

## A Novel Splice Variant of the Nuclear Coactivator p120 Functions Strongly for Androgen Receptor: Characteristic Expression in Prostate Disease

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**Abstract.** We cloned a novel splicing variant for nuclear coactivator p120( $\alpha$ ), designated as p120 $\beta$  and studied its function and expression in several human prostate diseases. Transfection assays demonstrated that p120 $\beta$  functions as a strong coactivator for androgen receptor (AR), but weakly for other nuclear receptors. GST-pull down assay showed that a glutamine-rich region of the p120 bound to the ligand-binding domain of AR. Interestingly, p120 $\beta$  mRNAs were expressed predominantly in the normal prostate, androgen-responsive prostate cancers and an androgen-sensitive prostate cancer cell line, LNCaP, but weakly in recurrent cancers and the androgen-insensitive prostate cancer cell lines PC3 and DU145. Furthermore, knockdown of p120 $\alpha$  by siRNA abolished coactivator activity on thyroid hormone receptors (TR) and PPAR $\gamma$ , but did not affect that of ARs in PC3 cells. In addition, competitive assay with other nuclear receptors demonstrated that TR and PPAR $\gamma$  did not inhibit p120 $\beta$ -induced stimulation. These findings suggested that while p120 $\alpha$  was essential for ligand-dependent stimulation of TRs and PPAR $\gamma$ , p120 $\beta$  acted as a coactivating protein predominantly for AR.

**Key words:** Androgen receptor, Coactivator, Prostate, Prostate disease

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**PROSTATE** cancer is the most common male malignancy in most Western countries, and the second leading cause of cancer death of men in the United States [1]. At the early stage of prostate cancer, the growth of prostate cancer cells is usually androgen-dependent and some tumor cells become androgen-independent in 6–18 months [2]. Androgen ablation through agonistic analogs of LHRH and antiandrogens, given either alone or in combination, can effectively treat prostate cancer [3]; however, hormone ablation therapy

only causes temporary regression of prostate cancer. We have very few prognostic markers for prostate cancer recurrence because the molecular mechanism by which prostate cancer becomes androgen-independent is not clear. Therefore, the development of new diagnostic approaches to detect androgen-dependent or -independent tumor cells is important for improving the treatment and prediction of prognosis.

Androgen receptor (AR) was the first factor highlighted as a cause of prostate cancer. Many investigations found mutation, amplification, overexpression, and a shorter CAG repeat sequence of the AR gene in androgen-independent prostate cancer [4–8]. The AR is a member of the steroid hormone superfamily of nuclear transcriptional factors. In the absence of ligand, AR is kept in a repressed state. In the presence of ligand, recruitment of coactivators such as SRC-1 [9]

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and ARA70 [10] causes activation of gene transcription. While ARA70 was initially identified as a specific co-activator for AR, several subsequent experiments revealed that ARA70 functioned as a coactivator for other nuclear receptors and functioned as a key mediator of androgen-oestrogen synergism [11]. Several findings have demonstrated the significance of transcriptional mechanisms of AR, including co-factors interacting with AR in prostate cancer [12, 13]. In addition, recent molecular methodologies could be used to discover consistent gene expression patterns associated with carcinogenesis or androgen sensitivity in prostate cancer [14, 15]; however, the mechanisms for the development of androgen resistance in regrowth prostate cancer are not clear.

We have cloned a nuclear general receptor co-activator p120 (p120 $\alpha$ ) using a yeast two-hybrid system and the ligand-binding domain of the thyroid hormone receptor (TR)  $\beta$ 1 as bait [16]. The p120 protein was recently reported as one of the components of the TRRAP/TIP60 HAT complex [17]. We report here the cloning and characterization of a novel splicing variant, p120 $\beta$  for p120 $\alpha$ . In addition, we revealed the determination of the strong coactivator function of p120 $\beta$  for AR and the expression characteristics of p120 $\beta$  in prostate disease samples and cell lines.

## Materials and Methods

### *Cell lines*

PC3, LNCap, and DU145 cells were obtained from the American Type Culture Collection and grown in DMEM, or RPMI1640 supplemented with 10% fetal bovine serum, 0.25 mg/ml streptomycin, and 100 mg/ml penicillin. CV-1 cells have been described previously [18]. Parallel incubations were performed for flasks of cells approaching confluence in humidified air with 5% CO<sub>2</sub>.

### *Tissues and samples*

Prostate cancer tissues were obtained from newly-diagnosed patients who underwent 18-gauge core-needle biopsy of the prostate. To avoid any influence from normal epithelium or stroma, the samples were confirmed to contain only tumor tissues by histological examination [19]. Total RNA was extracted from the

remainder of cut specimens. Two benign prostate hyperplasia (BPH) samples were obtained during operation at Gunma University Hospital. All samples were examined after informed consent had been obtained.

### *RT-PCR and 5' RACE*

Primers were designed to the exon6/exon7/exon8 boundary. p120 cDNA was amplified using the following primer (p120 sense 5'-GAGGAGGCTGAAGTAAAGAGG-3', antisense 5'-AGGTGTGGTGAAGTGTAGG-3',  $\beta$ -actin sense 5'-GCTCGTCGTCGACACGG-CTC-3', antisense 5'-CAAACATGATCTGGGTCATCTTCTC-3'). PCR was performed with 25 ng of primers and 3  $\mu$ l of cDNA from two different cell lines (PC3, LNCaP cells) to determine that amplification was in the linear range. PCR of p120 was carried out for 40 cycles ( $\beta$ -actin for 20 cycles) of 1 min at 94°C, 2 min at 60°C, 2 min at 72°C. To obtain the 5' portion of human p120 $\alpha$  and p120 $\beta$  cDNA, 5' RACE was performed using human liver Marathon Ready cDNA (Clontech, USA).

### *Plasmids*

MMTV-Luc contains the murine mammary tumor virus promoter in pA3 Luc [4]. Positive TRE (PAL) constructs two copies of a palindromic element ligated upstream of the TK109 promoter in the vector pA3 Luc [16]. PPARE or RXRE constructs two copies of a DR1 element in the pA3Luc. Human AR was cloned into the SV40-driven expression vector pECE (a gift of Dr. Steven P. Balk, Harvard Medical School, Boston) [4]. Human TR $\beta$ 1, RXR (a gift of Dr. Ronald M. Evans, The Salk Institute, La Jolla) [20], estrogen receptor (ER) and mouse PPAR $\gamma$ 2 were cloned into the SV40-driven expression vector pKCR2 [18]. Full-length human glucocorticoid receptor (GR)  $\alpha$  cDNA was amplified by PCR using human liver cDNA as a template (Marathon cDNA, Clontech, USA). p120 $\alpha$  or  $\beta$  was cloned into pKCR2.

### *Transfection*

Transfection assays were performed as previously described [16]. Cells were transfected with the following amounts of DNA per 6 wells (10  $\mu$ g of pA3 Luc-TK reporter constructs, 100 ng of each receptor, and 3  $\mu$ g of either pKCR2-p120 $\alpha$ ,  $\beta$  or pKCR2). Sixteen

hours after transfection, cells were cultured in the absence or presence of appropriate ligands for 24 h and then harvested for luciferase assays.

#### *GST pull-down Assay*

Restriction and PCR fragments of p120 cDNA (Fig. 3A) and AR cDNA (Fig. 3B) were ligated in frame into pGEX4T1 or pGEX4T2 (Pharmacia Biotech Inc.). AR or p120 labeled with [<sup>35</sup>S] methionine was synthesized by the TNT-coupled *in vitro* translation system (Promega, Madison, WI.). This labeled protein was incubated with GST-Sepharose or GST-fusion proteins-Sepharose for 2 h at 4°C in the presence or absence of 10 nM DHT.

#### *Knockdown of p120 $\alpha$ gene by short interfering (si) RNA*

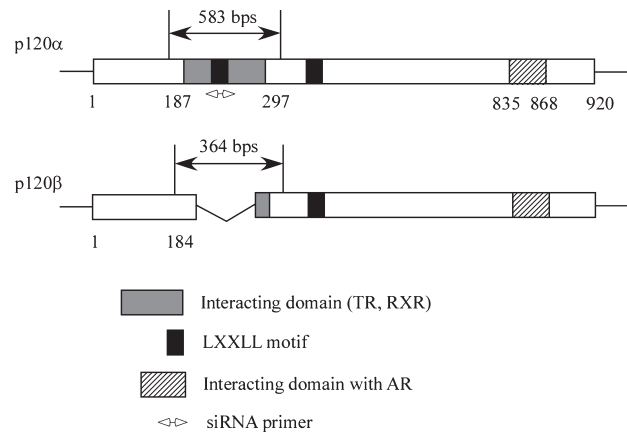
A siRNA targeting p120 $\alpha$  was designed and synthesized by Dharmacon (Lafayette CO) (shown in Fig. 1). The nucleotide sequence of sip120 $\alpha$  was 5'-GAT GATTCACCCCAGAAAAAG-3'. As a negative control, control duplex VIII (Dharmacon) was transfected in parallel. Two hundred nM of sip120 $\alpha$  was transfected into PC3 cells using Lipofectamine 2000 (Invitrogen). After 48 h of siRNA transfection, total RNA was isolated and the expression of p120 mRNA was quantitated by RT-PCR analysis as described above. In separate experiments, 24 h after transfection of siRNA, MMTV-Luc and pECE-AR, pal-TK-Luc and pKCR2-TR, DR-1 Luc and pKCR2-PPAR $\gamma$  were cotransfected using the same reagent in the absence or presence of each ligand, and luciferase assay was performed after 24 h.

#### *Competitive transfection assay*

PC3 were transfected with the following amounts of DNA per 6 wells (10  $\mu$ g of pA3 MMTV-Luc reporter constructs, 100 ng of AR plus TR, AR plus PPAR $\gamma$ , and AR plus RXR, and 3  $\mu$ g of either pKCR2-120 $\alpha$  or  $\beta$ ). Sixteen hours after transfection, cells were cultured in the absence or presence of appropriate ligands (DHT alone or DHT plus either T3 or TZ or 9cis-RA) for 24 hours and then harvested for luciferase assays.

#### *Statistical Analysis*

Results are expressed as mean  $\pm$  SEM. Statistical



**Fig. 1.** Schema of p120 $\alpha$  and p120 $\beta$ . Numbers correspond to amino acid numbers of p120 $\alpha$ . Arrows show the products of RT-PCR used in this experiment.

analysis was performed by ANOVA followed by Student's *t*-test or the Wilcoxon/Kruskal-Wallis test using JMP (SAS Institute Inc., Cary NC). A *p*-value less than 0.05 was considered statistically significant.

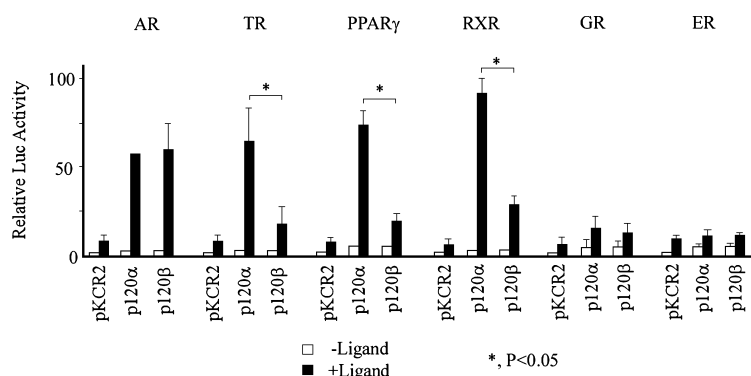
## Results

#### *Cloning of p120 $\beta$*

We obtained p120 $\alpha$  and  $\beta$  cDNA from a human fetal brain cDNA library, and its 5'-end sequence was determined by 5'-RACE. Fig. 1 shows that 73 amino acids were deleted in p120 $\beta$  from number 184 to 256 of p120 $\alpha$  because exon 7 is missing. This deleted region contained one LXXLL motif [21] that was required for binding TR, RAR, and RXR [18]. p120 $\beta$  still contained one LXXLL motif in its middle portion; however, we demonstrated that this LXXLL motif was not important for interaction with any nuclear receptors by GST-pull down assays and a yeast-two hybrid system (data not shown).

#### *Functional characterization of p120 $\beta$*

To study the functions of p120 $\beta$ , we performed transient transfection studies using CV1 cells. Shown in Fig. 2, experiments revealed that p120 $\alpha$  enhanced the ligand-induced transcription of TR, AR, GR, PPAR $\gamma$  and RXR but not the estrogen receptor (ER). In contrast, p120 $\beta$  lost ligand-inducible enhancement by TR, PPAR $\gamma$ , and RXR, and p120 $\beta$  had a tendency toward



**Fig. 2.** CV1 cells were transfected as described in “Materials and Methods”, with the respective ligands (10 nM DHT, 100 nM T3, 1  $\mu$ M 9-cis retinoic acid, 10  $\mu$ M TZ, 10  $\mu$ M deoxycorticosterone and 10 nM estradiol). Data are quantified as fold activity, where 1 equals activity in the absence of ligand and in the presence of the same amount of pKCR2 or p120 $\alpha$  or p120 $\beta$ . The results shown are the means  $\pm$  S.E. of three separate experiments. \* $P < 0.05$  compared to ligand-induced activation without p120.

reduced function as a co-activator for GR compared with p120 $\alpha$ , while p120 $\beta$  retained the ability of AR-related gene transcription (Fig. 2). These findings suggested that p120 $\beta$  was a strong co-activator of AR functionally.

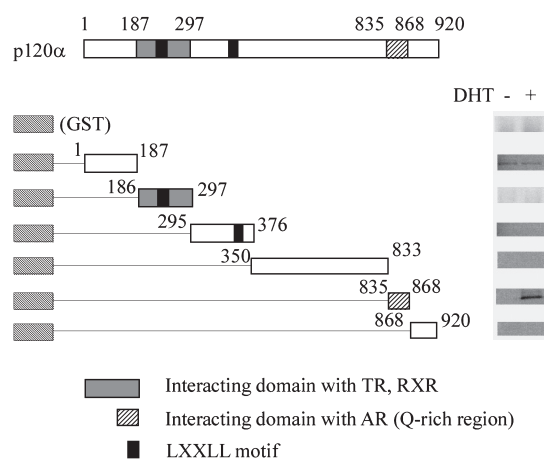
#### Binding characterization of p120 $\beta$

Binding studies determined that p120 $\beta$  has two binding areas, amino acids 1–187 and its carboxyl-terminus (Fig. 3A). These areas did not contain the LXXLL motif. Amino acids 1–187 alone have significant ligand-independent binding to interact with AR. This same phenomenon was observed in the interaction experiment used for TR [16]. These findings suggest that amino acids 1–187 may be related to interaction stability with nuclear receptor irrespective of the absence or presence of ligand. The other binding area, the carboxyl terminus of p120, did not contain the LXXLL motif, and was a glutamine-rich region. This area interacted with AR in a ligand-dependent manner (Fig. 3A). The other LXXLL motif was found not to be a binding site of AR in the GST pull-down assay. Furthermore, the region of AR interaction with p120 was the carboxyl terminus, including the AF-2 region of AR (Fig. 3B).

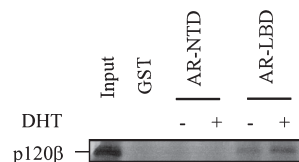
#### Expression of p120 $\alpha$ and p120 $\beta$ in human tissues

Next, to clarify whether p120 $\alpha$  and  $\beta$  were expressed in various tissues, we performed RT-PCR analysis (Fig. 4). Our previous Northern blot study showed that the 3.5 kb size of p120 mRNA appeared

A

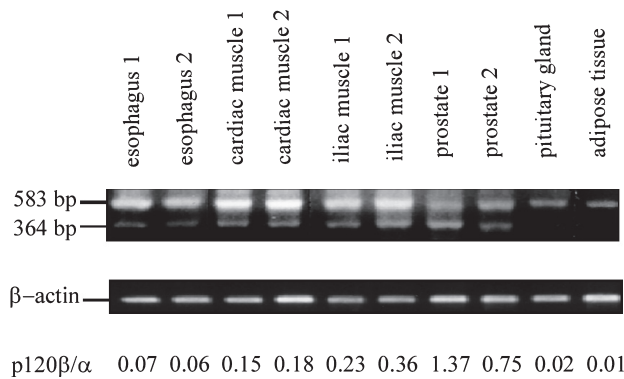


B



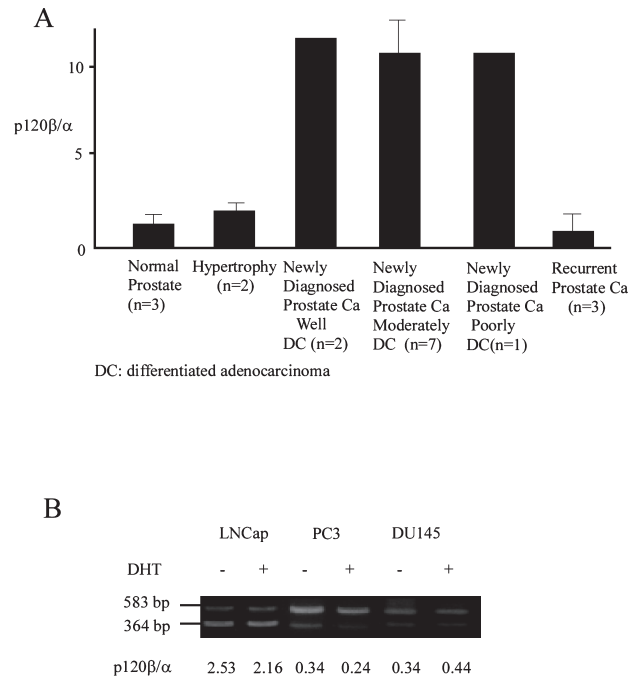
**Fig. 3.** **A:** GST fusion proteins of p120 derivatives were incubated with radiolabeled *in vitro* translated AR, either in the presence or absence of 10 nM DHT. **B:** GST fusion proteins of AR derivatives (NTD (NH2-terminal domain): amino acid 1–604, LBD (ligand binding domain): amino acid 614–919 of human AR) were incubated with radiolabeled *in vitro* translated p120 $\beta$  either in the presence or absence of 10 nM DHT.

to be expressed ubiquitously [16]. In the present study, the Northern blot method was not useful to



**Fig. 4.** Expression of p120 $\alpha$  and  $\beta$  by RT-PCR in several normal tissues. Ratios of p120 $\beta$  mRNA to p120 $\alpha$  mRNA were calculated from the density of mRNA bands on agarose gels by densitometry.

compare the expression levels of p120 $\alpha$  and  $\beta$  because the predicted sizes of mRNA for p120 $\alpha$  and  $\beta$  were similar. Furthermore, as p120 $\beta$  had only one portion deleted from p120 $\alpha$  cDNA, we could detect two specific bands using the primer design shown in Fig. 1. When we performed RT-PCR analysis using these sets of primers, a 583 bp band for p120 $\alpha$ , and a 364 bp band for p120 $\beta$  could be detected simultaneously, and then we calculated the p120 $\beta$ / $\alpha$  ratio measured from band density. Although the 364 bp band might include BRD7 cDNA [17], which has a unique c-terminus with the c-terminus region of p120 $\beta$  and functionally has HAT activities by making the components of the TRRAP/TIP60 HAT complex, we confirmed that LNCap and PC3 cells expressed no BRD7 by RT-PCR experiments under the same conditions using the specific primers for BRD7 (data not shown). First, considering androgen response, we explored the expression levels of p120 in several samples of normal muscle, prostate, pituitary gland, and adipose tissue as androgen-responsive organs. p120 $\alpha$  was expressed in all tissues examined, as shown in Fig. 4. In contrast, p120 $\beta$  was not expressed in the pituitary gland or adipose tissue. In addition, p120 $\alpha$  was expressed more predominantly than p120 $\beta$  in the esophagus, cardiac muscles, and iliac muscles. In contrast, in the normal prostate, p120 $\beta$  was expressed equivalently or higher than p120 $\alpha$  (Fig. 4). These findings suggested that p120 $\beta$  may have specific functions or may be regulated by specific transcriptional factors in the prostate.



**Fig. 5.** **A:** Expression ratio of p120 $\beta$ / $\alpha$  of 3 normal controls, 2 hypertrophies, 10 newly diagnosed (2 well, 7 moderately and 1 poorly differentiated) prostate cancers and 3 recurrent prostate cancers. **B:** Expression of p120 $\alpha$  and  $\beta$  by RT-PCR in prostate cancer cell lines LNCap, PC3, and DU145 cells in the presence or absence of DHT.

### Expression of p120 $\alpha$ and $\beta$ in prostate diseases and cultured prostate cancer cells

Next, to examine the expression levels of p120 in prostate disease, we performed a similar experiment on specimens of benign prostate hyperplasia (BPH) and prostate cancer. We developed a new technique to obtain prostate specimens using an 18-gauge core-needle [19]. p120 $\beta$  was predominantly expressed compared with p120 $\alpha$  in BPH, as in a normal prostate (Fig. 5A). Similarly, the expression levels of p120 $\beta$  in all newly diagnosed prostate cancers (these cancer cells were androgen-dependent because hormonal treatment was effective in these patients after diagnosis), were significantly higher than those of p120 $\alpha$ . On the other hand, the three recurrent cancer samples showed lower expression patterns of p120 $\beta$  rather than p120 $\alpha$  (Fig. 5A). Furthermore, we confirmed the expression of intact AR in these cases by RT-PCR (data not shown). This finding suggested that the androgen-sensitive status was related to the predominant expression of p120 $\beta$  rather than p120 $\alpha$ . We should accumu-

late data on more cases of hormone refractory cancer to obtain more conclusive findings.

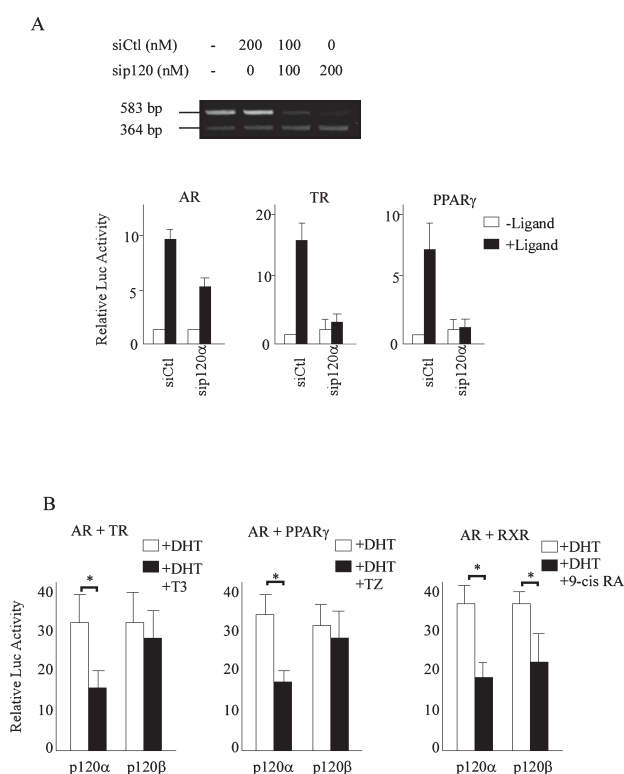
To confirm the relation between p120 expressions and androgen response, we compared expression levels of p120 in several prostate cancer cell lines, androgen-resistance prostate cancer PC3 and DU145, and androgen-sensitive prostate cancer LNCaP [29] in the presence or absence of dehydrotestosterone (DHT). As shown in Fig. 5B, RT-PCR analysis demonstrated that p120 $\beta$  was expressed more highly than p120 $\alpha$  in LNCaP cells, while PC3 and DU145 cells showed lower expression of p120 $\beta$  than p120 $\alpha$ . In addition, incubation with DHT did not affect the expression levels in any cell lines tested here.

*Expression of p120 in cultured prostate cancer cells, and knockdown of p120 $\alpha$  but not p120 $\beta$  retains androgen-responsible activation*

To determine whether p120 is directly involved in androgen-mediated transactivation *in vivo*, the effects of silencing of p120 $\alpha$  gene was observed by using siRNA targeting the endogenous p120 $\alpha$  gene in PC3 cells. Transfection of sip120 $\alpha$  reduced p120 $\alpha$  mRNA but did not affect p120 $\beta$  mRNA. In these transfected PC3 cells, TR- and PPAR $\gamma$ -mediated ligand-dependent activation were completely diminished, revealing that p120 $\alpha$  may be essential for thyroid hormone and PPAR $\gamma$  ligand-inducible transactivation; however, AR-mediated activation became weak but was retained (Fig. 6A). These findings indirectly but not slightly suggested that p120 $\beta$  may be involved in AR activation, specifically *in vivo*.

*p120 $\beta$  functions as a relatively specific co-activator in androgen-independent prostate cancer PC3 cells*

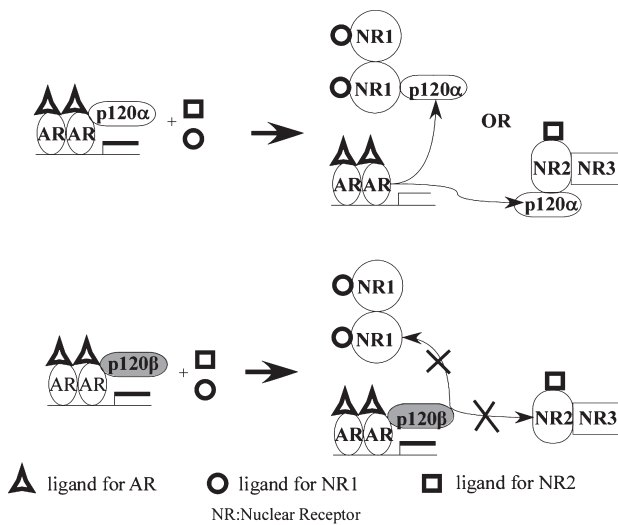
To investigate the role of p120 $\beta$  in PC3 cells, we performed a competitive transient transfection study. Expression of intact AR alone showed only 1.5-fold activation in the presence of DHT; however, co-expression of AR and p120 $\beta$  induced 3-fold activation in the presence of DHT (data not shown). Previous findings that showed that endogenous ARs in PC3 cells were not functional [22] were supported by the present results. Overexpression of both p120 $\beta$  and AR together recovered the androgen response of PC3 cells (Fig. 6B). PC3 cells were transfected with AR, p120 $\alpha$  or p120 $\beta$ , and TR or PPAR $\gamma$ . Sixteen hours after



**Fig. 6.** **A:** Knockdown of p120 $\alpha$  gene by siRNA experiments. Transfected 200 nM sip120 $\alpha$  completely diminished p120 $\alpha$  mRNA expression by RT-PCR and completely decreased TR- and PPAR $\gamma$ -mediated transactivation; however, AR-mediated transcription was retained (Sic1; control SiRNA). **B:** Competitive transfection assay with other nuclear receptors shows that T3 and TZ inhibited androgen induced-activation by p120 $\alpha$ , but did not alter the androgen response by p120 $\beta$  in PC3 cells. Results represent the mean  $\pm$  S.E. of 3 experiments. \* $P < 0.05$  compared to DHT-induced fold activation without other ligands.

transfection, cells were cultured in the absence or presence of appropriate ligands (none vs 1 nM DHT or 100 nM T3 + 1 nM DHT, and none vs 1 nM DHT or 10  $\mu$ M TZ + 1 nM DHT) for 24 h. Incubation with T3 or TZ significantly inhibited DHT-induced transactivation by p120 $\alpha$  (Fig. 6B). T3 and TZ did not influence p120 $\beta$  functions. This phenomenon may explain the mechanism by which p120 $\alpha$  may pull to other nuclear receptors in the presence of certain ligands, implying that p120 $\beta$  but not  $\alpha$  may be a specific co-activator of AR (Fig. 7); however, 9-cis RA for RXR ligand significantly inhibited both p120 $\alpha$  and p120 $\beta$  transactivation induced by DHT. It should be noted that previous studies have shown that liganded RXR can function as a repressor to suppress the AR target





**Fig. 7.** Schematic models of functional interaction between p120 and AR. Although p120 $\alpha$  may be pulled from liganded-AR to other liganded nuclear receptors, p120 $\beta$  may not be recruited from liganded-AR.

gene [23]. The decreasing p120 $\beta$  function in the presence of 9-cis RA may be due to this direct protein-protein interaction between RXR and AR.

## Discussion

The findings of the present investigation suggested two novel aspects of p120 as a coactivator protein. One was that p120 changed to a strong co-activator for AR from a general nuclear co-activator by splicing. To confirm that p120 $\beta$  is a strong coactivator of AR, we performed competitive assays using PC3 cells. Fig. 7 reveals the mechanism by which p120 $\beta$  functioned as an AR coactivator although in the presence of other ligands, p120 $\alpha$  did not. This finding demonstrated the specificity of p120 $\beta$  in AR transactivation.

The second was that the glutamine-rich region of p120 but not LXXLL motifs was addressed at the binding region to AR. It has been reported that the glutamine-rich region of SRC-1 was essential for binding to the amino-terminal region of AR in a ligand-independent manner [24]. On the other hand, from the viewpoint of the binding site of the nuclear receptor, strong AF-2 functions were determined in the ligand-binding domain of RXR, PPAR $\gamma$ , GR, ER and TR, because the binding surface of the liganded-AF-2 domain recruits a complex, including coactivators. However,

AR is reported to differ from other nuclear receptors in its interaction with coactivators. Namely, interaction between the AF-1 domain and the liganded-AF-2 domain of AR is required for binding p160 coactivator families [25–27]. Moreover, coactivators such as ARA160 [28], cyclin E [29], BRCA1 [30] and ART-27 [31] have been characterized to interact with the AF-1 domain of AR. Our investigation revealed that p120 interacted with the AF-2 of AR in a ligand-dependent manner, but not with the AF-1 domain. Our findings and others suggested that other cofactors that bridge p120 and the AF-1 domain may be necessary for enhancing transcriptional activity in the presence of ligand.

Only a few studies on the expression of coregulators of AR in prostate cancer have been published [13, 15, 32–35]. The best characterized coactivator, SRC-1, and its mRNA levels appeared to be expressed at an equivalent amount in normal and tumour tissues [15, 32]. In addition, expressions of ARA160, ARA54 and TRAP220 were not changed in prostate cancers [26]; however, Tekur *et al.* found reduced levels of ARA70 expression in prostate cancer cell lines (LNCap, TSU-Pr1, DU145, PC3) compared with levels in normal prostatic epithelial cells [33]. Moreover, ARA55 expression levels in recurrent prostate cancers were significantly lower than in untreated prostate cancers [34]. A recent study indicated that the expressions of SRC-1 and PIAS1 were significantly lower in hormone-refractory prostate cancers than in untreated prostate cancers [35]. On the other hand, in functional studies, BRCA1 resulted in the increase of androgen-induced cell death in prostate cancer cells [36]. However, the definitive role of cofactors in prostate cancer is still ambiguous, and the mechanisms of androgen-independent growth of prostate recurrent cancer have not been resolved.

In our expression studies, p120 $\beta$  was predominantly expressed only in the normal prostate, while in other tissues, p120 $\alpha$  showed higher expression levels in RT-PCR. From these findings, we hypothesized that there might be a strong link between androgen sensitivity and the predominant expression of p120 $\beta$ . In further experiments, we addressed the predominant expression of p120 $\beta$  over p120 $\alpha$  in BPH tissues and newly-diagnosed untreated prostate cancers. In addition, our findings showed that recurrent hormone-refractory cancers appeared to express p120 $\alpha$  more predominantly than p120 $\beta$ , although we should accumulate data on

more cases of hormone refractory cancer to obtain more conclusive findings. The present study suggested although further study is required, that the ratio of p120  $\beta/\alpha$  may be linked to androgen sensitivity, suggesting that androgen-independent factors in prostate cancer may modulate the expression levels of p120 $\alpha$  and  $\beta$ .

To further confirm the relation between androgen sensitivity and the expression ratio of p120 $\alpha$  and  $\beta$ , we performed several experiments. First, the p120  $\beta/\alpha$  ratio is strongly related to androgen sensitivity, as shown by the findings in prostate cancer cell lines, LNCap, PC3 and DU145 cells. p120 $\alpha$  was predominantly expressed in androgen-independent cell lines, PC3 and DU145 cells while, in contrast, the expression of p120 $\beta$  was stronger in the androgen-dependent cell line, LNCap. Second, androgen did not affect the expression ratio of p120  $\beta/\alpha$  in all cultured cells tested in the current experiments. This phenomenon suggests that the p120 gene may not be regulated by androgen. We confirmed that androgen response elements (AREs) do not exist in the promoter region of the p120 gene by estimated gene bank data. When our findings

are taken together, the change in the p120  $\beta/\alpha$  ratio may be related to the change in the character of prostate cancer androgen sensitivity.

Our attempts to identify whether the change in the p120 expression pattern is a cause or result of changes of androgen sensitivity in prostate disease are still in progress. In conclusion, the expression ratio of p120 may contribute not only to the clinical characteristics of androgen resistance of prostate cancer but also to the better understanding of the mechanisms of prostate cancer from androgen-dependent to androgen-independent growth.

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