) and the endocervical crypts (Fig. 2, o-r and w-z), respectively. P2X7 staining was uniform, both in endometrial tissues of women ages <50 (Fig. 2, a-d) and in women ages >51 (e.g., Fig. 2, e and f; age 67) and in endocervical tissues of women ages <50 (Fig. 2, o-r and w-z) and in women ages >51 (data not shown).

In normal uteri, strong P2X7 staining was found at the transition zones of the columnar-endocervical and ectocervical-squamometaplastic epithelia (Fig. 2, y and z). In the endometrium and endocervix, the P2X7 antibody stained the endometrial glands (Fig. 2, a-f) and the endocervical crypts (Fig. 2, o-r and w-z), respectively. P2X7 staining was uniform, both in endometrial tissues of women ages <50 (Fig. 2, a-d) and in women ages >51 (e.g., Fig. 2, e and f; age 67) and in endocervical tissues of women ages <50 (Fig. 2, o-r and w-z) and in women ages >51 (data not shown).

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**Uterine Cancers.** Endometrial (Fig. 2, g-n), endocervical (Fig. 2, s-v), and ectocervical cancers (Fig. 2, zs-zv) lacked P2X7 staining. The endogenous positive controls were sections comprising endometrial cancers plus normal glands in the same slice (Fig. 2, k-n). In those cases, the anti-P2X7 antibody stained positively the normal glands but did not stain the cancer tissues (Fig. 2, k and m).

Interesting results were obtained in tissues from women with ectocervical dysplasia. Analysis of tissues with different grades of ectocervical dysplasia showed that the dysplastic lesions per se lacked P2X7 staining (Fig. 2, zi-zr). In addition, when the cross-section was evaluated in its entirety, there was gradual decrease in staining as the degree of abnormality progressed. Thus, in mild dysplasia, about one third of the epithelium in the basal side lacked P2X7 staining (Fig. 2, zi-zf). In moderate dysplasia, about one half of the epithelium extending from the basal side lacked P2X7 staining (Fig. 2, zm-zp), and pockets lacking staining could be seen projecting more superficially (Fig. 2, zm and zo). This was in contrast to the rather uniform graded staining in normal ectocervical epithelium (Fig. 2, zc). In severe dysplasia, about two thirds of...
the epithelium extending from the basal side lacked P2X7 staining, and staining was observed only in the most superficial layer composed of superficial cells and envelops (Fig. 2, zq and zr). Some cases of severe dysplasia lacked P2X7 staining entirely (data not shown), similar to cases of cervical squamous cell carcinoma (i.e., Fig. 2, zs and zu).

Collectively, the data in Fig. 2 indicate that (a) the P2X7 is expressed predominantly in the epithelial components of the uterus, (b) uterine epithelial cancerous lesions lack expression of the P2X7, and (c) in ectocervical dysplasia the dysplastic cells lacked P2X7 staining and the total P2X7 staining across the epithelium correlated reciprocally with the severity of the dysplasia.

**P2X7 mRNA Expression: In situ Hybridization.** The technique of in situ hybridization was used to determine the cellular distribution of P2X7 mRNA in normal and cancer uterine tissues. The experiments focused on the expression of the full-length P2X7 using a cDNA template (probe) designed to hybridize specifically with the full-length P2X7. The sensitivity of the method was tested using human keratinocytes, which express endogenously the full-length P2X7. In preparations of cells not exposed to the probe (Fig. 3A) or in preparations of cells hybridized with the sense P2X7 probe (Fig. 3B), no staining was obtained with the anti-digoxigenin antibody. In contrast, hybridization with the antisense P2X7 probe elicited significant perinuclear and cytoplasmic staining (Fig. 3C).

The specificity of the P2X7 probe to distinguish P2X7 and P2X7- mRNA in situ was tested using Madin-Darby canine kidney cells that lack endogenous expression of the receptor. Madin-Darby canine kidney cells expressing tetracycline repressor regulating system (to prevent potential toxic effects of the interest genes) were transfected with either the P2X7 or P2X7- cDNAs. Cells were grown in the presence or absence of doxycycline [to induce expression of the P2X7 or P2X7- proteins (9)], and cell preparations were hybridized either with the sense or with the antisense P2X7 probe. The results in Fig. 3 (d-k) show that specific hybridization of the P2X7 probe was seen only in doxycycline-treated cells transfected with the P2X7 cDNA and hybridized with the antisense P2X7 probe (Fig. 3, g). Collectively, the results in Fig. 3 (a-k) validate the usefulness of the P2X7 cDNA template for detection of the full-length P2X7 mRNA using the in situ hybridization technique.

In cross-sections of human normal uterine tissues, specific intense perinuclear and cytoplasmic P2X7 mRNA staining decorated the epithelial components of the endometrial glands (Fig. 3, i and m), the endocervical crypts (Fig. 3, p and q), and

**Figure 2.** Immunostaining of endometrial (a-n), endocervical (o-z), and ectocervical (w-zv) normal (a-f, o-r, and w-zh), dysplasia (zr-zv), and cancer tissues [endometrial (g-n) and endocervical (s-v) adenocarcinomas and squamous cell carcinoma (zs-zv)] with the anti-P2X7 antibody (a, c, e, g, i, k, m, o, q, s, u, w, y, za, zc, ze, zg, zi, zk, zm, zo, zg, zs, and zu) or with the anti-E-cadherin antibody (to show the epithelial components of the tissues; b, d, f, h, j, l, n, p, r, t, v, x, z, zb, zd, zf, zh, zj, zl, zn, zp, zr, zt, and zv), zi to zl, mild cervical dysplasia; zm to zp, moderate cervical dysplasia; zg and zr, severe cervical dysplasia. a to d, o to r, and zi to zr, obtained from women ages 35 to 47; e, f, w to zb, zg, zh, and zs to zt, obtained from women ages 55 to 67. In sections containing normal endometrial glands plus endometrial cancer (k-n), the anti-P2X7 antibody stained the normal glands (k, four glands in the middle; m, two glands at the left bottom corner) but not the cancerous tissues. y, arrowhead, endocervical columnar (upward)–squamometaplastic (downward) junction. za, arrowhead, squamometaplastic (upward)–squamous (downward) junction. ze and zf, hyperkeratosis. The experiments were repeated two to four times with similar trends. Magnification, ×4 to ×20.
Figure 3. Expression of the P2X7 mRNA: in situ hybridization. a to c. Cultures of normal human foreskin keratinocytes were either not hybridized (a), hybridized with the sense P2X7 probe (b), or hybridized with the antisense P2X7 probe (c). Nuclei were counterstained as described in Materials and Methods. Specific hybridization of the P2X7 probe in c is seen as intense blue perinuclear and cytoplasmic reaction with the anti-digoxigenin antibody. The experiment was repeated twice with similar trends. d to k. Tetracycline repressor–expressing Madin-Darby canine kidney cells (MDCK) were transfected with either the P2X7 cDNA or the P2X7-j cDNA, grown in the absence (Dox−) or presence (Dox+) of doxycycline, and hybridized either with the sense P2X7 probe (S) or with the antisense P2X7 probe (AS). Nuclei were not stained. g. Specific hybridization of the P2X7 probe was seen only in doxycycline-treated cells transfected with the P2X7 cDNA and hybridized with the antisense P2X7 probe. The experiment was repeated twice with similar trends. l to x. In situ hybridization with the antisense P2X7 probe of cross-sections of human endometrial (l-o), endocervical (p-s), and ectocervical (t, u, w, and x) tissues. l, m, p, q, and t to v: normal tissues; n, o, r, s, w, and x: cancer tissues. Magnification: ×4 (l, n, p, r, t, v, and w) or ×20 (m, o, q, s, u, and x); v: cross-section of the normal ectocervix hybridized with the sense P2X7 probe. l, m, p, q, t, and u: specific hybridization of the antisense P2X7 probe was seen in the epithelia of the normal tissues. The experiment was repeated three to five times with similar trends.
the ectocervix (Fig. 3, t and u). The subepithelial regions of these tissues stained significantly less.

In cross-sections of the corresponding cancers, no P2X7 mRNA staining was found (Fig. 3, n, o, r, s, w, and x). These results confirm the data in Fig. 1C that epithelial uterine cancer cells express minimal amounts of the full-length P2X7 mRNA.

Quantification of P2X7 Expression in Normal and Cancer Uterine Tissues. The data in Figs. 1-3 indicate that uterine epithelial cancer cells, in contrast to normal uterine epithelial cells, express low amounts of the full-length P2X7 mRNA and protein. These data were the basis for the semiquantitative analysis shown in Fig. 4 of full-length P2X7 mRNA levels (top) and protein levels (bottom) in normal (white columns) and cancer uterine tissues (black columns). P2X7 mRNA expression was determined in terms of P2X7 mRNA quantitative PCR levels normalized in each tissue to the constitutive glyceraldehyde-3-phosphate dehydrogenase (e.g., Fig. 1C). P2X7 protein levels were determined in terms of densitometry of the P2X7 65 to 85–kDa band in Western blots normalized in each tissue to the constitutive tubulin (e.g., Fig. 1A). Included in the analysis for P2X7 mRNA were a total of 50 tissues from 36 women, and in the analysis for P2X7 protein a total of 54 tissues from 41 women. The data in Fig. 4 show that P2X7 mRNA and protein levels were higher in normal endometrial endocervical and ectocervical tissues than in endometrial, endocervical, and ectocervical cancers. In all cases where normal and cancer tissues were obtained from the same woman, P2X7 mRNA and protein levels were higher in the normal than in the cancer tissues (Fig. 4).

Figure 5 shows the combined data of Fig. 4, grouped into P2X7 mRNA and protein results. Cutoff levels of P2X7 mRNA and protein were defined as those separating normal (“positive”; empty circles) from cancer tissues (“negative”; filled circles). The cutoff values (in arbitrary units) were established from Fig. 5 as P2X7 mRNA ≥1,000 and P2X7 protein ≥15. The data of the “positive” and “negative” P2X7 mRNA and protein levels grouped by these categories were evaluated using χ² analysis (Table 1A and B). The results indicate that, for the sample studied in Fig. 4, the variables of P2X7 mRNA and P2X7 protein were sensitive (92%, 100%) and specific (100%, 90%) in differentiating normal and cancer uterine tissues.

The results in Fig. 4 were not affected by the age, race, and ethnic origin of the woman or by the type, stage, or grade of the cancer (data not shown).

Discussion

We found higher levels of expression of P2X7 mRNA and protein in human uterine epithelial normal tissues than in cancer tissues. The mRNA quantitative PCR and in situ hybridization data as well as the protein Western blot results were specific for the full-length P2X7, whereas the immunostaining results most probably represent P2X7 plus P2X7 junta data. In view of the similar results using the four different end points, it is suggested that the immunostaining results also reflect contributions made primarily by the full-length P2X7. The greater expression of P2X7 in normal versus cancer tissues was not affected by women’s age, suggesting that menopause and estrogen status did not significantly affect the expression of the P2X7 in uterine epithelial cells. It was previously shown that estrogens do exert antiapoptotic effects in the uterus by blocking P2X7-mediated apoptosis (7). However, those effects were unrelated to the expression of the P2X7 receptor and involved modulation of events distal to the receptor (7).

In the normal uterus, most of the full-length P2X7 was expressed in the epithelial tissues of the endometrium, endocervix, and ectocervix. In the ectocervix, P2X7 staining was found mainly in cells of the germinative layers. Some staining was also found in superficial layers of the ectocervix, but it probably represents receptor trapped in the flat superficial cells or in the anucleated squames (envelops; ref. 18). The abundance of expression in the epithelial components of the uterus and particularly in the germinative layers of the ectocervix is in accordance with the presumed role of the P2X7 as cell growth regulator (7). Epithelial tissues of the uterus, similar to other surface epithelia, are continuously exposed to stimuli that could alter cell growth. In addition, uterine epithelial cells are also exposed to seminal fluid and sperm cells, and endometrial tissues are exposed to the migrating and implanting blastocyst. The abundance of P2X7 receptor in the epithelial tissues could be a phylogenetic mechanism to control cell growth of uterine epithelial tissues through apoptosis (4, 11) and to allow sperm...
migration through the uterine canal and implantation of the blastocyst in the endometrium without undue mitogenic reaction of the maternal epithelial compartment.

The comparative experiments of the present study focused on common epithelial uterine cancers, which compose >95% of total uterine cancers (19). Because the results were similar regardless of tumor type, adenocarcinomas, and squamous cell carcinomas, it is possible that the differences in the expression of P2X7 in normal versus cancer tissues are not limited to uterine tissues and that similar effects occur also in other types of epithelial neoplasia. Unpublished preliminary experiments from our laboratory support this statement and have shown similar trends in skin, breast, and prostate tissues.

The differences in the expression of P2X7 in normal versus cancer tissues are interesting in view of the known compartmentalization of the receptor. The functional P2X7 receptor resides in the plasma membrane, whereas cytoplasmic staining reflects newly synthesized receptor in transit to the membrane, unprocessed receptor, or postactivation internalized receptor (14). One of the mechanisms by which cells regulate P2X7 activity is by modulating membrane expression of the receptor (4). However, the amount of receptor present in the membrane is relatively small (4, 14), and a more likely explanation for the present results is regulation of P2X7 activity through the regulation of P2X7 mRNA levels and protein translation. This conclusion is supported by the P2X7 data. mRNA and protein levels of the truncated P2X7 were similar in normal and cancer cells; in contrast, mRNA and protein levels of the full-length P2X7 were higher in normal versus cancer cells. Because both P2X7 and P2X7 isoforms share a similar promoter (9, 20), differences in transcription rates are unlikely to explain the findings. An alternative explanation is enhanced degradation of the P2X7 mRNA in cancer cells or greater stability of the P2X7 mRNA in the normal cells (21).

These speculations raise the possibility that uterine cancer cells have developed mechanisms to escape growth control by down-regulation of P2X7 mRNA. Whether the effect precedes the transformation process (i.e., it is associated with or causes cancer development) or whether it occurs as a result of the neoplastic process is at present unknown. However, regardless of which hypothesis prevails, the present results show that loss of the P2X7 occurs early on in the neoplastic process because P2X7 mRNA and protein were absent already in mild cervical dysplasia (Fig. 2, zi and zj). Therefore, better understanding of the biology and oncology of the P2X7 receptor may contribute to our understanding of uterine cancer development and progression.

The present results suggest that cellular levels of the full-length P2X7 can be used as a novel biomarker to differentiate normal and cancer uterine epithelial tissues. The biochemical migration through the uterine canal and implantation of the blastocyst in the endometrium without undue mitogenic reaction of the maternal epithelial compartment.

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Table 1. Data of Fig. 5 were categorized by 2 × 2 contingency tables (A) and significance of differences between groups was estimated by χ² test (B).

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<tbody>
<tr>
<td>Sensitivity</td>
<td>(24/26) 92%</td>
<td>(25/25) 100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>(24/24) 100%</td>
<td>(26/29) 90%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>(24/24) 100%</td>
<td>(25/28) 89%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>(24/26) 92%</td>
<td>(26/26) 100%</td>
</tr>
<tr>
<td>False-positive ratio</td>
<td>(0/24) 0</td>
<td>(3/28) 11%</td>
</tr>
<tr>
<td>False-negative ratio</td>
<td>(2/26) 8%</td>
<td>(0/26) 0</td>
</tr>
<tr>
<td>Likelihood ratio*</td>
<td>[92 / (100 – 92)] = 110</td>
<td>[100 / (100 – 90)] = 10</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>3.43-49.25</td>
<td>–∞ to +∞</td>
</tr>
<tr>
<td>χ² (Fisher’s exact test)</td>
<td>3.39</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative.

*Likelihood of having a normal tissue if the test is positive.
assays using minced tissues to determine P2X7 mRNA and/or protein levels used blocks of tissues that were processed without preseparation of the epithelial components. In the cancer tissue blocks, most of the tissue mass contained cancerous elements; however, in the normal tissue blocks, the epithelial components (endometrium, endocervix, or ectocervix) comprised only small part of the tissues and the remaining mass contained subepithelial stromal components. Because the latter does not contain significant amounts of the P2X7 (Fig. 2) and because the results were normalized to tissue total glyceraldehyde-3-phosphate dehydrogenase (for P2X7 mRNA) and tissue total tubulin (for P2X7 protein), the determinations in Fig. 4 may have underestimated the P2X7 mRNA and protein determinations in normal tissues. Accordingly, assays that will aim at collecting samples of surface epithelia may provide more accurate results by retrieving lower amounts of stromal elements. Therefore, the P2X7 marker could potentially provide a robust method for screening/detection of uterine cancers.

An additional potential advantage of using the P2X7 as a biomarker could be to differentiate cervical dysplasia lesions. The data shown in Fig. 3 (21-27) suggest that specimens obtained from mild-moderate dysplasia would yield positive values for P2X7 mRNA and protein levels (contributed by the normal component of the ectocervical epithelium), whereas severe dysplasia and cancers would be negative. Therefore, theoretically, the P2X7 method could differentiate between mild/moderate and severe dysplasia. Such a distinction may be of value because this cutoff is often used clinically in the decision-making process of conservative versus surgical management (22) of these cases.

In summary, the present results indicate that levels of the full-length P2X7 are lower in uterine epithelial cancer tissues than in the corresponding normal tissues. The data suggest that tissue analysis of P2X7 mRNA and protein levels could be used as a novel biomarker to differentiate normal and cancer uterine epithelial tissues. Future prospective blinded studies are being planned and may be able to determine the usefulness of this biomarker in the clinical setting.

References
The P2X7 Receptor: A Novel Biomarker of Uterine Epithelial Cancers

Xin Li, Lingying Zhou, Ying-Hong Feng, et al.


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