Prostate Cancer: The Id1 Story

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Id (inhibitor of differentiation or DNA binding) gene encodes a helix-loop helix protein which dimerizes and blocks the basic HLH protein from binding DNA. It expresses mainly in actively dividing cells and was first reported to be involved in hormone-induced prostate cancer in the Noble rat model. It was subsequently confirmed also in human prostate cancer. Through functional studies under in vitro system, they have further demonstrated the role of Id1 in prostate cancer progression. They have shown that Id1 transfection stimulated prostate cancer cell proliferation through downregulation of p16/Rb, while inducing activation of MAPK and NFkB pathways. Activation of the latter pathways by ectopic transfection of Id1 to LNCaP cells, an androgen dependent line, also resulted in reduced sensitivity of prostate cancer cells to androgen on the one hand and upregulating the expression of EGFR and PSA on the other hand, which are both hallmarks of androgen independent prostate cancer. This review shows the crucial role played by Id1 in the conversion of prostate cancer from androgen dependent to androgen independent stage.

Key words: androgen independent prostate cancer, Id1 gene

I. Introduction

Prostate cancer is one of the most commonly diagnosed cancers and is the second killer in men in the United States especially amongst African Americans [26]. In oriental population, the incidence is much lower. However, with the changing life styles in recent years, its incident has increased in Hong Kong and mainland China [10]. A number of factors are believed to be related to development of prostate cancer including genetic, dietary and environmental factors. Despite extensive research worldwide no significant breakthrough has been made either in the mechanism or treatment of prostate cancer. There is also a lack of reliable factor/marker which could accurately predict the metastatic ability of the prostate cancer before it breaks away from the site of origin. For treatment of advanced prostate cancer, especially the metastatic ones, androgen ablation approach (either chemical or surgical) remains the gold standard of treatment since it was first established by Charles Huggins over half of a century ago [1, 18]. The approach is effective initially as the source of androgens is removed. However, usually after a period of 1–3 years, the tumor often re-emerges and become androgen independent (AI). There is no effective treatment for the AI prostate cancer by then.

II. The Nature of Id Family of Proteins

The Id family of genes has four members, i.e. Id1 to Id4. Id1 is located in 20q11; Id2, 2p25; Id3 1q36.1 and Id4 6p21.3. Id1 protein belongs to the 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 [3]. Id1 has been shown to play a role in cell proliferation [5], differentiation [19] and senescence [2], and recent studies suggest that Id1 may serve as an oncogene. It has been found to be upregulated in several types of human cancer such as cervical [27, 32], colon [31], breast [14] and prostate cancer [22, 24].

III. Discovery of Id1 in Prostate Cancer in Animal Model

In order to study the mechanism of prostate cancer in detail, we established an animal model based on the earlier approach of Noble by implanting a combination of testosterone (T) and 17β-estradiol (E2) subcutaneously for up to 12 months [30]. In this modified model, we established that T+E2 was effective in inducing prostate cancer in the Noble rat. Microarray study revealed that during the prostate carcinogenesis in this hormone-induced prostate cancer, Id1 gene activity was overexpressed [22]. Immunohistochemical examination of prostate tissue revealed that Id1 protein was overexpressed only in prostate carcinoma but not in hyperplasia or normal prostate tissue. More interestingly, the level of expression of Id1 was correlated with the tumor phenotype; that is, the more poorly differentiated the tumor, the higher the Id1 overexpression [22]. This is interesting as it may indicate that the level of Id1 expression may be related to the malignancy of prostate cancer. Is this also the case in human prostate cancer?

IV. Confirmation in Human Prostate Cancer

In order to find out the status of Id1 in human prostate cancer, we carried out an immunohistochemical study on human prostate tissue and found that the results were comparable to those of the Noble rat. Id1 protein was detected in prostate adenocarcinoma while absent in both normal and BPH [23]. More importantly, the level of expression was correlated with the clinical (Gleason) grading, i.e. the higher the Gleason grading, the higher the Id1 expression. Thus, Id1 expression level in human prostate cancer, like those in the animal model, is directly correlated with the malignancy of prostate cancer.

V. Functional Studies

Having identified the importance of this Id1 in prostate carcinogenesis both in animal model and in human prostate cancer, our next aim was to examine the role of this gene in prostate cancer progression and the molecular mechanisms involved. It would be very difficult, if not impossible, to dissect the role of this gene in prostate carcinogenesis under in vivo condition. We therefore resorted to study in detail the functional role of Id1 in prostate carcinogenesis under in vitro system using several well known prostate cancer cell lines such as LNCaP, PC3 and DU145 cell lines.

VI. Selection of Cell Lines for In Vitro Studies

We first screened the three lines for expression of Id1 and found that both PC3 and DU145 lines expressed Id1 constitutively. Both cell lines are well known prostate cancer lines which are hormone-independent. The LNCaP cell which is androgen dependent/responsive, expresses Id1 protein only at very low or barely detectable level in the presence of serum, but become complete negative when cultured in serum free condition for 24 hours. We thus selected this line for our functional studies, by transfecting a full length human Id1 gene cDNA into LNCaP cells. After drug selection, 10 clones were selected which expressed various levels of Id1 proteins under serum free condition for our studies [24].

VII. Transfection of LNCaP Cells with Id1 Gene and Its Effect on Cell Proliferation through Inactivation of p16/Rb

We first examined the effect of ectopic expression of Id1 gene on cell proliferation. The results showed that the cell proliferation rate was increased in all lines positively expressing Id1 protein, and that the proliferation rate was directly correlated with levels of Id1 protein expression. We further found that increase in cell proliferation was through downregulation of p16 with a concurrent increase in phosphorylated form of Rb protein indicating that Id1 induced inactivation of p16/Rb pathway may be responsible for the increased cell proliferation observed in prostate cancer cells (Fig. 1) [24].

VIII. Involvement of MAPK in Stimulation of Cell Proliferation

Recently, Id1 has been shown to facilitate human fibroblasts to escape senescence through the Ras-Raf-MEK signaling pathway [21]. In addition, one of the downstream effectors of the MAPK pathway, early growth response-1 (Egr-1) has been reported to interact with Id1 [29]. Since activation of MAPK and activation of one of its downstream effectors, Egr-1, are associated with advanced cancer [7, 9], we further investigated the involvement of MAPK in Id1 induced prostate cancer cell proliferation [15].

We first examined the effect of ectopic expression of Id1 on Raf-1, MEK1/2 and Egr-1 expression. Using several selected clones, we determined the possible contribution of MAPK in Id1-induced serum independent cell proliferation in LNCaP cells. Western blotting data revealed that there was an increase in expression in the phosphorylated form of Raf-1 and MEK1/2 while the total Raf-1 and MEK1/2 proteins was not affected. The level of expression of phosphorylated Raf-1 and MEK1/2 was much lower in the controls. Since activation of Raf-1 and MEK1/2 signaling is through phosphorylation, these data indicated that exogenous Id1 expression in LNCaP cells resulted in a direct activation of Raf/MEK1/2 kinases (Fig. 1) [15]. We also examined the
Egr-1 signals at mRNA and protein levels and found that both parameters were increased in Id1 transfected clones. Using specific inhibitor of MEK1/2, PD098059, it effectively decreased the phosphorylation of MEK1/2 together with a concurrent decrease in Egr-1, thus resulting in a decreased MAPK signaling transduction activity. These results support the involvement of MAPK signaling pathway in Id1-induced serum independent LNCaP cell growth (Fig. 1) [15].

IX. Involvement of Id1 in Apoptosis

Growth of tumors can be achieved by either increase in cell proliferation or decrease in apoptosis or both. In the previous sections we have provided information on ectopic expression of Id1 in relation to cell proliferation. In the following passages, we would like to provide information on the exogenous Id1 in protection of LNCaP cells from TNFα-induced apoptosis [16]. When LNCaP cells were treated with an apoptosis inducer, TNFα, apoptosis occurred in both Id1 negative and positive cells. However, the apoptotic rate was much higher in the parental LNCaP cells than in the Id1 transfectants, indicating a protective role of Id1 expression against apoptosis. In addition, increased Bax and caspase 3 and PARP fragmentation was also observed in the parental LNCaP (Id1 negative) cells but it was less evident in the Id1 expressing cells. Inactivation of Id1 by treatment with antisense oligonucleotides in Id1 expressing DU145 cells resulted in the increased apoptosis rate. These results clearly indicate that Id1 expression plays a crucial role in the protection of prostate cancer cells against apoptosis (Fig. 2) [16].

X. Involvement of Id1 in the Activation of NFκB in Protection of Cells from TNFα-induced Apoptosis

It was reported that NFκB was inactive in LNCaP cells [25]. In this study we examined whether activation of NFκB in the Id1 transfected was responsible for the protection of TNFα-induced apoptosis in LNCaP cells. We studied the expression of the two most abundant forms of NFκB, p65 and p50, in the cell nuclear extract by Western blotting. The results showed that increased p65 and p50 protein expression was found in the transfectant clones compared to the controls [16]. Furthermore, the transcriptional activity of
promoter was also greatly increased indicating that exoge-

uous expression of Id1 protein has resulted in NFκB activa-
tion in LNCaP cells.

In order to confirm these findings, the two most imme-
diate downstream effectors of NFκB, Bcl-xL and ICAM-1
(intercellular adhesion molecule-1) [13, 28], were also ex-
amined. The results showed that both proteins were much
higher in the Id1 transfectant clones compared to controls
[16]. Since activation of NFκB is found to parallel and
depend on degradation of IκBs [6], we thus examined one
of the three major IκBs, IκB-α. The results showed that a
decrease in IκB-α in all transfectant clones compared to
controls. These results show clearly that Id1 expression in
LNCaP cells led to activation of NFκB signaling pathway
(Fig. 2) [16].

To further confirm these results, we treated DU145
cells with antisense Id1. Unlike LNCaP cells, DU145 ex-
presses Id1 protein constitutively together with activation of
NFκB [25]. Downregulation of Id1 activity by antisense Id1
in DU145 cells, resulted in the downregulation of NFκB and
its downstream effectors, Bcl-xL and ICAM-1 [16]. These
results indicate that suppression of Id1 expression not only
reduced the expression of NFκB but also suppressed its
signaling transduction.

We next examined whether downregulation of Id1 ac-
tivity by antisense would subject DU145 cells to TNFα-
induced apoptosis. We treated the DU145 cells by antisense
Id1 and TNFα and found that the apoptotic rate of treated
DU145 cells was much higher than the controls (i.e. DU145
cells without treatment by antisense Id1) [16]. Concurrent
to this, there was a significant reduction in level of NFκB and
increased cleavage of caspase-3 and PARP indicating an
activation of apoptotic pathway. The results showed that
suppression of Id1 expression by antisense Id1 resulted in
reduced protection of DU145 cells against apoptosis in-
duced by TNFα (Fig. 2) [16].

XI. Role of Id1 in Transformation of Androgen
Dependent to Androgen Independent
Prostate Cancer

One of the most crucial and unsettled issues in prostate
cancer research is the ability of prostate cancer cells to trans-
form from androgen-dependent (which can be easily con-
trolled by androgen-ablation either chemically or surgically)
to androgen-independent stage (which is not responsive to

![Fig. 2.](image-url) This figure summarizes the results for Id1 transfection in the activation of NFκB and its protection against TNFα-induced apoptosis [16]. The left side represents ectopic expression of Id1 in LNCaP cells which resulted in activation of NFκB, thus resulting in blocking the apoptotic pathway activated by TNFα, thus reducing apoptosis. The right side represents treatment of DU145, an androgen independent cell line which expressed Id1 protein constitutively, with Id1 antisense resulting in downregulation of NFκB, thus allowing TNFα-induced apoptotic pathway to proceed, resulting in increased apoptosis of DU145 cells.
hormonal manipulation therapy or other approaches, with consequential death). The mechanism surrounding this transformation is little known to date.

In our earlier studies, using LNCaP line, which is a well known hormone-dependent (or responsive) prostate cancer cell line, we have shown that transfection of Id1 gene could enhance the growth of these cells in serum free condition together with an activation of NFκB, and MAPK pathways as well as to reduce the sensitivity of the cells to androgen stimulation (Figs. 1, 2) [15, 16]. All these features are reminiscent of androgen-independent prostate cancer cells. On the other hand, DU145 and PC3, the two well known androgen-independent lines, are both characterized by their expressing high levels of Id1 and NFκB signals and are more resistant to TNFα-induced apoptosis. Downregulation by antisense Id1 resulted in a concurrent reduced expression of NFκB and its downstream effectors, together with an activation of apoptotic pathway, thus is more amenable to TNFα induced apoptosis (Fig. 2) [16]. The latter features are more closely akin to androgen-dependent prostate cancer cells. Could Id1 gene be central to this crucial transformation?

XII. Involvement of Id1 in EGFR Upregulation

It is well known that androgen action is through androgen receptor (AR) and that upregulation of AR has been suggested to reduce the growth response of LNCaP cells [12]. We therefore examined the AR level in LNCaP and Id1 transfectant clones, and found that the level of AR was not affected by Id1 overexpression, indicating that in this case, AR expression was not changed. It has been reported that upregulation of EGFR to facilitate androgen dependent prostate cancer to overcome the androgen dependency and to proceed to androgen independent stage [8, 11]. In our earlier studies, we found that Egr-1 which induces EGFR at transcriptional level [20] was increased in Id1 transfectant clones. We therefore investigated the EGFR expression in Id1 transfected clones. The results showed that EGFR expression was increased significantly indicating that Id1 transfection resulted in increased expression of EGFR [17]. Treatment of DU145 cells with antisense Id1 resulted in downregulation of EGFR indicating the relationship of Id1 and expression of EGFR [17].

XIII. Involvement of Id1 in PSA Secretion

The other hallmark of androgen independent prostate cancer is increase in PSA secretion in the absence of androgens. We found that while PSA was not detectable in parental LNCaP and vector transfected cells, the Id1 transfected clones expressed PSA significantly. Further the level of PSA expression was directly correlated with level of expression of Id1 protein indicating a direct correlation of Id1 level and PSA secretion in LNCaP cells [17]. The increased expres-
sion of PSA was also found in LNCaP cells transiently over-expressed with Id1 gene indicating that the results observed was not due to clonal variation in the transfected clones [17].

It has been reported recently that four NFkB binding sites were identified in the core enhancer of the PSA gene and activation of NFkB has been shown to induce the expression of PSA protein [4]. In the earlier sections we have demonstrated that Id1 can activate NFkB signaling pathway and protect prostate cancer cells from apoptosis. It is therefore possible that increased PSA expression in the Id1 clones may be associated with induction of NFkB activity. We therefore inactivated the NFkB activity by specific inhibitor, Bay 11-7085, which has been shown to inhibit nuclear translocation of p65 and p50 subunits. The results showed that upon treatment of this specific inhibitor, both subunits were decreased which was correlated with the decrease in expression of PSA. The results indicate that upregulation of PSA in Id1 transfectants may be mediated through NFkB signaling pathway [17]. Treatment of DU145 cells, with antisense Id1 resulted in downregulation of PSA in these cells indicating that expression of Id1 is closely linked to PSA secretion [17].

Finally, in order to show that upregulation of Id1 may be associated with the development of androgen independence, we examined the Id1 expression in both androgen dependent and androgen independent prostate cancer specimens using immunohistochemical approach. The results showed that in human prostate xenografts the Id1 level was much higher in CWR22R (hormone refractory line) than the CWR22 (androgen dependent line) indicating that Id1 may be directly related to androgen independent prostate cancer development [17].

XIV. Conclusions

With the foregoing discussion, we would like to summarize the results in Figure 3. Starting from LNCaP, a well known androgen dependent prostate cancer cell line, we have demonstrated that transfection of this line with an Id1 gene, could enhance the growth of LNCaP cells in serum free condition together with suppression of p16/Rb, activation of MAPK and NFkB pathways [15, 16, 22]. We have further demonstrated that, via activation of MAPK, it resulted in overexpression of EGFR, a feature of androgen independent prostate cancer (Fig. 3) [17]. Through its activation of NFkB, Id1 could also stimulate the secretion of PSA while the AR was not changed [17]. A combined activation of MAPK and NFkB together with inhibition of p16/Rb pathways, the LNCaP cells has been transformed from a typically androgen dependent phenotype to a new line reminiscent of androgen independent prostate cancer like DU145 or PC3 (Fig. 3). We thus succeeded in transforming the LNCaP cells from an androgen dependent to androgen independent stage through transfection of Id1 gene. It is clear from our studies that Id1 is one of the crucial factors regulating the transformation of androgen dependence to androgen independence. Inactivation of Id1 may thus be considered as a novel strategy for treatment of androgen independent prostate cancer.

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XVI. References


