Id-1 expression induces androgen-independent prostate cancer cell growth through activation of epidermal growth factor receptor (EGF-R)

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The failure of prostate cancer treatment is largely due to the development of androgen independence, since the androgen depletion therapy remains the front-line option for this cancer. Previously, we reported that over-expression of the helix–loop–helix protein Id-1 was associated with progression of prostate cancer and ectopic expression of Id-1 induced serum-independent proliferation in prostate cancer cells. In the present study, we investigated if exogenous Id-1 expression in the androgen sensitive LNCaP cells had any effect on androgen-dependent cell growth and studied the molecular mechanisms involved. Using stable Id-1 transfectants, we found that expression of Id-1 was able to reduce serum-stimulated growth and S phase fraction of the cell cycle in LNCaP cells, indicating that Id-1 may be involved in the development of androgen independence in these cells. The Id-1-induced androgen-independent prostate cancer cell growth was correlated with up-regulation of EGF-R (epidermal growth factor receptor) and PSA (prostate specific antigen) expression, as confirmed by western blotting analysis and luciferase assays. In contrast, down-regulation of Id-1 in androgen-independent DU145 cells by its antisense oligonucleotides resulted in suppression of EGF-R expression at both transcriptional and protein levels. In addition, the results from immunohistochemistry study showed that Id-1 expression was significantly elevated in hormone refractory prostate cancer tissues when compared with the hormone-dependent tumours. Our results suggest that up-regulation of Id-1 in prostate cancer cells may be one of the mechanisms responsible for developing androgen independence and this process may be regulated through induction of EGF-R expression. Inactivation of Id-1 may provide a potential therapeutic strategy leading to inhibition of androgen-independent prostate cancer cell growth.

Introduction

Prostate cancer is one of the leading causes of cancer death in the developed countries and the incidence is increasing around the world. Although most of the patients respond initially to the androgen ablation therapy, many of them will inevitably progress to the androgen-independent stage when tumours re-grow regardless of the androgen levels (1). Unfortunately, there is no effective treatment available once the tumour reaches androgen-independent stage. Understanding molecular mechanisms responsible for the development of androgen-independent prostate cancer is thus crucial for improving the survival of prostate cancer patients.

Id-1 (inhibitor of differentiation/DNA binding) protein belongs to the Id family of helix–loop–helix proteins. It lacks the basic domain for DNA binding and functions mainly as a dominant inhibitor of the bHLH transcription factor through heterodimerization (2). Id-1 has been shown to play a critical role in the regulation of cell proliferation (3), differentiation (4) and senescence (5), and recent studies suggest that Id-1 may also function as an oncogene. For example, ectopic expression of Id-1 has been shown to result in immortalization of human keratinocytes (6). Id-1 has been found to be up-regulated in several types of human cancer, such as pancreatic (7), cervical (8), colon (9) as well as prostate cancers (10). In addition to its potential oncogenic action, Id-1 has also been suggested to take part in the malignant progression of human cancer. For example, in breast cancer, Id-1 was found to be constitutively expressed in the highly aggressive but not the non-aggressive cancer cells (11). Moreover, Id-1 expression has also been shown to associate with tumour malignancy in endometric cancer (12). In our previous studies, we observed that increased Id-1 expression was associated with the progression from hyperplasia to prostate cancer in a Noble rat model (13). In human prostate cancer, Id-1 expression was increased with increased Gleason score of the tumours (10), suggesting that over-expression of Id-1 may also play a role in prostate cancer progression.

The previous findings that ectopic expression of Id-1 can mediate the hormone-induced growth stimulation in breast cancer cells indicate that over-expression of Id-1 may affect the hormone response of the cancer cells (14). Previously, we found that prostate cancer cell lines DU145 and PC-3, which are commonly used as in vitro models for androgen-independent prostate cancer, expressed Id-1 protein at a high level compared with LNCaP cells, which is an androgen-dependent prostate cancer cell line (15). Previously, we have generated stable LNCaP transfectants that ectopically expressed high levels of Id-1 (16). In the present study, using both stable and transient Id-1 expressing transfectants, we investigated the involvement of Id-1 in response to androgen-induced growth in prostate cancer cells. First, we examined if exogenous Id-1 had any effect on androgen response of the LNCaP cells in terms of cell growth. Then we investigated the molecular mechanisms responsible for Id-1 induced androgen-independent prostate cancer cell growth. Our results showed that Id-1 expression in LNCaP cells led to decreased sensitivity to androgen-induced cell growth, which was associated with up-regulation of epidermal growth factor-receptor (EGF-R) and prostate growth.
specific antigen (PSA) expression. Inactivation of Id-1 by its antisense oligonucleotides resulted in down-regulation of both EGFR and PSA protein expression. In addition, using human xenografts and tissue samples, we also studied the expression of Id-1 on hormone refractory human prostate cancer and the results showed that Id-1 expression was significantly elevated in hormone refractory prostate cancer as compared with the hormone-dependent tumours. Our results suggest that over-expression of Id-1 may be responsible for the progression of androgen-dependent prostate cancer cells to androgen-independent stage through up-regulation of EGF-R. Inactivation of Id-1 may provide a novel therapeutic strategy in the treatment of androgen-independent prostate cancer.

Materials and methods

Cell culture

Human prostate adenocarcinoma cell lines LNCaP and DU145 were obtained from American Type Culture Collection (Rockville, MD). The establishment of the stable LNCaP-Id-1 transfectants as well as LNCaP-pBabe was described previously (16) and all the cell lines were maintained in RPMI 1640 (Sigma, St Louis, MO) supplemented with 5% of fetal calf serum (FCS) (Invitrogen, Carlbad, CA) at 37°C in 5% CO2. Before performing experiments, the medium was changed to FCS-free RPMI 1640 (SFM). The androgen agonist mibolerone is a gift from Dr K.W.Chan (Department of Pathology, Queen Mary’s Hospital, HK) and was dissolved in ethanol at the stock concentration of 10 μM.

3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Cell growth rate was measured using an MTT proliferation assay kit and the concentration by Bio-Rad protein assay (Bio-Rad, Hercules, CA) before being washed with 1 ml of Buffer B (10 mM HEPES pH 7.6, 60 mM KCl, 1 mM EDTA) and then washed with PBS and then incubated with propidium iodide (50 μg/ml) and RNase (1 μg/ml) for 30 min. Cell cycle analysis was performed on a flow cytometer EPICS profile analyser and analysed using the ModFit LT2.0 software (Coultar, Miami, FL).

Antisense oligonucleotide treatment of DU145 cells

Phosphorothiolated oligonucleotide of the antisense Id-1 gene and the control oligonucleotide were synthesized (Invitrogen) and was dissolved in TE (10 mM Tris–Cl pH 7.4, 1 mM EDTA pH 8.0) as described previously (16). The cells were first cultured in SFM for 48 h and then treated with the Id-1 antisense or control oligonucleotide.

Luciferase assay

pER-1 (luciferase reporter containing the EGF-R promoter, kindly donated by Dr A.Johnson, NCI, MD, USA) and pRL-TK-Luc (internal control) was co-transfected with either the pcDNA, pcDNA-Id-1 or pcDNA-Id-1AS into the cells using Fugene 6 reagent (Roche Diagnostics, Indianapolis, IN). Cells were lysed 48 h after transfection and were assayed for luciferase activity using the Dual-luciferase reporter assay system (Promega, WI). Each experiment was repeated three times and each data point represented the mean and standard deviation.

Transfection

Retrovirus carrying the empty vector or the full length Id-1 gene was generated from the packaging cell line PG13 as described previously (16) and used to transfect the LNCaP cells. Forty-eight hours after the transfection, the cells were incubated with serum-free RPMI-1640 for a further 48 h and then lysed for western blotting analysis.

Immunohistochemistry study

CWR22 is an androgen-dependent human prostate cancer xenograft estabished from primary human prostate cancer by serial transplantations on nude mice (19). CWR22R is the recurrent tumour derived from CWR22 after castration of the host (20), which grows in an androgen-independent manner. In this study, these xenografts were maintained in intact or castrated nude mice, respectively, as described previously (19). Normal human prostate (n = 3), benign prostate hyperplasia (BPH) (n = 3) and prostate cancer tissues (n = 6) were obtained from the archival tissue bank of the Department of Pathology, West China University of Medical Sciences, Chengdu, China, from one of our previous studies (10). Three of the prostate cancer specimens were obtained from autopsies in patients who died of prostate metastasis or late stage prostate cancer due to the failure of androgen ablation therapy from the same hospital. Tissues from both the xenograft and human prostate cancer were fixed, paraffin embedded and sectioned at the thickness of 5 μm. Slides were used for immunohistochemistry study of Id-1 expression using rabbit polyclonal Id-1 antibody (Santa Cruz Biotechnology) with the protocol described previously (10).

Results

Effect of ectopic Id-1 expression on androgen response of LNCaP cells

To examine whether ectopic Id-1 expression had any effect on the growth response of the androgen sensitive LNCaP cells toward androgen stimulation, we studied four stable Id-1 transfectants generated previously (16). As shown in Figure 1A, under serum-free conditions, Id-1 was not detected in both LNCaP (parental) and pBabe (empty vector control) cells, but was present at different levels in the four Id-1 transfectants
It was found previously that under serum-free conditions, the growth of LNCaP cells could be stimulated by the synthetic androgen mibolerone at the concentration of 1 nM (21). We therefore investigated the growth response of LNCaP cells to mibolerone at concentrations of 0.01–1 nM.

After exposure to mibolerone (indicated as Mb in the figures), the Id-1 transfectants and the controls were subjected to cell cycle analysis. As shown in Figure 1B, in the absence of androgen (indicated as –Mb), >90% of the LNCaP or pBabe cells were arrested at G1 phase of the cell cycle, with only ~5% of cells entering S phase. In contrast, in the Id-1 transfectants, the proportion of S phase cells was much higher (ranging from 11.9 to 16%) when cultured in the same conditions, which is consistent with our previous findings (16). After exposure to mibolerone (indicated as +Mb, 0.01 nM), there was an ~3-fold increase in number of cells entering S phase in LNCaP (from 4.8 to 14.4%) and pBabe (from 5.8 to 14.3%) cells. In contrast, in Id-1 transfectants, addition of androgen did not result in a significant increase in S phase fraction as in the controls. For example, in clone C3, there was an increase of 3% S phase cells (from 16 to 19%) after exposure to mibolerone. These results indicate that Id-1 expression in LNCaP cells reduced their sensitivity to androgen-stimulated S phase fraction.

To confirm the results on cell cycle analysis, we studied the growth rate of the Id-1 transfectants and the controls after exposure to mibolerone using MTT proliferation assays. As shown in Figure 1C, all of the cell lines showed increased cell growth rate after exposure to mibolerone (0.01–1 nM) in a time- and dose-dependent manner. For example, after treatment with 0.01 nM mibolerone, there was a 34% increase in cell viability in LNCaP cells at 24 h post-exposure time compared with the untreated control, and the increment reached 119% after exposure to 1 nM mibolerone at the same time point. The increased cell growth became more evident at a later time point (i.e. 72 h post-exposure time) in LNCaP and pBabe cells (287 and 305% increase, respectively, after exposure to 1 nM mibolerone). In contrast, the androgen-induced cell growth was much less significant in the Id-1 transfectants. For example, treatment of C3 or C4 with 1 nM of androgen for 72 h resulted in an increment of <80%.

Taken together, these results indicate that expression of Id-1 in the androgen sensitive LNCaP cells reduced their sensitivity to androgen-induced cell growth.

Fig. 1. Effect of ectopic Id-1 expression on growth response of LNCaP to androgenic stimulation. (A) Id-1 expression in control (LNCaP and pBabe) and Id-1 transfectants (C1–C4) was analysed by western blotting analysis and expression of actin was measured as a loading control. (B) Effect of androgen on S-phase entry of the cells. LNCaP, pBabe and Id-1 transfectants were first grown in SFM for 48 h and were then treated with 1 nM of mibolerone for 24 h. Cells were then subjected to flow cytometry analysis and cell cycle distribution was presented as the percentage of cells at each phase. (C) Growth stimulation of androgen on the control (LNCaP and pBabe) and Id-1 transfectant (C1–C4) cells. Cells were cultured in SFM for 48 h and then treated with mibolerone (0.01–1 nM) or solvent only. Viable cells were labelled with MTT reagent at 24 h intervals and quantified spectrophotometrically at 570 nm. Results represented the OD ratio between the treated and untreated cells at indicated time points. Each data point represented the mean and standard deviation. Note that Id-1 expression induces decreased sensitivity to androgen-stimulated S phase fraction and growth in LNCaP cells.
Effect of Id-1 on the expression of AR, EGF-R and PSA in LNCaP cells

The action of androgen is through binding with the AR and up-regulation of AR expression has been suggested to reduce the growth response of LNCaP cells to androgen stimulation (22). To investigate the molecular mechanisms responsible for Id-1-induced androgen de-sensitization, we first studied whether Id-1 expression in LNCaP cells had any effect on AR protein expression. As shown in Figure 2A, using western blotting, we found that AR expression levels were similar between the controls and the Id-1 transfectants, indicating that decreased sensitivity to androgen in Id-1 transfectant may not be due to alterations of AR expression.

Up-regulation of EGF-R has been suggested to facilitate the androgen-dependent prostate cancer cells to surpass the androgen dependency and progress to androgen-independent stage (23,24). Previously, we found that Egr-1 (early growth response factor 1), which induces EGF-R expression at the transcription level (25), was increased in Id-1 transfectants (Figure 2B) (15). In this study, we then investigated if Id-1 expression in LNCaP cells could affect the expression of EGF-R protein. As shown in Figure 2C, expression of EGF-R was increased (up to 500% compared with the controls) in all of the Id-1 transfectants, suggesting that ectopic Id-1 expression resulted in up-regulation of EGF-R protein expression.

Expression of PSA is androgen dependent in normal prostate and androgen-dependent prostate cancer cells, but at androgen-independent stage, high levels of PSA are usually present in the absence of androgen, although the mechanism of this elevation is not clear (26). As shown in Figure 2D, we found that while PSA was undetectable in the control LNCaP and pBabe cells, all the Id-1 transfectants showed different levels of PSA protein. In addition, the highest level of PSA was found in C2 cells, which also expressed the highest level of Id-1, suggesting that PSA protein expression in the Id-1 transfectant is associated with the Id-1 levels. Since AR expression levels were similar among the control and Id-1 transfectants, the difference in PSA expression between the control and Id-1 transfectants may not be due to changes of AR levels.

To eliminate the possibility that the observed alterations in EGF-R and PSA expression in Id-1 transfectants were due to clonal variation during the generation of stable transfectants, we repeated the above experiments by transiently transfecting LNCaP cells with an Id-1 expression vector. As shown in Figure 2E, increased expression of Egr-1, EGF-R and PSA proteins was found in the transient Id-1 transfected cells while there was no significant difference in AR expression between the control (LNCaP and pBabe) and Id-1 transfected cells (pBabe-Id-1). These results support the findings in the stable transfectants that ectopic expression of Id-1 increases the expression of Egr-1, EGF-R and PSA proteins. In addition, to examine if the increased EGF-R expression was at transcriptional level, we also transiently co-transfected an EGFR-reporter gene construct with the Id-1 expression vector. As shown in Figure 2F, co-transfection of the EGFR-reporter construct with the Id-1 expression vector resulted in up to 3.5-fold increase in EGF-R transactivation activity in LNCaP cells, which agrees with the findings at protein level (Figure 2E). These results are consistent with the results generated from stable transfectants, further indicating that ectopic Id-1 expression results in up-regulation of EGF-R and PSA expression in LNCaP cells.

Effect of NF-κB inactivation on PSA expression in stable Id-1 transfectants

Recently, four NF-κB binding sites were identified in the core enhancer of the PSA gene and activation of NF-κB has been shown to induce the expression of the PSA protein (27). Previously, using the same cell lines, we have found that Id-1 can activate NF-κB signaling pathway and protect prostate cancer cells from apoptosis (17). It is possible that increased PSA expression in the Id-1 transfectants in the present study may be associated with induction of NF-κB activity. To examine if
NF-κB activation was involved in the induction of PSA expression in the Id-1 transfectants, we treated the Id-1 transfectants (C3 and C4) with a NF-κB specific inhibitor Bay 11-7085, which has been shown to inhibit nuclear translocation of the NF-κB subunits, p65 and p50, through suppressing phosphorylation of IkappaB-α (28). As shown in Figure 3A, treatment of C3 and C4 with 20 μM of Bay 11-7085 resulted in the reduction of the nuclear p50 and p65 proteins, indicating that Bay 11-7085 treatment sufficiently reduced the activity of NF-κB. Interestingly, the expression of PSA in both transfectants was also decreased by the treatment (Figure 3B). These results showed that expression of PSA was associated with activation of NF-κB and inhibition of NF-κB activity led to decreased PSA expression in Id-1 transfectants, indicating that up-regulation of PSA in the Id-1 transfectants may be mediated through NF-κB signalling pathway.

**Effect of Id-1 antisense oligonucleotides on the expression of EGF-R and PSA in LNCaP cells transfected with Id-1**

To further confirm the association between Id-1 and EGF-R and PSA expression, we next studied whether blockage of Id-1 expression in the stable Id-1 transfectants could inhibit the expression of EGF-R and PSA in LNCaP cells by treating the cells with antisense oligonucleotides targeting to the Id-1 gene (indicated as Id-1AS). Two of the representative Id-1 transfectants clones, C3 and C4, were exposed to two concentrations (10 and 20 μM) of the Id-1AS for 72 h and western blotting was used to examine the Id-1 expression. As shown in Figure 4, expression of Id-1 was decreased after Id-1AS treatment in a dose-dependent manner while Id-1 protein levels remained high in the untreated and control oligo treated cells, indicating a sufficient suppression of Id-1 expression by Id-1AS. Interestingly, while AR expression was not affected by the Id-1AS treatment, EGF-R expression was decreased and PSA protein expression was completely abolished in both clones after exposure to Id-1AS. These results suggest that down-regulation of Id-1 protein expression leads to decreased EGF-R and PSA expression, further supporting previous results that ectopic Id-1 expression induces up-regulation of PSA and EGF-R in LNCaP cells.

**Effect of Id-1AS on EGF-R expression in DU145 cells**

High levels of EGF-R expression in DU145 have been suggested as a possible mechanism for its androgen-independent growth (29) and high levels of Id-1 were also found in DU145 cells compared with the LNCaP cells in our previous studies (16). To further establish the association between Id-1 and EGF-R in prostate cancer cells, we treated DU145 cells with two concentrations of Id-1AS (10 or 20 μM) and measured EGF-R expression levels. As shown in Figure 5A, after treatment with Id-1AS for 72 h, the level of Id-1 protein was decreased with increased concentrations of Id-1AS (up to 50% reduction), which was associated with down-regulation of EGF-R protein levels in DU145 cells (up to 70% reduction). In addition, the EGF-R reporter activity was also decreased when the Id1-AS containing vector (pcDNA-Id-1AS) was co-transfected with an EGF-R reporter construct into these cells, compared with the vector control (pcDNA) (Figure 5B). These results indicate that suppression of Id-1 in DU145 cells has led to down-regulation of EGF-R expression at both transcriptional and protein levels, suggesting that the association...
Evidence of increased Id-1 expression in hormone refractory human prostate cancer

In order to confirm our hypothesis that up-regulation of Id-1 may be associated with the development of androgen independence, we next studied Id-1 expression in both androgen-dependent and -independent prostate cancer specimens using immunohistochemistry. First, we used the human xenograft CWR22 established from primary human prostate cancer that requires androgen for tumour growth in nude mice (19). In addition, we used its derived xenograft, CWR22R, which is the CWR22 established from primary human prostate cancer that independent specimens were studied and primary prostate immunohistochemistry. First, we used the human xenograft dependent and -independent prostate cancer specimens using oligonucleotide (20

Discussion

In this study, we found that exogenous expression of Id-1 protein in androgen sensitive prostate cancer cells promoted androgen-independent cell growth, which was associated with up-regulation of EGF-R and PSA expression. The fact that inhibition of Id-1 expression by its antisense oligonucleotides led to down-regulation of EGF-R at both transcriptional and protein levels further indicates that Id-1 may be an upstream regulator of the EGF-R. In addition, we also demonstrated that the expression of Id-1 increased significantly in hormone refractory tumours compared with the hormone-dependent tumours. These lines of evidence support the hypothesis that over-expression of Id-1 may be responsible for the progression of prostate cancer cells from androgen dependence to an androgen-independent stage and this process may be mediated through up-regulation of EGF-R expression. Targeted inactivation of the Id-1 gene may provide a novel therapeutic value against androgen-independent prostate cancer.

Previously, we have demonstrated that ectopic Id-1 expression induces proliferation of LNCaP cells in serum-free conditions, which is mediated through activation of Raf/MEK pathway (16), indicating that Id-1 expression may provide autocrine signals required for activation of the mitogenic signalling pathway. This, to a certain extent, is similar to what occurs in the androgen-independent stage, when tumour cells grow in the absence of certain mitogenic factors (i.e. androgen). In the present study, the results that expression of Id-1 in LNCaP cells reduced their growth response to androgenic stimulation demonstrated by the reduced S phase fraction and decreased cell growth rate (Figure 1) also suggest a role of Id-1 as a possible autocrine signal for androgen-independent prostate cancer cell growth.

Progression to hormone refractory disease is often associated with over-expression of growth factors and receptors. It has long been suggested that up-regulation of EGF-R is involved in the development of androgen-independent prostate cancer (23,24,30). Although EGF-R has been detected in both primary and metastatic prostate cancer, a higher proportion of EGF-R positive cells have been detected in tumour samples after hormone ablation treatment (24). In addition, androgen-independent prostate cancer cell lines PC-3 and DU145 were found to express higher levels of EGF-R when compared with that of LNCaP (31,32). Recently, EGF-R expression has also been shown to correlate with the progression of prostate cancer to androgen refractory stage (33). These findings provide additional evidence to support the hypothesis that EGF-R expression may play a role in the development of androgen-independent prostate cancer. However, the molecular
Fig. 6. Immunohistochemical staining of Id-1 protein in androgen-dependent and hormone refractory prostate cancers. (A) Id-1 expression in androgen-sensitive human prostate cancer xenografts CWR22 (panel 2) and androgen-independent derivative CWR22R (panel 3). Panel 1, negative control of the CWR22 with omission of primary antibody. Note that Id-1 staining intensity is much stronger in CWR22R than in the CWR22, while the negative control shows no positive staining. (B) Expression of Id-1 in human normal prostate, BPH and prostate cancer specimens. Panel 1, normal prostate tissue; panel 2, BPH; panel 3, primary prostate cancer; panels 4–6, androgen-independent prostate cancers. Note that all prostate cancer specimens show positive staining for Id-1 protein expression (panels 3–6), while non-malignant tissues are stained negative (panels 1 and 2). Positive Id-1 staining is the strongest in androgen-independent tumour samples compared with the androgen sensitive tumours.
mechanisms responsible for the activation of EGF-R in prostate cancer are not clear. In this study, we found that EGF-R expression was increased after Id-1 was stably transfected into LNCaP cells (Figure 2C). These results were further confirmed by transient transfection experiments which showed that EGF-R expression was increased at both transcriptional and protein levels after transfection of Id-1 (Figure 2E and F). In addition, inhibition of Id-1 expression in the stable Id-1 transfectants led to reduction of EGF-R expression (Figure 4) and down-regulation of Id-1 expression in the androgen-independent DU145 cells also resulted in transcriptional suppression of EGF-R (Figure 5). These results strongly suggest Id-1 as an upstream regulator of the EGF-R. Recently, it is reported that constitutive activation of the MAPK pathway reduces androgen requirement of prostate cancer proliferation (34) and attenuation of the MAPK signalling pathway is able to restore androgen sensitivity in prostate cancer cells (35). Our previous studies also showed that up-regulation of Egr-1, which is one of the downstream effectors of the MAPK pathway and an upstream regulator of EGF-R (25), was essential for Id-1 induced prostate cancer cell proliferation (15). It is possible that the ability of Id-1 protein in reducing androgen dependency of the prostate cancer cells is through providing autocrine signals that activate the MAPK pathway, which in turn induces EGF-R expression.

Expression of PSA is regulated by androgen and in prostate cancer patients, the PSA level decreases sharply after androgen ablation therapy (26). After progression to the androgen-independent stage, the PSA level increases in the absence of androgen (26). Several mechanisms have been suggested to account for the high levels of PSA expression in androgen-independent prostate cancer. For example, amplification of AR, which results in hypersensitivity to residue androgen after androgen ablation (36), or mutations of AR that allow activation by factors other than androgen (37). Recently, the transcription factor NF-κB is shown to induce PSA expression at transcription level (27). In the present study, we did not observe any significant changes in AR expression in the Id-1 transfectants (Figure 2A), therefore, the increased PSA expression in these cells may not be due to alterations of the androgen signalling pathway. Instead, we found that expression of PSA in the Id-1 transfectants was abolished by treatment by a NF-κB inhibitor (Figure 3), suggesting that increased PSA expression in Id-1 transfectants may be due to activation of NF-κB induced by Id-1. Previously, we have shown that Id-1 expression induces the nuclear translocation of NF-κB leading to transactivation of this pathway, which provides survival signals against apoptosis in LNCaP cells (17). It is thus possible that the Id-1-induced PSA expression in the present study is regulated through NF-κB pathway in prostate cancer cells. We are currently studying the molecular mechanisms responsible for NF-κB-induced PSA expression and its role in androgen-independent prostate cancer cell growth.

In addition to our previous findings that increased Id-1 expression is correlated with higher Gleason grades in prostate cancer (10), the results from the present study using prostate cancer specimens also demonstrated that Id-1 expression was much higher at the hormone refractory stage, although relatively small number of samples were studied (Figure 6). These in vivo results further support the hypothesis that expression of Id-1 may facilitate the growth of prostate cancer cells in the absence of androgen and up-regulation of Id-1 may play a crucial role in the development of androgen independence in prostate cancer cells.

In summary, the present study demonstrates that Id-1 expression in androgen sensitive prostate cancer cells leads to decreased androgen response and this process may be mediated through up-regulation of EGF-R expression. Our evidence suggests Id-1 as a novel upstream regulator of EGF-R and provides a scientific basis for the development of new therapeutic strategies against androgen-independent prostate cancer through inactivation of Id-1.

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References

Id-1 induces androgen-independence


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