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Paracrine regulation of talin mRNA expression by androgen in human prostate

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Abstract Androgens are essential for normal prostate physiology and are intimately associated with the growth and progression of prostate cancer. However, few androgen regulated genes in the prostate have been identified. Using the mRNA differential display technique a 164-bp cDNA fragment was identified as being androgen regulated in the human prostate. Nucleotide sequence analysis of this fragment revealed 84% homology with the gene encoding the cytoskeletal protein talin. Confirmation of the androgen regulation of this gene was carried out using Northern analysis. Primary prostatic stromal cells treated with conditioned medium (CM) from androgen-treated primary prostatic epithelial cells showed an approximate 2-fold reduction in talin mRNA levels compared with stromal cells treated with CM from epithelial cells not exposed to androgens. Expression of talin mRNA in human prostatic tissue was confirmed by in situ hybridisation. The highest levels of expression were present in the epithelial cells, with lower levels of expression in the stroma. Thus, androgen regulation of talin expression may play a role in normal and/or aberrant growth and development of the prostate.

Key words: Differential display; Talin; Androgen regulation; Prostate

1. Introduction

Prostate cancer is the second most common cause of cancer-related death in American and European males, with more than 317 000 new cases diagnosed and over 41 000 deaths occurring every year in the USA [1]. Androgens are intimately involved in the regulation of normal growth and differentiation of the prostate and therefore androgen regulated genes are likely to contribute to the development and progression of prostate cancer. Androgen responsive genes such as prostate specific antigen (PSA) [2] and prostate specific membrane antigen [3] have been characterised and PSA is commonly used as a clinical marker for prostate cancer [4].

We have previously used the differential display technique to identify androgen responsive genes in human prostatic cells [5]. Here we report the identification of talin as an androgen responsive gene. Talin is a cytoskeletal protein which provides a link between the the intracellular actin cytoskeleton and membrane bound integrins, which mediate cell adhesion by binding to extracellular adhesive proteins [6]. The protein is composed of two major domains, an N-terminal domain of 47 kDa which interacts with the cell membrane, and a larger C-terminal domain of 190 kDa which interacts with actin, vinculin and other cytoskeletal elements [7]. Talin is usually concentrated at focal contacts [8] and is likely to be important for anchorage dependent growth. Oncogenesis is associated with anchorage-independent growth and it is possible that talin may play a role in this process. For instance, down-regulation of talin using antisense technology impairs the folding and processing of integrins and dramatically reduces the kinetics of cell spreading [9]. Furthermore, studies on the Wistar-Furth rat have suggested that a point mutation in the talin gene may contribute to the high incidence of tumours in this strain [10]. In this study we show the regulation of talin gene expression by androgens in prostatic cells and describe the localisation of talin mRNA in human prostatic tissue.

2. Materials and methods

2.1. Cell culture

Primary prostatic stromal and epithelial cell cultures were prepared from tissue obtained from patients undergoing transurethral prostatectomy for benign prostatic hyperplasia as previously described [11]. Approval for the use of the tissue was obtained from the local Ethical Committee. Stromal cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 1% glutamine, penicillin (100 units/ml) and streptomycin (100 μg/ml). Epithelial cells were cultured in WAJC 404 medium supplemented with HEPES, pH 7.6 (25 mM), sodium hydrogen carbonate (15 mM), zinc-stabilised insulin (2.5 μg/ml), cholera toxin (10 ng/ml), dexamethasone (1 μM), epidermal growth factor (10 ng/ml), 0.5% bovine pituitary extract, heparin (4 units/ml), sodium selenite (10 ng/ml), transferrin (10 μg/ml), penicillin (100 units/ml) and streptomycin (100 μg/ml).

For androgen exposures primary epithelial cells were washed twice with phosphate-buffered saline then exposed to RPMI medium supplemented with 10% steroid depleted, dextran-coated charcoal (DCC) treated FCS for 72 h. The cells were then incubated with DCC medium or DCC medium containing 10 nM mibolerone for 0–96 h and the culture medium was collected. Prostatic stromal cells which had previously been exposed to DCC medium for 72 h were then incubated with the collected culture medium from the epithelial cells for 96 h before harvesting and the preparation of total RNA.

2.2. Differential display

Differential display was performed essentially as described [12] with the following modifications. Total RNA was extracted from cells as previously described [13]. Poly(A)$^+$ RNA was purified using Dynabeads Oligo(dT)$_{25}$ according to the manufacturer's protocol (Dynal), and 0.25 μg was reverse transcribed using T_{12}VC as primer and AMV reverse transcriptase (400 units/ml). Differential display was performed in duplicate using primers T_{12}VC and 1019 (GGTACTCCAC), [α-^{32}P]dATP (500 μCi/ml) and AmpliTaq DNA polymerase (50 units/ml). Samples were subjected to 30 cycles of PCR comprising 94°C for 30 s, 40°C for 20 s and 72°C for 30 s. PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels for 20 h at 500 V. DNA from bands thought to be differentially expressed was extracted by elution into 200 μl water and the cDNA recovered by ethanol precipitation. Eluted cDNA species were re-amplified using 30 cycles of PCR with the appropriate primers and cloned into the pCRII vector (Invitrogen) according to the manufacturer's protocol. The DNA sequences of the isolated clones were determined using the

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Thermo-Sequenase cycle sequencing kit (Amersham Life Sciences) and homologies to known genes determined using the GenBank database.

2.3. Northern blotting

RNA samples were electrophoresed as previously described [14]. Briefly, RNA (5 μg) was fractionated on an agarose gel, transferred to a nylon membrane (Hybond N^+; Amersham), fixed by heating at 80°C for 2 h and stained with methylene blue to assess the integrity of the RNA. Probes were generated from cloned cDNAs by restriction endonuclease digestion and radiolabelled with [α-^{32}P]dATP using random-primed labelling mixture according to the manufacturer's protocol (Boehringer Mannheim). Hybridisation and washing were carried out as previously described [15]. Blots were analysed after exposure to a phosphor storage screen using a Phosphorimager (Molecular Dynamics) and subsequently re-probed with radiolabelled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as a control for RNA loading.

2.4. In situ hybridisation

Digoxigenin-labelled antisense and sense talin probes were generated using a 164-bp talin cDNA cloned into the pCRII vector as template. Linearisation of the plasmid was carried out using the appropriate restriction endonuclease and probes were then synthesised using the digoxigenin labelling kit (Boehringer Mannheim) according to the manufacturer's protocol.

Deparaffinised, rehydrated prostatic tissue sections (5 μM) on silane-coated microscope slides were permeabilised by incubation in proteinase K (20 μg/ml) for 30 min at 37°C and acetylated in PBS containing 0.25% acetic anhydride and 0.1 M triethanolamine for 10 min at room temperature. Pre-hybridisation was carried out in 50% formamide, 4×SSC, 1×Denhardt's solution, tRNA (125 μg/ml) and freshly denatured salmon sperm DNA (100 μg/ml) for 30 min at 42°C. Hybridisation was performed using pre-hybridisation solution containing denatured talin antisense or sense probes (5 ng per slide) for 16 h at 42°C. Washes were then carried out at 52°C (2×SSC, 50% formamide, 30 min; 1×SSC, 50% formamide, 30 min; 0.5×SSC, 50% formamide, 30 min). The slides were then washed in buffer 1 (150 mM NaCl, 100 mM Tris-HCl, pH 7.5) and incubated in buffer 1 containing 5% BSA and 0.3% Triton X-100 for 30 min at room temperature. The sections were incubated in alkaline phosphatase-conjugated antidigoxigenin antibody diluted 1:500 in buffer 1 containing 5% BSA and 0.3% Triton X-100 for 2 h at room temperature. After washing in buffer 1, the slides were briefly immersed in buffer 2 (100 mM NaCl, 50 mM $MgCl_2$, 100 mM Tris-HCl, pH 9.5) and incubated in buffer 2 containing 0.34 mg/ml nitroblue tetrazolium and 0.18 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in the dark for 16 h at 4°C. The slides were then immersed in TE buffer, pH 8.0, rinsed in water, counterstained with Mayer's haematoxylin and mounted in Glycergel mounting medium.

3. Results

3.1. Identification of talin as an androgen responsive gene

Differential display was carried out on poly(A)$^+$ RNA from primary prostatic stromal cells which had previously been incubated for 96 h with conditioned medium taken from primary prostatic epithelial cells treated with 10 nM mibolerone for 0–48 h. A representative autoradiograph using a single primer set is shown in Fig. 1. Band patterns between individual samples were similar and bands were usually present across all lanes. However, several bands were shown to be reproducibly differentially expressed, one of which (arrowed)

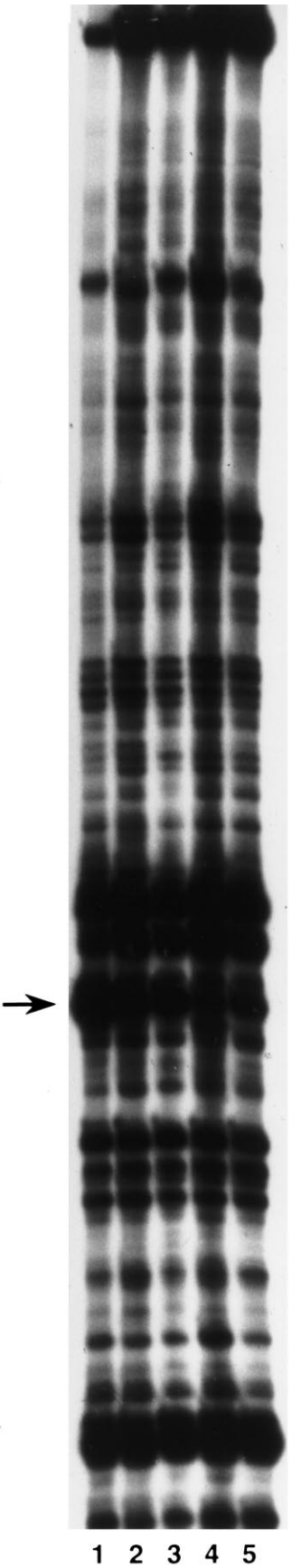

Fig. 1. Differential display was performed on poly(A)$^+$ RNA from primary prostatic cells exposed for 96 h to conditioned medium from untreated primary prostatic epithelial cells (lane 1) or from cells exposed to 10 nM mibolerone for 4, 8, 24 or 48 h (lanes 2–5, respectively). A representative experiment carried out using primers T_{12}VC and 1019 is shown. An arrow indicates a clone that was subsequently confirmed as differentially expressed (talin).

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                    10                  30                  50
Clone 9308      GGTACTCCAC CCTGCCAGCA GCTTCCAGCC AGTCCCCACA GCCTCATCAG
                | | |||||  |||||||||| |||||||||| |||||||||  ||| | ||||
Talin (mouse)   GCTGCTCCAG CCTGCCAGCA GCTTCCAGCC AGTCCCCACG GCCACGTCAG

                          70                  90
Clone 9308      CTCTCTTCAC CGTTTTTTGA TACTATCCCC CACCCCCAGC TACCCATGGG
                |||   |||  |  |||||||| ||||||  ||  |   |||||| |||| |||||
Talin (mouse)   CTCAACTCAT CCCTTTTTGA TACTATATCC CCTACCCAGC TACCTATGGG

                   110                 130                 150
Clone 9308      GCTGCAGAGT TATAAACCCA AACAGGTCAT GCTCCAATAA AAATGATTCT
                |||  || ||  | ||||||||| |||||||||   |||||||||  |  |||||||
Talin (mouse)   GCTTGAGGGT TGTAAACCCA AACAGGTCAG ACTCCAATAA AGGTGATTCT


Clone 9308      GCAAAAAAAA AAAA
                 ||||||||| ||||
Talin (mouse)   ACAAAAAAAA AAAA
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Fig. 2. Nucleotide sequence of differentially expressed clone 9308 and homology with the 3′ region of murine talin cDNA. Vertical lines represent identical bases, flanking primer sequences (1019 and $T_{12}VC$) are in boldface and the putative polyadenylation signal is underlined.

was down-regulated after exposure to conditioned medium from cells treated with androgen. This band was excised from the gel, cloned into the PCRII vector and three clones were fully sequenced (data not shown). All three clones contained a 164-bp cDNA insert with the correct flanking primer sequences ($T_{12}VC$ and 1019). A search of the GenBank database revealed an 84% homology with the 3′ end of the murine talin gene (Fig. 2).

3.2. *Effect of androgen on talin mRNA levels in primary prostatic stromal cells*

To confirm the putative androgen regulated expression of talin and to determine the temporal pattern of talin down-regulation, primary prostatic stromal cells were incubated with conditioned medium from primary prostatic cells which had previously been treated with 10 nM mibolerone for varying lengths of time. The levels of talin mRNA were then assessed using Northern analysis. A probe was designed for human talin using sequence information from a series of ESTs with high homology to the murine talin gene. An 833-bp cDNA was amplified using RT-PCR and the identity confirmed by sequencing. This probe identified an 8.5-kb mRNA by Northern analysis similar to the 8.3-kb mRNA for murine talin [16]. Talin mRNA levels were shown to be decreased to approximately 50% of control levels following exposure to conditioned medium from primary epithelial cells exposed to 10 nM mibolerone for 96 h (Fig. 3).

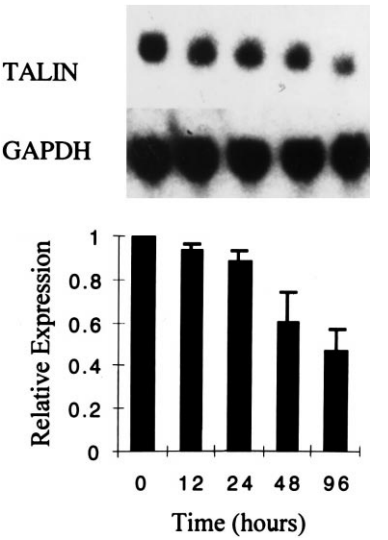

Fig. 3. Northern analysis was performed using total RNA from primary prostatic stromal cells exposed for 96 h to conditioned medium from untreated primary epithelial cells or from cells exposed to 10 nM mibolerone for the times indicated. Blots were probed with a talin cDNA then stripped and reprobed with a GAPDH cDNA as a control for gel loading. The expression of talin was normalised to GAPDH and the expression relative to untreated control cells was calculated. Results are expressed as mean ± S.D. ($n = 3$).

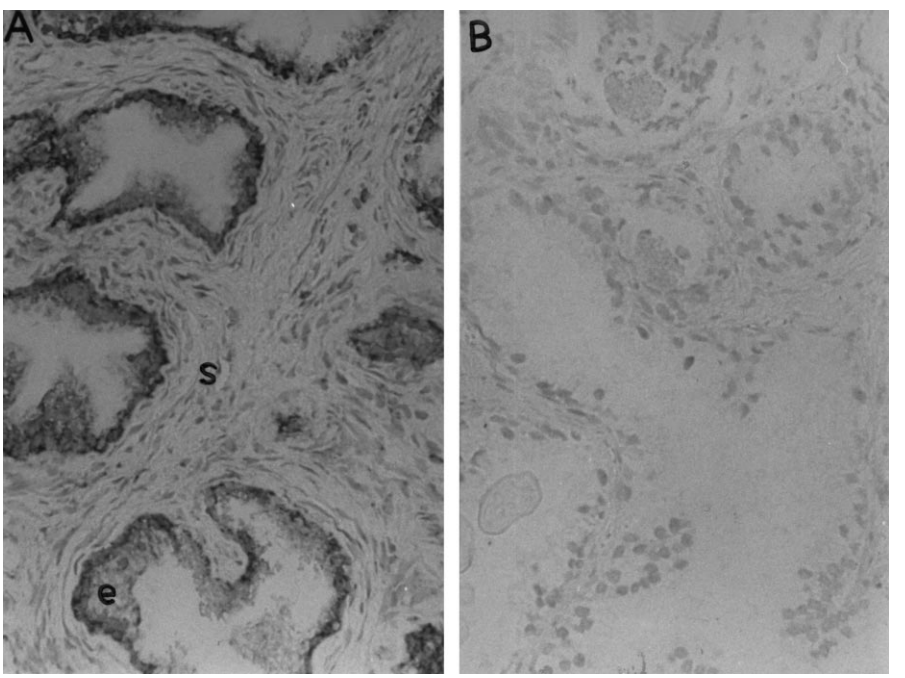

Fig. 4. In situ hybridisation was carried out using digoxigenin-labelled antisense (A) or sense (B) riboprobes on sections of benign prostatic tissue. Talin mRNA, denoted by a purple-blue colouration, was present in epithelium (e) and at lower levels in the stroma (s).

3.3. In situ hybridisation

Hybridisation of digoxigenin-labelled riboprobes to talin mRNA in representative sections of benign prostatic tissue is shown in Fig. 4. The antisense probe hybridised strongly to prostatic epithelial cells, the strongest expression being confined to the basal cells, with the surrounding stroma showing lower levels of expression. No signal was detectable when sections were hybridised with the corresponding sense probe.

4. Discussion

In the present study we have identified a gene in cultured human prostatic stromal cells that is down-regulated by the addition of conditioned medium (CM) from prostatic epithelial cells treated by androgens which was not affected by CM from epithelial cells not treated with androgens. We identified this gene by means of the differential display technique and confirmed its expression in human prostatic tissue by in situ hybridisation.

We have modelled stromal-epithelial interactions in the human prostate by exposing one prostatic cell type to conditioned medium from another. Similar methods have previously been used to show stimulated LNCaP cell proliferation when treated with conditioned medium from prostatic and bone fibroblasts [17]. In our study, talin mRNA expression was found to be decreased in primary prostatic stromal cells in response to an exposure to conditioned medium from androgen treated epithelial cells. Direct exposure of stromal cells to androgen had negligible effect on talin expression (result not shown) and therefore this suggests that the action of androgens on talin expression in prostatic stromal cells can be mediated indirectly by a secreted factor produced by prostatic epithelial cells; a paracrine factor(s) produced by prostatic epithelial cells is the likely agent of this androgen-mediated down-regulation. It is true, however, that androgen is still present in the conditioned medium and it is possible that the changes in talin expression seen are the result of paracrine factors acting in synergy with androgen. The kinetics of down-regulation suggest that the action of androgen is relatively slow. For instance, the most significant decrease in talin expression is after treatment of cells with conditioned medium from cells incubated with androgen for 96 h. However, genes such as nm23, c-*myc* or EGRα show a 2–4-fold change in expression after direct exposure to androgen within 6–8 h [18,19]. Similar kinetics of expression to those seen with talin have been previously reported for orphan receptor TR2 and the cDNA tag TL35 [20,21].

Recent research has implicated the regulation of expression of cell adhesion molecules and cytoskeletal proteins as a key factor in the progression of cancer. For example, reduced or aberrant expression of E-cadherin and α-catenin are predictors of poor outcome in prostate cancer [22]. In addition, the actin-filament binding protein gene, TMe1, identified using differential display, is down-regulated in prostate cancer when compared to the normal prostate [23]. Although little is known about the human talin gene, it has been localised to chromosome 9p [24] and deletions on this chromosome in primary untreated prostate cancers have been reported [25]. Furthermore, studies on differential gene expression between normal muscle and rhabdomyosarcoma identified reduced or absent talin mRNA levels in tumour cells compared with normal cells [26]. Also, in invasive breast carcinoma, down-regulation of talin and other cytoplasmic components of focal contacts such as α-catenin, vinculin, α-actinin and pp125 (focal adhesion kinase) has been demonstrated [27]. We have shown that androgen mediates a down-regulation in talin expression in prostatic stromal cells. There have been no previous reports on the regulation of talin by androgens; however, androgen regulation of other cytoskeletal proteins has been demonstrated in rat motoneurons [28]. Furthermore, sex hormone regulation of talin expression has been demonstrated previously in a study in which estradiol was shown to decrease both the overall number and size of talin- and vinculin-rich cell-matrix adhesion plaques in MCF-7 human mammary carcinoma cells [29]. The androgen-mediated reduction in talin gene expression that we have observed in primary prostatic stromal cells could form part of a mechanism leading to loss of cell adhesion in epithelial cells mediated through androgen action. Our in situ hybridisation studies show that talin mRNA is not only expressed in the stroma of prostatic tissue sections but also at higher levels in the epithelial cells. It is possible, therefore, that talin expression in these cells may also play an important role in the regulation of cell adhesion in both benign and malignant prostate.

Further studies on talin expression in benign and malignant prostate will be required to further delineate its possible role in the development and progression of prostatic disease.

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References

[1] Parker, S.L., Tong, T., Bolden, S. and Wingo, P.A. (1996) Ca Cancer J. Clin. 46, 5–27.
[2] Wolf, D.A., Schulz, P. and Fittler, F. (1992) Mol. Endocrinol. 6, 753–762.
[3] Israeli, R.S., Powell, C.T., Fair, W.R. and Heston, W.D. (1993) Cancer Res. 54, 1807–1811.
[4] Seregni, E., Botti, C., Ballabio, G. and Bombardieri, E. (1996) Tumori 82, 72–77.
[5] Betts, A.M., Waite, I., Neal, D.E. and Robson, C.N. (1997) FEBS Lett. 405, 328–332.
[6] Knezevic, I., Leisner, T.M. and Lam, S.C.T. (1996) J. Biol. Chem. 271, 16416–16421.
[7] Mugurama, M., Nishimuta, S., Tomisaka, Y., Ito, T. and Matsumura, S. (1995) J. Biochem. 117, 1036–1042.
[8] Depasquale, J.A. and Izzard, C.S. (1991) J. Cell Biol. 113, 1351–1359.
[9] AlbigesRizo, C., Frachet, P. and Block, M.R. (1995) J. Cell Sci. 108, 3317–3329.
[10] Jackson, C.W., Hutson, N.K., Steward, S.A. and Rees, D.J.G. (1993) Blood 82, A340.
[11] Collins, A.T., Zhiming, B., Gilmore, K. and Neal, D.E. (1994) J. Endocrinol. 143, 269–277.
[12] Liang, P. and Pardee, A.B. (1992) Science 257, 967–971.
[13] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[14] Williams, J.G. and Mason, P.J. (1987) Nucleic Acid Hybridisation: A Practical Approach (Hames, B.D. and Higgins, S.J., Eds.) pp. 139-160, IRL Press, Oxford.
[15] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.
[16] Rees, D.J., Ades, S.E., Singer, S.J. and Hynes, R.O. (1990) Nature 347, 685–689.
[17] Gleave, M., Hsieh, J.T., Gao, C.A., von Eschenbach, A.C. and Chung, L.W. (1991) Cancer Res. 51, 3753–3761.
[18] Yoshimura, I., Wu, J.M., Chen, Y., Ng, C., Mallouh, C., Backer, J.M., Mendola, C.E. and Tazaki, H. (1995) Biochem. Biophys. Res. Commun. 208, 603–609.

[19] Blok, L.J., Grossmann, M.E., Perry, J.E. and Tindall, D.J. (1995) Mol. Endocrinol. 9, 1610–1620.
[20] Blok, L.J., Kumar, M.V. and Tindall, D.J. (1995) Prostate 26, 213–224.
[21] Ideta, R., Yeh, S., Lee, Y.F., Adachi, K., Takeda, H., Su, C.Y., Slatzman, A. and Chang, C.S. (1995) Endocrine 3, 277–283.
[22] Isaacs, W.B., Bova, G.S., Morton, R.A., Bussemakers, M.J.G., Brooks, J.D. and Ewing, C.M. (1995) Cancer 75, 2004–2012.
[23] Wang, F.L., Wang, Y., Wong, W.K., Liu, Y., Addivinola, F.J., Liang, P., Chen, L.B., Kantoff, P.W. and Pardee, A.B. (1996) Cancer Res. 56, 3634–3637.
[24] Gilmore, A.P., Ohanian, V., Spurr, N.K. and Critchley, D.R. (1995) Hum. Genet. 96, 221–224.
[25] Visakorpi, T., Kallioniemi, A.H., Syvanen, A.C., Hyytinen, E.R., Karhu, R., Tammela, T., Isola, J.J. and Kallioniemi, O.P. (1995) Cancer Res. 55, 342–347.
[26] Genini, M., Schwalbe, P., Scholl, F.A. and Schafer, B.W. (1996) Int. J. Cancer 66, 571–577.
[27] Glukhova, M., Koteliansky, V., Sastre, X. and Thiery, J.P. (1995) Am. J. Pathol. 146, 706–716.
[28] Matsumoto, A., Arai, Y., Urano, A. and Hyodo, S. (1994) Horm. Behav. 28, 357–366.
[29] Depasquale, J.A., Samsonoff, W.A. and Gierthy, J.F. (1994) J. Cell Sci. 107, 1241–1254.