Age-associated changes in the expression pattern of cyclooxygenase-2 and related apoptotic markers in the cancer susceptible region of rat prostate

Alaa F.Badawi1,4, Yingying Liu1, Mazen B.Eldeen1, Willard Morrow2, Zaineb R.Razak1, Marie Maradeo1 and Mostafa Z.Badr3

1Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA 19111, 2Department of Basic Medical Science, School of Medicine and 3Division of Pharmacology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64108, USA
4To whom correspondence should be addressed
Email: af_badawi@fccc.edu

Senescence-associated changes in the prostate are believed to play an important role in the genesis of prostate cancer. In order to provide further information on how aging increases the prostate susceptibility to cancer, we examined the pattern of cyclooxygenase (COX)-2 expression and the concomitant alterations in prostaglandin E2 (PGE2) synthesis in the prostate glands of 4-, 10-, 50- and 100-week-old Fischer 344 rats. This was carried out in the prostatic areas where hormone-induced tumors arise, namely the periurethral ducts of the dorsolateral prostate (DLP). Age-associated changes were also evaluated for pro- and anti-apoptotic factors linked to COX-2 signaling and known to be involved in the normal development of the prostate gland as well as in carcinogenesis. COX-2 expression was increased in the DLP in an age-dependent manner where senescent rats had >3-4-fold higher COX-2 mRNA and protein levels than their juvenile counterparts (P < 0.05). The age-related changes in COX-2 were accompanied by a similar up-regulation in the PGE2 synthesis. Evaluation of mediators of apoptotic signaling showed a significant (P < 0.05) decline in the expression levels of the pro-apoptotic BAX (>6-fold) and peroxisome proliferator-activated receptor γ (>3-fold) and in caspase-3 activity (>2-fold) and an up-regulation of the anti-apoptotic Bcl2 (>8-fold), PKCα (>2-fold) and pAkt (>4-fold) in the 100-week-old rats versus the 4-week-old animals. There was an ~15-fold age-dependent decrease in the pro-apoptotic ratio BAX:Bcl2 and an increase in the anti-apoptotic variable PKCα2′Bcl2/BAX in the senescent rats compared with the juvenile ones. These results suggest that increased COX-2 expression can be linked to the decline in the pro-apoptotic signaling in the prostate gland during aging. Subsequently, COX-2 inhibitors can be considered as a promising class of agents to attenuate the increased cell survival and, hence, protect against tumorigenesis in the aging prostate.

Introduction

Incidence of prostate cancer increases almost exponentially with age. Over 80% of prostate cancer cases are diagnosed in men aged 65 years or older (1). Therefore, the predictable increase in prostate cancer cases as the population ages renders the disease as a critical public health problem. Senescence-associated changes in the prostate are believed to be involved in the genesis of prostate cancer. Apart from the altered hormonal milieu (2,3), senescent animals or elderly men exhibit decreased apoptotic potentials (4–6), loss of antioxidant enzyme activities (7) and accumulation of DNA damages (8) in their prostates compared with their younger counterparts. Although these changes are all implicated in carcinogenesis, the precise mechanism underlying the increased rates of prostate cancer at senescence remains poorly understood. Recently, among several factors thought to play a key role in prostate cancer, cyclooxygenase-2 (COX-2) has received particular attention as a feasible target for early detection and prevention (9).

Two COX isoforms, COX-1 and COX-2, have been identified. COX-1 is constitutively expressed and mediates ‘housekeeping’ functions while COX-2 is undetectable in most tissues but can be rapidly induced in response to cytokines, growth factors and tumor promoters (10). In the absence of any activation, prostate exhibits the highest COX-2 levels among human tissues (11). COX-2 is also the predominant COX isoform in the adult rat male reproductive system (12). Increased COX-2 expression was demonstrated in human prostate cancer compared with non-cancer tissues (13). Furthermore, elevated levels of intracellular prostaglandin E2 (PGE2), produced primarily by COX-2, have been widely reported in prostate tumors in humans (14–18) and animal models (19). PG synthesis was further increased as prostate cancer advanced to worse prognosis (17). Although the role of COX-2 (and PGE2) in the susceptibility of aging prostate to cancer is not known, evidence suggests that COX-2 can be involved in, or associated with, the process of aging as observed, e.g. in human skin (20) and murine macrophages (21).

A possible mechanism by which COX-2 influences carcinogenesis is by increasing cell resistance to apoptosis (9). Apoptotic cell death plays a critical role in the homeostasis of the normal prostate as well as in cancer development (22). A major pathway implicated in apoptosis in the normal and cancerous prostate is the intrinsic or mitochondrial pathway triggered and mediated primarily by Bcl2 family members, e.g. BAX and Bcl2 (22). The link between COX-2 and Bcl2 in prostate cancer was substantiated from studies showing that LNCaP, human prostate cancer cells that over-express COX-2, exhibited induction in apoptosis and down-regulation in Bcl2 gene expression when treated with NS-398, a COX-2 inhibitor (23). In these cells, COX-2 inhibitors also induce apoptosis by inactivating the Akt-dependent pathway (24). Factors such as Akt and PKCα mediate cell survival by influencing the phosphorylation of Bcl2 (25). Modulation of the Bcl2-mediated apoptotic signaling can also occur via activation of the peroxisome proliferator-activated receptors γ (PPARγ), a member of the nuclear hormone receptors family and a lipid-activated transcriptional factor (26). Moreover, induction

Abbreviations: ADU, arbitrary density units; COX, cyclooxygenase; DLP, dorsolateral prostate; EIA, enzyme immunoassay; PGs, prostaglandins; PPARγ, peroxisome proliferator-activated receptor γ; VP, ventral prostate.
COX2 inactivates PPARγ (27,28) and both subsequently down-regulate the pro-apoptotic signaling (26).

In order to provide further information on the senescence-associated changes in the prostate that may explain its higher susceptibility to cancer, the present study examines Fischer 344 rats for the age-related changes in the pattern of COX-2 expression and the concomitant alterations in PGE2 synthesis in the prostatic regions susceptible to hormone-induced cancer, namely the periurethral ducts of the dorsolateral prostate (DLP) and anterior prostate in the proximal region of the DLP (29). Ventral prostate (VP) is characterized, however, as a region not susceptible to hormone-induced prostate cancer (29). Age-associated changes were also evaluated for pro- and anti-apoptotic factors linked to COX-2 signaling and known to be involved in normal development of the prostate gland and its susceptibility to cancer.

Materials and methods

Experimental animals

Male Fischer 344 rats of various age groups (4–100 weeks old) were obtained from Charles River (Raleigh, NC). The age groups of 4, 10, 50 and 100 weeks, represent a major portion of the rats’ life span as juvenile (n = 8), post-pubertal (n = 8), middle-aged (n = 14) and senescent (n = 12) animals, respectively. Upon arrival, animals were kept in an AAALC-accredited facility at the University of Missouri-Kansas City, on a daily cycle of alternating 12-h light–dark periods. Rats received rodent chow diet #5001 (Purina Mills, St Louis, MO) and drinking water ad libitum. Animals were killed within 1 week beyond their stated age. All procedures were performed in compliance with relevant regulations. Upon death, the urogenital system (seminal vesicles, prostate lobes and bladder) was removed and placed in a Petri dish containing PBS buffer (137 mM NaCl, 3 mM KCl, 4 mM Na2HPO4 and 1.5 mM KH2PO4, pH 7.4) with 0.05%Tween-20 and centrifuged to separate cell debris and the fat layer. Supernatants were termed as prostatic regions susceptible to hormone-induced cancer.

Analysis of mRNA and protein expression

**RT–PCR**. Total RNA, isolated by TRI reagent (Sigma, St Louis, MO), was subjected to RT–PCR reaction using Titanium® One-step RT–PCR protocol (Clontech, Palo Alto, CA) as described previously (27). Briefly, RT–PCR reactions were carried out in a buffer containing 0.5–1 μg RNA, Moloney murine leukemia virus-reverse transcriptase (MMLV-RT), 20 μM oligot(dT) primers, 40 mM Tricine, 20 mM KCl, 3 mM MgCl2, 0.2 mM each dNTP, 1× Taq enzyme mix provided by the manufacturer, 20 U recombinant RNase inhibitor and 45 μM PCR primer mix (Table I) in a total volume of 50 μl. Using a hot-lid Gene-Amp 9700 thermocycler (Perkin Elmer, Norwalk, CT), RNA was reverse transcribed at 50°C for 30 min followed by 5 min at 94°C. Table I shows the PCR cycling programs of the examined genes. Under each cycling condition, the yield of the amplified products was linear with respect to the input RNA and cycle number. The assay resolved a 2-fold difference in the amount of input RNA. Furthermore, the yields were linear when the PCR reactions were carried out for 20, 25 or 30 cycles. PCR products were separated in 1.5% agarose gels and visualized by ethidium bromide staining. Appropriate negative controls were carried out as described previously (27) to rule out contamination of RNA with genomic DNA, and to ensure that no cDNA synthesis would occur prior to RT inactivation. Digitized images of the stained cDNA products were captured as 8-bit digital TIFF files using a DC290 Digital Camera (Eastman Kodak, Rochester, NY). Band intensity was measured using Kodak Digital 1D-Image Analysis 3.6 software. Transcripts were normalized to the corresponding β-actin and expressed as arbitrary density units (ADU).

**Western blot analysis**. Prostate tissues (50 mg) were lysed in 1.5 ml lysis buffer (1% SDS, 1.0 mM sodium orthovanadate, 10 mM Tris pH 7.4) and centrifuged at 16 000 g for 10 min at 4°C. The protein content of the supernatant was quantified by Bio-Rad reagent (Bio-Rad, Hercules, CA), using the method of Bradford (30). Supernatants were incubated for 5 min at 100°C with Laemmli sample buffer (Bio-Rad) and 10–20 μg protein were loaded per well in 7.5–12% SDS-acrylamide gels, separated by electrophoresis and electro-transferred to nitro-cellulose membranes (Bio-Rad). Membranes were blocked with 3%–10% non-fat dry milk in TBS buffer containing 0.02–0.05% Tween-20 and probed for 1–2 h with primary antibodies (diluted 1:100–1:1000 in 5% non-fat dry milk or BSA for pAkt). COX-2 antibody was purchased from Cayman Chemicals (Ann Arbor, MI) whereas other primary and all secondary antibodies were obtained from Santa Cruz Biotech (Santa Cruz, CA). After incubation with peroxidase-conjugated anti-rabbit or anti-goat Ab (1:5000), signals were detected by chemiluminescence (Perkin Elmer Life Science, Boston, MA), normalized to the β-actin and expressed as ADU. Protein expression

Analysis of the tissue levels of caspases-3 activity was carried out in prostate tissues using Caspase-3 Colorimetric Assay kit (Assay Designs, Ann Arbor, MI). Tissue samples (100 mg) were homogenized in the caspase-3 assay buffer, and centrifuged to separate cell debris and the fat layer. Supernatants were subjected to caspase-3 activity analysis according to the manufacturer’s instructions using Ac-DEVD-NA as a substrate and a reaction time of 3 h at 37°C. Color intensity was measured at 405 nm using SpectraMax-250 microplate reader. Standard curves and reaction-related positive and negative controls were carried out and assayed with the samples.

**Analysis of PGE2 synthesis**

Tissue levels of PGE2 were examined by enzyme immunoassay (ELISA) as described earlier (27). ELISA for PGE2 was carried out using a Correlate-EIA kit (Assay Designs, Ann Arbor, MI) according to the manufacturer’s instructions. The cross-reactivities of the ELISA for a number of eicosanoids were described earlier (27). EIA for PGE2 was carried out using a Correlate-EIA kit (Assay Designs, Ann Arbor, MI) according to the manufacturer’s instructions. The cross-reactivities of the ELISA for a number of eicosanoids were described earlier (27).

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**Table I. Primers and PCR thermocycling parameters**

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<th>Primer</th>
<th>Sequence</th>
<th>PCR conditions</th>
<th>RT–PCR (bp)</th>
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<tbody>
<tr>
<td>COX2</td>
<td>5′-CTGTATCCCGCCCTGCTGTTG-3′</td>
<td>25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>3′-ACTCTGCTGGTGCGCCCTC-5′</td>
<td>Final extension: 68°C, 2 min</td>
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</tr>
<tr>
<td>PPARγ</td>
<td>5′-TGTGTGACAGGAAACACTTACAGCATG-3′</td>
<td>30 cycles: 94°C, 30 s; 65°C, 30 s; 72°C, 1 min</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>3′-ATGAGCCTGGTAAACAGCAGGACT-5′</td>
<td>Final extension: 72°C, 2 min</td>
<td></td>
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<tr>
<td>BAX</td>
<td>5′-ACAAAGAATGTCGCGCCGGTCC-3′</td>
<td>25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min</td>
<td>429</td>
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<td></td>
<td>3′-GGTATCCAGGATGGCAGAGCG-5′</td>
<td>Final extension: 68°C, 2 min</td>
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<td>Bcl2</td>
<td>5′-CTCAGATCTACCCAGGGCAG-3′</td>
<td>25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>3′-AGAGGGCTAAGTGGGTGAGAT-5′</td>
<td>Final extension: 68°C, 2 min</td>
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</tr>
<tr>
<td>Akt-1</td>
<td>5′-TACCTGGAAGTCACTGCGCAAG-3′</td>
<td>35 cycles: 93°C, 30 s; 56°C, 45 s; 74°C, 45 s</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>3′-GAGAAGAAGGTTGACGACAGGACA-5′</td>
<td>Final extension: 74°C, 10 min</td>
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<tr>
<td>PKCa</td>
<td>5′-TGACCCCTCGATTACGATTTT-3′</td>
<td>35 cycles: 93°C, 30 s; 60°C, 45 s; 74°C, 45 s</td>
<td>325</td>
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<td>3′-GGCTGTTCTGCTTTGCTGAA-5′</td>
<td>Final extension: 74°C, 10 min</td>
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<tr>
<td>β-actin</td>
<td>5′-GTGGGCGCCTGCTAGGCACCAC-3′</td>
<td>25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>3′-CTTCTTGTGTCAGCGACATTTC-5′</td>
<td>Final extension: 68°C, 2 min</td>
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Molecular Devices, Sunnyvale, CA). Levels of PGE2 were expressed as ng/g wet tissue. Standard curves and reaction negative and positive controls were generated for PGE2 and assayed simultaneously with the samples.

**Statistical analysis**

Statistical differences within age groups were determined by one-way ANOVA. To make an account for the ordering of the age categories, correlation analysis was carried out between age and examined markers and between individual markers across the different age groups by determining the coefficients of correlation \( r^2 \) using linear regression model. Since BAX and Bcl2 mediate opposing forces in the net apoptosis where BAX mediates apoptosis while Bcl2 mediates survival (31), a ratio of BAX:Bcl2 was created as a pro-apoptotic measure. Higher ratios implicate higher apoptotic gains. Since enhanced PKCa results in more active Bcl2 and less BAX, i.e. cell survival, the multiplicative variable \((\text{PKCa})(\text{Bcl2}/\text{BAX})\) was calculated as an anti-apoptotic measure, higher values indicate higher pro-survival processes. Expression levels of the apoptotic markers, the ratio of BAX:Bcl2 and the variable of \((\text{PKCa})(\text{Bcl2}/\text{BAX})\) were evaluated in response to age in the rat prostate and compared between groups by one-way ANOVA. All tests were performed using the SAS System 8.0 (SAS Institute, Cary, NC).

**Results**

**COX-2 expression and PGE2 synthesis**

The expression levels of COX-2 mRNA and protein were examined in the aged rat DLP (Figure 1A and B) and VP (Figure 1C and D) lobes. COX-2 was constitutively expressed in the prostate tissues of young rats (4-week-old). In the DLP, but not VP, COX-2 expression increased in an age-dependent manner \( r^2 = 0.89, \ P < 0.05 \) where in senescent rats (100-week-old) mRNA levels were 4-4 fold higher than those in the juvenile rats (4-week-old). These age-associated changes of COX-2 in the DLPs were accompanied by significant increases in the PGE2 synthesis (Figure 2A). Although COX-2 levels in the young rats (baseline) were comparable between DLP and VP, DLP contained ~2-6 fold higher PGE2 than VP at the various age stages (Figure 2).

**Expression of apoptotic markers**

Levels of Bcl2 and BAX expression (mediators of the intrinsic apoptotic signaling, Figure 3), caspase-3 activity (a downstream effector pro-apoptotic caspase, Figure 4) and PPARγ,

Fig. 1. Age-related changes in COX-2 expression in rat prostate. Representative blots are shown for COX-2 mRNA (left panel) and protein (right panel) in the rat DLP (A) and VP (C). The average levels of COX-2 mRNA (± SD, \( n = 3 \) animals) were quantified as arbitrary density units (ADU) relative to β-actin and plotted as a function of age in the DLP (B) and VP (D). Statistical differences between age groups were evaluated using one-way ANOVA test. \( P \) values showed significant differences in the expression levels of COX-2 in the DLP \( P < 0.05 \) but not VP. Correlation \( r^2 \) between age and mRNA levels was consistent with differences between groups and was significant in the DLP (B) but not VP (D).

Fig. 2. Age-related changes in the levels of PGE2 in the rat DLP (A) and VP (B). Results are mean ± SD (\( n = 3 \) animals). Statistical differences between age groups were evaluated using one-way ANOVA test. \( P \) values showed significant differences in the levels of PGE2 in the DLP \( P < 0.05 \) but not VP. Correlation \( r^2 \) between age and PGE2 levels was consistent with differences between groups and was significant in the DLP but not VP.

PKCo and Akt expression (modulators of the activity of Bcl2-family members, Figure 5) were examined in the DLP, the region in which COX-2 (and PGE2) was altered in an age-dependent manner. There was an age-related decline in
BAX mRNA and protein expression (Figure 3A) where senescent rats contained 4–6-fold lower BAX in the DLPs than juvenile (4-week-old) rats. In contrast to the inverse relationship between BAX and age ($r^2 \approx 0.56$, $P < 0.05$, Figure 3B), Bcl2 expression was significantly up-regulated as the animals progressed to older ages ($r^2 \approx 0.98$, $P < 0.05$). Analysis of the downstream caspase-3 showed a slight, but not significant, increase in the activity in rats at their post-puberty stage (10-week-old) with a decrease as the animals age (Figure 4). Senescent rats had 4–2-fold lower caspase-3 activity compared with young rats.

Analysis of modulators of Bcl2-mediated apoptotic signaling (PPARγ, PKCa and Akt) is shown in Figure 5. Significant down-regulation in the PPARγ ($r^2 = -0.86$) and up-regulation in the PKCa ($r^2 = 0.65$) was observed in the 100-week-old animals compared with the 4-week-old ones. Although total Akt was not changed with age, phosphorylated (active) Akt (pAkt) protein levels increased in an age-dependent manner ($r^2 = 0.84$, $P < 0.05$). Correlation analysis between COX-2 and apoptotic markers (across age) is shown in Table II. COX-2 was correlated directly with Bcl2, PKCa and pAkt and inversely with caspase-3 activity. A moderate, but marginally significant inverse correlation was obtained between COX-2 and PPARγ.

**Fig. 3.** Age-related changes in BAX and Bcl2 expression in the rat DLP. Representative blots are shown for BAX and Bcl2 mRNA (left panel) and protein (right panel) in the rat DLP (A). The average levels of BAX and Bcl2 mRNA ($\pm$ SD, $n = 3$ animals) were quantified (ADU) relative to β-actin and plotted as a function of age (B). Statistical differences between age groups were evaluated using one-way ANOVA test. $P$ values showed significant differences in the BAX and Bcl2 mRNA expression ($P < 0.05$). Correlation ($r^2$) between age and mRNA levels was consistent with differences between groups. BAX was inversely correlated with age while Bcl2 was directly correlated ($P < 0.05$).

**Fig. 4.** Age-related changes in caspase-3 activity in the rat DLP. Results are mean ± SD ($n = 3$ animals). Statistical differences between age groups were evaluated using one-way ANOVA test. $P$ values showed significant differences in caspase-3 activity in the DLP ($P < 0.05$). Correlation ($r^2$) between age and caspase-3 activity was consistent with differences between groups and the two factors were inversely correlated ($P < 0.05$).
The ratio of BAX:Bcl2 was created as an indicator of the overall age-related pro-apoptotic processes whereas the variable PKCα·Bcl2/BAX was generated as an indicator of the anti-apoptotic signaling (Figure 6). There was a >16-fold lower BAX:Bcl2 ratio in senescent rats (100-week-old) compared with their younger counterparts (4-week-old). This down-regulation started to emerge during the post-pubertal stage where the 10-week-old and the 50-week-old rats exhibited ~4–5-fold lower BAX:Bcl2 ratios than the 4-week-old animals. On the other hand, young animals had 14-fold lower PKCα·Bcl2/BAX values compared with the senescent

![Fig. 5. Age-related changes in the expression levels of PPARγ, PKCα, Akt and pAkt in the rat DLP. Representative blots are shown for mRNA (left panel) and protein (right panel) in the rat DLP (A). The average levels of PPARγ, PKCα and Akt mRNA or pAkt protein (± SD, n = 3 animals) were quantified (AU) relative to β-actin and plotted as a function of age (B). Statistical differences between age groups were evaluated using one-way ANOVA test. P values showed significant differences in the expression of PPARγ, PKCα and pAkt (P < 0.05) but not Akt. Correlation coefficient between age and the expression levels of the apoptotic markers are shown and were consistent with differences between groups.

Table II. Correlational analyses between COX-2 and apoptotic markers in rat DLP

<table>
<thead>
<tr>
<th>Marker</th>
<th>( r^2 )</th>
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<tr>
<td>PPARγ</td>
<td>-0.61</td>
</tr>
<tr>
<td>BAX</td>
<td>-0.28</td>
</tr>
<tr>
<td>Bcl2</td>
<td>0.82(^b)</td>
</tr>
<tr>
<td>PKCα</td>
<td>0.99(^b)</td>
</tr>
<tr>
<td>P Akt</td>
<td>0.96(^b)</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>-0.91(^{b,d})</td>
</tr>
</tbody>
</table>

\(^a\)Values were calculated across the different age groups using linear regression model and represent the coefficient of correlation (\( r^2 \)) between the mRNA levels of COX-2 and the examined markers except as shown.

\(^b\)Statistically significant \( r^2 \) at \( P < 0.05 \).

\(^c\)Correlation was calculated between COX-2 and pAkt protein levels.

\(^d\)Correlation was evaluated between COX-2 mRNA and caspase-3 activity.

Fig. 6. Age-related changes in the pro-apoptotic BAX:Bcl2 ratio and the anti-apoptotic variable PKCα·Bcl2/BAX in the rat DLP. Pro- (solid lines) and anti-apoptotic (dashed lines) variables were created from mRNA levels of Bcl2, BAX and PKCα at the different age groups. Data (mean ± SD of 3 animals) represent the ratio of ADU for BAX:Bcl2 or for PKCα·Bcl2/BAX adjusted for the corresponding β-actin mRNA signals. Statistical differences between experimental groups were evaluated using one-way ANOVA test. P values showed significant differences in the pro- and anti-apoptotic values among the different age groups (\( P < 0.05 \)).

The increased anti-apoptotic processes with age emerged at post-puberty (10 weeks of age). Rats at this stage had 3-fold lower PKCα·Bcl2/BAX ratios than the 100-week-old senescent rats.
Discussion

Senescence plays an important role in the genesis of prostate cancer. Evolution of age-related pathological changes in the prostate, such as benign prostatic hyperplasia and prostate cancer are frequently associated with changes in the hormonal milieu, e.g. decrease in the serum androgen to estrogen ratio (32). DLP, but not VP, is susceptible to steroid hormone-associated cancer and has less capacity to prevent the formation of carcinogenic estrogen metabolites and DNA damage (33). Age-dependent overgrowth and pathological changes leading to cancer usually arise in the DLP but not the VP. Moreover, several studies have shown that prostatic hyperplasia develops only in the DLP (34), which has lower rates of apoptotic rates than VPs (34,35). The higher survival rates in the DLPs may confer its vulnerability to pathological changes and can be exaggerated as the animals age (3,4,34,35).

The present study provides the first insight into the age-related changes in the expression pattern of COX-2 in rat prostate. DLP, the cancer susceptible region, contained higher COX-2 expression than the VPs (Figure 1) with a concomitant increase in PGE2 expression (Figure 2). COX-2 was expressed in young animals in the apparent absence of any inducing factor and was increased with age, primarily in the DLP. In general, prostate exhibits the highest levels of COX-2 among other tissues (11). COX-2 product, PGE2, was shown to be involved in the mitogenic action of steroid hormones in the DLP and in the homeostasis of the VP (36,37). Therefore, although COX-2 may be critical for the normal prostate growth, it can play a multifunctional role in controlling tumorigenesis and host immune responses (14–19,38–40). For example, induction of COX-2 (9,13) and increased PG synthesis were reported in both human (14–18) and animal (19) prostate cancers and was linked to worse prognosis (17). The age-associated increases in COX-2 expression in the DLP may be related to several factors. Steroid hormone receptor expression decreases with age in the VP but increases in the DLPs as observed in Brown Norway rats (4). Since exposure to steroid hormones induces COX-2 (41), increased expressions with age are expected to take place in the DLP more than the VP. Furthermore, increased oxidative stress (7,8) and elevated infiltration of phagocytic cells into the prostates, factors that induce COX-2 (9), occur as the animals age (42).

Aging of the VP, in Noble rats and in the Fischer F344 rats, is characterized morphologically by widespread atrophy of acini, increased accumulation of concretions in glandular lumen, infiltration of inflammatory cells and focal epithelial atypia. Although our results suggest a role of COX-2 in the pathological disorders in the DLP, it does not implicate such an effect in the VP. A recent study (42), using cDNA microarray, reported that VPs in Noble rats exhibit an age-dependent decline in the expression of genes involved in protein synthesis, protein fidelity maintenance, anabolism, growth inhibition and energy metabolism, and an increase of genes implicated in cell survival. This observation may help explain the susceptibility of aged VP to atypical hyperplastic lesions by mechanisms other than COX-2-related signaling.

Rat intestinal epithelial cells stably transfected with COX-2 (43) and human prostate cancer cells that over-express COX-2 (23,43,44), e.g. LNCaP, display elevated levels of Bcl1 expression and increased resistance to apoptosis (43). Upon treatment with COX-2 inhibitors, these cells exhibited induction in apoptosis and down-regulation in Bcl2 (23). In line with several recent studies that established a direct role of COX-2 and PGE2 in rendering cells resistant to apoptosis (45–49), COX-2 induction could be linked to the decreased apoptotic processes (this study) and the development of hyperplasia observed in the aged DLP region of the Sprague-Dawley (34) and Brown Norway (35) rats.

The age-related changes in BAX (decline) and Bcl2 (increase) reported in the present study were noted previously in Brown Norway rats and were confined to the DLP but not the VP (4). Moreover, in the animals examined in the present study, we observed a similar trend in the hepatic expression of BclXL, where mRNA levels of this anti-apoptotic factor increased by up to 6-fold in the senescent rats, compared with their 4-week-old counterparts (50). In aging Brown Norway rat prostate, higher anti-apoptotic processes are known to occur in the DLP, but not VP (35). VP contains 5-fold lower Bcl1 and 20-fold higher BAX than the DLP (35). Bcl2 was detected in the epithelial, but not stromal, cells whereas BAX was localized in both cell types (35). This pattern of lobe- and cell-specific expressions of BAX and Bcl2 may be linked to the increased susceptibility of DLP to cancer. BAX and Bcl2 interaction was implicated in carcinogenesis and in tumor response to therapy (51). The BAX:Bcl2 ratio declined by >16-fold in 100-week-old rats compared with the 4-week-old ones (Figure 6). The correlation between COX-2 and Bcl2 expression (Table II) suggests that the age-related up-regulation in Bcl2 can be related to the induction in COX-2. In the rat mammary glands, celecoxib, a COX-2 inhibitor, induced 20-fold increase in the BAX:Bcl2 ratio, an effect that was linked to the anti-tumorigenic actions of COX-2 inhibitors (52). Members of Bcl family influence apoptosis either directly or via a caspase-dependent pathway. Alterations in Bcl2 and BAX activity/expression influence caspase-9 activity, which, subsequently, activates caspase-3 to induce apoptosis. The inhibition of caspase-3 activity with age can be due to alterations in the Bcl2/BAX expression and should reduce the overall apoptotic gains, further contributing to the age-related pathology of the DLP.

In addition to the interaction between BAX and Bcl2 (31,51), their activity can be affected by post-translational modification with phosphorylation (25,53), e.g. via PKCo and pAkt. Forced expression of PKCo in human cells increased Bcl1 phosphorylation (54) and caused >10-fold higher cell resistance to apoptosis (25). Therefore, over-expression of PKCo and pAkt in the DLP of senescent versus young rats may contribute to the reduced apoptotic rates by further enhancing the Bcl1-mediated anti-apoptotic effects. The multiplicative factor PKCoBcl2/BAX can predict the effect of chemotherapeutic agents on the PKC-Bcl2-mediated cell survival (55,56). In human prostate cancer cells, inhibition of COX-2 down-regulates the phosphorylation of Akt, blocking its anti-apoptotic activity (24). This effect occurs independently of the phosphatidylinositol-3-kinase activity and is not reversed by inhibitors of protein phosphatases 1 and 2A. This observation suggests that COX-2 promotes cell survival by activating the Akt-dependent pathway (57) and is supported by the direct relationship between COX-2 and pAkt noted in the DLP (Table II). On the other hand, activation of PKCo and Akt transcriptionally up-regulates COX-2 (58). Therefore, activation of Akt/PKCo and the subsequent induction of COX-2/PGE2 synthesis may combine to enhance cell survival at senescence and explain why prostate overgrowth is confined to DLP and occurs in an age-dependent manner (4).
Induction of COX-2 (and increased PGE2 synthesis) inactivates PPARγ while PPARγ influences COX-2 transcription (26). PPARγ activation attenuates cell proliferation and induces apoptosis by modulating the transcription of genes involved in cell growth. The interaction between COX-2 and PPARγ has been substantiated by an inverse relationship between the two molecules that was observed in various types of cancer (26,27,52) and was noted in the present study in the rat DLP during senescence (Table II). The down-regulation of PPARγ expression during aging is perhaps due to the COX-2 induction and the overproduction of PGE2 that inactivate and transcriptionally down-regulate PPARγ, contributing further to the aging-associated inhibition of apoptotic cell death (59).

Apoptosis is a physiological process critical for organ development, tissue homeostasis and elimination of defective or pre-neoplastic cells in complex organisms. Defects in homeostasis of apoptotic mechanisms play a major role in the pathogenesis of various cancers and attempts to activate apoptosis provide a therapeutic approach to the treatment of these malignancies. Results of the present study implicate a role of COX-2 in the age-related decline in apoptosis. This assumption is based on numerous reports demonstrating a possible link between COX-2 induction and enhanced cell survival. If a relationship can be established between COX-2 and anti-apoptotic processes in the prostate gland, treatment with COX-2 inhibitors might serve to protect against cell survival in the aged prostates. Further studies are being carried out in our laboratory in order to investigate the modulating effects of COX-2 inhibitors on the apoptotic signaling in the aging rat prostate.

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