Original Article

PI PROSTATE INTERNATIONAL

Prostate Int 2013;1(1):10-15 • http://dx.doi.org/10.12954/PI.12004

Androgen receptor coactivator p120 subtype β is highly expressed in prostate cancer

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Purpose: The β form of p120 is reported to be a strong coactivator of the androgen receptor. We investigated the gene expression profiles of the α and β forms of p120 in prostate cancer cell lines, benign prostatic hyperplasia (BPH), nontreated prostate cancer (NTPC), and prostate cancer after androgen deprivation therapy (PCA-ADT).

Methods: We obtained 154 prostate needle biopsy specimens (81 in BPH, 51 in NTPC, and 22 in PCA-ADT). Levels of p120α and β expression were determined by multiplex real-time polymerase chain reaction.

Results: Prostate cancer cell lines, LNCaP, PC-3, DU-145, and LNCaP-LA, which is a derivative of LNCaP under androgen deprivation, expressed both p120α and p120β. p120α expression levels were significantly higher than those of p120β in all cell lines examined. In human prostate tissues, p120α expression was significantly higher than that of p120β in BPH and NTPC. p120α expression in BPH was significantly higher than in other groups. In contrast, p120β expression was significantly higher in NTPC and PCA-ADT than in BPH. Expression of the two forms of p120 was not correlated with age, prostate-specific antigen, or Gleason score.

Conclusions: The expression profiles of p120α and p120β significantly differ in cancerous and benign prostatic tissues.

Keywords: Androgen receptor, Nuclear coactivator, Prostatic neoplasms

INTRODUCTION

Prostate cancer is one of the most common types of cancer, and is second only to lung cancer as the most common non-cutaneous cancer diagnosed in males in the United States [1]. In Japan, the incidence of prostate cancer is increasing rapidly [2]. Curative options for prostate cancer include surgery, radiotherapy, and androgen deprivation therapy (ADT). Huggins and Hodges [3] treated a patient with progressive prostate cancer by castration in 1941, and administration of ADT improved his condition. ADT has since been widely used as the gold standard for prostate cancer.

Prolonged treatment is, however, limited by the development of resistance. ADT with luteinizing hormone-releasing hormone agonist, alone or combined with an antiandrogenic agent, is the most widely performed hormonal therapy. Various mechanisms of reduced susceptibility to ADT have been proposed. These include over-expression of androgen receptors (AR) [4], variations in AR [5], activation of ARs that do not bind androgens and other hormones [6], and cofactor abnormalities involving androgen production within prostate cancer cells [7]. AR associates with coactivator or corepressor proteins that modulate its activation in the presence of ligand. Alterations in AR coactivator expression or function in prostate cancer include colocalization with AR in aggregates (mutated AR) [8], potentiation of the agonistic effect of hy-

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Submitted: 7 October 2012 / Accepted after revision: 3 February 2013

http://p-international.org/
pISSN: 2287-8882 • eISSN: 2287-903X

droxyflutamide [9], and potentiation of various AR activations [10]. Increased expression of steroid receptor coactivator-1 (SRC-1) and transcriptional intermediary factors-2 (TIF-2), which are AR coactivators, has been reported in castration-refractory prostate cancer (CRPC) tissue, and RAC-3 (SRC-3) expression is reportedly increased in poorly differentiated carcinomas and advanced tumors. Other cofactors (e.g., competitive protein binding, androgen receptor activation [ARA]70, ARA55) showing altered expression in prostate cancer have also been identified [7].

A nuclear general receptor coactivator, p120, was originally cloned as a coactivator of thyroid receptor (TR) [11]. p120 consisted of 920 amino acids and showed significant homology to skeletal muscle abundant protein. Various human tissues express p120 mRNA, including the heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. A novel splice variant of p120 (p120α), p120β was cloned in our university [12], and has been reported to be the strong coactivator of AR. In most tissues, p120β expression levels were lower than those of p120α, whereas prostate tissues express predominantly p120β. The authors suggested that p120β affects hormone sensitivity in prostate cancer. However, analysis of the expression patterns of p120α and p120β were based on semiquantitative real time polymerase chain reaction (PCR) using as a small number of prostate samples. In the present study, we developed a more accurate quantification method, using multiplex real time PCR, and evaluated the expression profiles of p120α and p120β in large numbers of samples to confirm the role of p120 in prostate cancer.

MATERIALS AND METHODS

1. Cells and chemicals

The human prostate cancer cell lines LNCaP, PC-3, and DU145 were purchased from the American Type Culture Collection (Manassas, VA, USA). DU145 were cultured in Dulbecco's modified eagle medium (Sigma-Aldrich Co., St. Louis, MO, USA), and PC-3 and LNCaP in RPMI-1640 (Sigma-Aldrich Co.), supplemented with 10% fetal bovine serum (FBS; Moregate Biotech, Bulimba, Australia). LNCaP-LA was used as an *in vitro* model of CRPC, and were derived from LNCaP cells cultured with 10% charcoal-stripped FBS for 2 years. The RNeasy Mini kit (QIAGEN, Chatsworth, CA, USA) was used for RNA isolation and cDNA was synthesized using moloney murine leukemia virus reverse transcriptase and random primers (Invitrogen, Life Technologies Co., Carlsbad, CA, USA) according to the manufacturer's protocols [13].

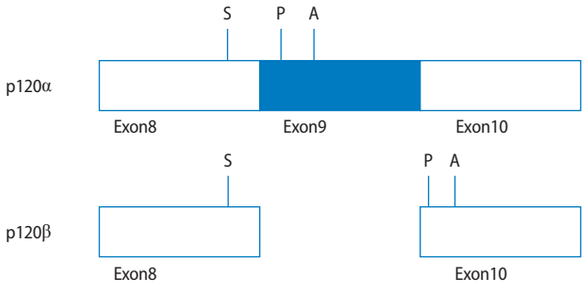

Fig. 1. Schema of p120α and p120β. In p120α, we designed an antisense primer and probe on exon 9, while probe p120β was designed on the junction of exons 9 and 10. S, sense primer; A, antisense primer; P, probe.

2. Multiplex real time PCR for p120α and p120β detection

We designed specific primers for detection of p120α and p120β (Fig. 1). As an internal control, 18S RNA copy numbers were determined. The p120α and p120β 18S RNA primer sets are shown in Table 1. The 18S1 set was for p120α, and the 18S2 set for p120β. Multiplex real time PCR was performed using 2× iQSupermix (Bio-Rad Laboratories Inc., Hercules, CA, USA), using 300 nM of each p120 primer, 200 nM p120 probe, 300 nM of each 18S primer, 200 nM 18S probe, 2.4 μL of water, and 1 μL of the template. PCR was carried out for 40 cycles at 95°C for 5 seconds, 60°C for 10 seconds, and 95°C for 10 seconds. The real time PCR assays were carried out in 96-well plates using CFX96 (Bio-Rad Laboratories Inc.). Standard curves for each transcript are shown in Fig. 2.

Table 1. Primer and probe sequences

Primer	Sequence
p120α	
Sense primer	5′-CCACTATGGAAGAGGCTA-3′
Antisense primer	5′-TCAGGAATCCCAGGAAAC-3′
Probe	5′-FAM-ACTTTGCCG-ZEN-AGTACCCCAGTCA-BkFQ-3′
P120β	
Sense primer	5′-CCAACCACTATGGAAGAG-3′
Antisense primer	5′-GACACCTGTACTGTTCAG-3′
Probe	5′-FAM-AGCCACAGC-ZEN-CATTTCACTCTCA-BkFQ-3′
18s1	
Sense primer	5′-CCATCACTGCCATTAAGG -3′
Antisense primer	5′-AGGTCAATGTCTGCTTTC -3′
Probe	5′-Cy5-ACACCACATGAGCATATCTTCGGC-IAbRQ-3′
18s2	
Sense primer	5′-CGAAGATATGCTCATGTG -3′
Antisense primer	5′- CATCCTTCTGTCTGTTCA-3′
Probe	5′-Cy5-AAGCAGACATTGACCTC-ACCAAGA-IAbRQ-3′

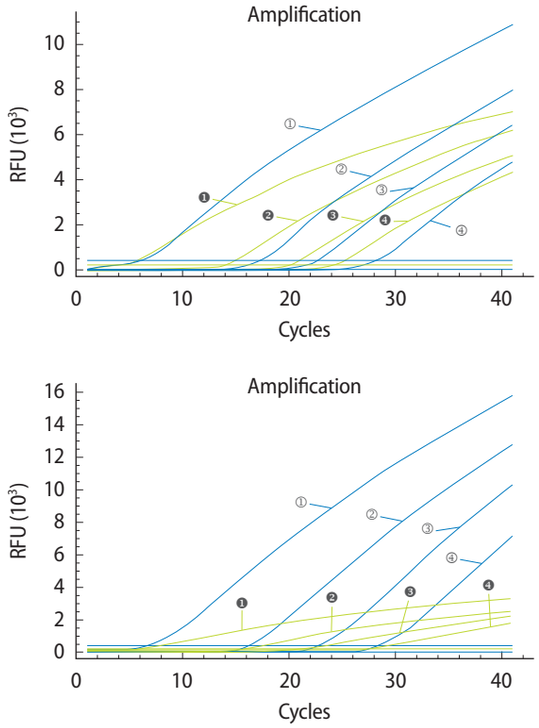

Fig. 2. Standard curves for real-time polymerase chain reaction. (A) p120α ❶5.32E+8 copies, ❷5.32E+6 copies, ❸5.32E+4 copies, ❹5.32E+2 copies, and 18s rRNA; ① 6.12E+8 copies, ② 6.12E+6 copies, ③ 6.12E+4 copies, and ④ 6.12E+2 copies. (B) p120β ❶5.27E+8 copies, ❷5.27E+6 copies, ❸5.27E+4 copies, ❹5.27E+2 copies, and 18srRNA; ① 4.83E+8 copies, ② 4.83E+6 copies, ③ 4.83E+4 copies, and ④ 4.83E+2 copies. RFU, relative fluorescence units.

3. Prostate biopsy specimens

All prostate biopsy specimens were obtained at the Gunma University Hospital between 2002 and 2009, as described previously [14]. Written informed consent was obtained from all patients. Fifty one nontreated prostate cancer (NTPC) samples, 81 benign prostatic hyperplasia (BPH) samples, and 22 prostate cancer samples that had many viable cells remaining after ADT for 6 months prostate cancer after androgen deprivation therapy (PCA-ADT) were obtained. Prostate biopsy after 6 months of ADT was intended for pathological evaluation of ADT. Table 2 shows the characteristics of the patients. NTPC and PCA-ADT samples were selected based on their mulitple positive cores.

4. Statistical analyses

Differences between the two groups were evaluated using the Student's *t*-test. A two-sided *P*-value of less than 0.05 was considered to indicate statistical significance.

Table 2. Patients' characteristics

Characteristic	BPH	NTPC	PCA-ADT[a]
No. of patients	81	51	22
Age (yr)	64.8 (46–85)	70.2 (55–86)	68.0 (50–78)
Prostate-specific antigen	7.28 (1–40.8)	91.1 (0.5–1,408)	386.1 (0.1–4,620)
Gleason score			Unknown 6
6		11	4
7		11	10
8		8	1
9		10	4
10		11	0

Values are presented as mean (range).
BPH, benign prostatic hypertrophy; NTPC, nontreated prostate cancer; PCA-ADT, prostate cancer after androgen deprivation therapy.
[a]Prostate cancer samples with many viable cells remaining after ADT for 6 months.

Table 3. Relative expression levels of p120α and p120β in prostate cancer cell lines

	p120α	p120β	β/α ratio
LNCaP	1.000±0.936[a]	0.089±0.109	0.138±0.121
LNCaPLA	3.136±5.152	0.057±0.010	0.099±0.085
DU145	0.535±0.833	0.007±0.003	0.064±0.075
PC3	0.304±0.277	0.021±0.026	0.144±0.221

Values are presented as mean±standard deviation. Values of p120α, p120β and β/α are presented as relative ratios vs. those of p120α in LNCaP (n=3).
[a]Reference.

RESULTS

1. p120α and β expression profiles in prostate cancer cell lines (Table 3)

Both p120α and β were expressed in all four cell lines. p120α, p120β, and β/α expression values are expressed as relative ratios vs. those of p120α in LNCaP. The expression levels of p120α showed no significant differences among the four cell lines. LNCaP-LA expressed p120β at a significantly higher level than PC-3 ($P<0.05$) or DU145 ($P<0.01$). β/α ratios were not significantly different among the four cell lines.

2. p120α and p120β expression profiles in human prostate tissues

In prostate cancer cell lines, p120α was expressed at a significantly higher level than p120β. Next, we compared the expression levels in human prostate cancer tissues. As shown in Fig. 3, expression levels of p120α were significantly higher than those of p120β in the BPH and NTPC groups, while there was no significant difference in the PCA-ADT group.

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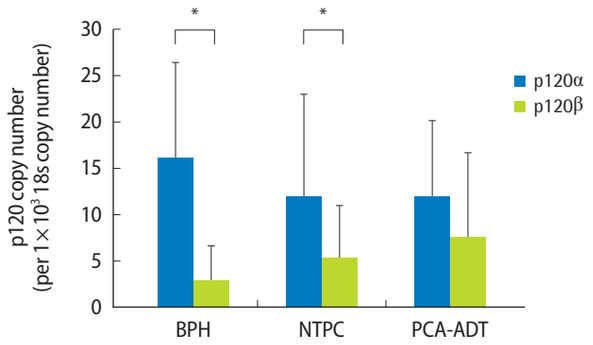

Fig. 3. Comparison of p120α and p120β among the three groups. The expression of p120α was significantly higher than those of p120β in BPH and NTPC. Bars are expressed as mean±standard deviation. BPH, benign prostatic hyperplasia; NTPC, nontreated prostate cancer; PCA-ADT, prostate cancer after androgen deprivation therapy. $^{*}P<0.05$.

Next, we compared expression levels of p120α and p120β, and β/α ratios among three groups. As shown in Fig. 4, expression levels of p120α in the BPH group were significantly higher than those in the NTPC, but not in the PCA-ADT group. In contrast, p120β expression levels were significantly lower in the BPH group than in the other two NTPC and PCA-ADT groups. Thus, the β/α ratios were significantly lower in the BPH group compared with the NTPC and PCA-ADT groups. No significant difference in the expression levels of each variant or the ratios in any group in terms of age, prostate-specific antigen levels, or Gleason score were observed.

DISCUSSION

The nuclear coactivator, p120, has at least two splicing variants; p120β was cloned and characterized at our university [12]. Although p120α acts as a coactivator of many nuclear receptors, including AR, TR, peroxisome proliferator-activated receptor γ, retinoid X receptor, glucocorticoid receptor and androgen receptor, the affinity of p120β for AR is strong [12]. In the present study, we quantified and compared the expression levels of both isoforms among prostate cancer cells and human prostate tissues. Hosoya et al. [12] using real time PCR reported that in prostate tissues p120β expression was predominant. They also showed that prostate cancer tissues expressed high levels of p120β and that β/α ratios were higher in newly diagnosed prostate cancer tissues than in recurrent prostate cancer. In the current study, p120α was found to predominate in BPH and NTPC samples. These results differ from previous reports. One reason for the discrepancy may be the quantification method. As mentioned earlier, relative expression levels were determined by the band intensity ob-

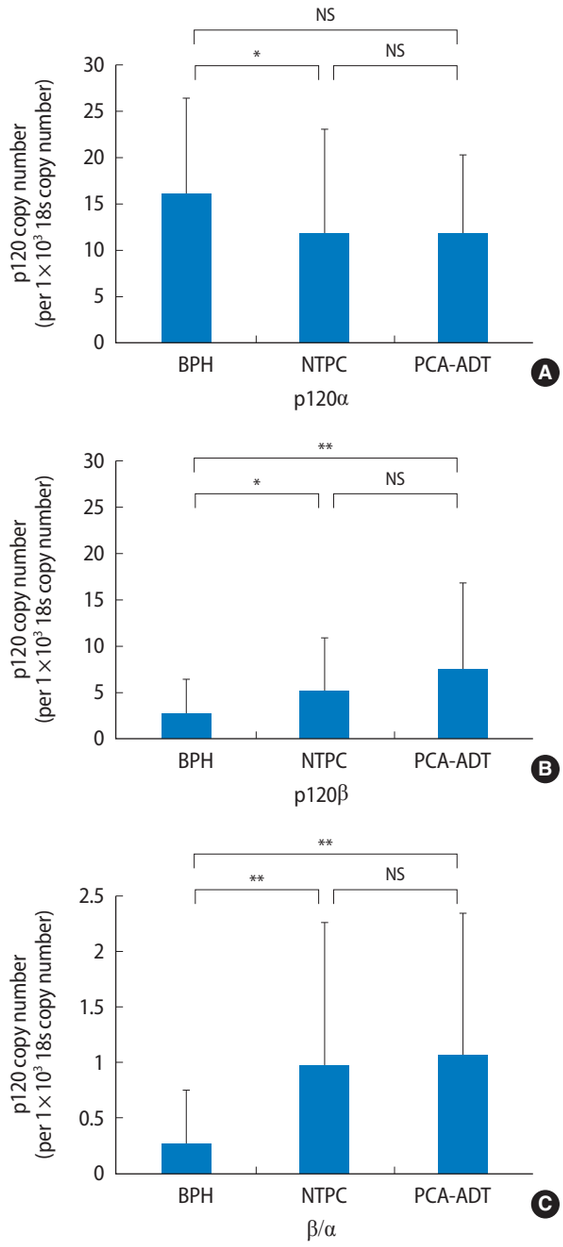

Fig. 4. Comparison of p120α, p120β, and β/α ratios among the three groups. The amount of p120α expressed was significantly higher in BPH than in the other two groups. p120β expression was significantly higher in NTPC and PCA-ADT than in BPH. (A) p120α copy number, (B) p120β copy number, and (C) β/α ratio. Bars are expressed as mean±standard deviation. BPH, benign prostatic hyperplasia; NTPC, nontreated prostate cancer; PCA-ADT, prostate cancer after androgen deprivation therapy; NS, not significant.

tained by conventional real time PCR, as reported previously [12]. For semiquantification of gene expression levels in real time PCR, selection of the optimal cycle number for PCR at which transcripts are amplified exponentially is necessary.

The previous study showed a PCR product profile after 40 cycles; thus we suggest that the quantification method might be the reason for the discrepancy. In the present study, p120α and p120β transcripts were detected separately with reference to the internal controls (Fig. 2). Another reason for the discrepancy may be the sample size. We used a large number of samples in this study. However, Hosoya et al. [12] evaluated only four non-cancerous tissues, 10 nontreated cancer tissues, and three recurrent prostate cancer tissues. To overcome this limitation of needle biopsy samples, we selected patients with multiple positive cores in biopsy samples.

Another finding of this study was that expression levels of p120β, a strong coactivator of AR, were significantly higher in the NTPC and PCA-ADT groups compared with the BPH group. Of the tested AR coactivators, SRC-1 and TIF-2 exhibited increased expression in CRPC. Protein inhibitor of activated STAT-1 and Ran/ara24 exhibited increased expression in NTPC. RAC-3 (SRC-3) expression was increased in poorly differentiated carcinomas and advanced tumors. Expression of ELE1/ARA70 in NTPC was decreased, and no changes in the expression of other coactivators, including ARA54, ARA55, TMF/ARA160, SRC1, and thyroid hormone receptor-associated protein 220, between benign prostate tissue and NTPC was apparent [15]. The expression profiles of p120α and p120β in prostate tissues we report here suggest that a nuclear coactivator, in particular, p120β, might play a role in prostate cancer. The expression levels of p120α in the BPH group were significantly higher than those in the NTPC, but not in the PCA-ADT group, while p120β expression levels were significantly lower in the BPH group than prostate cancer group regardless of previous ADT. These findings might suggest that p120β plays a role in the development of prostate cancer, while expression of p120α is primarily involved in the response of prostate cancer cells to ADT. To explore the exact mechanisms underlying the different expression profiles of p120, further studies are needed.

In conclusion, we developed a precise multiplex real time PCR method for quantification of p120α and p120β gene expression. Prostate tissues, noncancerous or cancerous, predominantly expressed p120α over p120β. p120β, which is a strong coactivator of the AR, was expressed at significantly higher levels in both nontreated and post-ADT prostate cancer tissues. These observations suggested that the expression profiles of p120α and p120β significantly differ in cancerous and benign prostatic tissues.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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