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Is EGR1 a potential target for prostate cancer therapy?

Delphine Gitenay, PhD and
The Vaccine Research Institute of San Diego, 10835 Road to the Cure, San Diego, CA 92121, USA
Tel.: +1 858 581 3960 Fax: +1 858 581 3970 delphinegitenay@yahoo.fr

Véronique T Baron, PhD, HDR†
The Vaccine Research Institute of San Diego, 10835 Road to the Cure, San Diego, CA 92121, USA
Tel.: +1 858 581 3960 Fax: +1 858 581 3970 vbaron@sdibr.org

Abstract

Prostate cancer is a major cause of cancer-related death in American men, for which finding new therapeutic strategies remains a challenge. Early growth response-1 (EGR1) is a transcription factor involved in cell proliferation and in the regulation of apoptosis. Although it has long been considered a tumor suppressor, a wealth of new evidence shows that EGR1 promotes the progression of prostate cancer. This review addresses the paradoxes of EGR1 function. While EGR1 mediates apoptosis in response to stress and DNA damage by regulating a tumor suppressor network, it also promotes the proliferation of prostate cancer cells by a mechanism that is not fully understood. Thus, EGR1 might be targeted for prostate cancer therapy either by ectopic expression in combination with radiotherapy or chemotherapy, or by direct inhibition for systemic treatment. Possible strategies to antagonize EGR1 function in a therapeutic setting are discussed.

Keywords

EGR1; oncogene; prostate cancer; transgenic mouse mode; tumor suppressor

Prostate cancer is the most commonly diagnosed cancer in men and the second highest cause of cancer-related death among men in the USA. In 2007, there were an estimated 218,890 new cases of prostate cancer and an estimated 27,050 deaths [1]. Routine use of prostate-specific antigen (PSA) screening enables a better diagnosis, but is still deficient in two ways. First, the correlation between PSA levels and the presence of cancer is indirect, so the presence of cancer must be confirmed by biopsy. Second, current biopsy methodology is diagnostic but not prognostic. Consequently, prostate cancer patients with minimally invasive forms of cancer needlessly undergo an aggressive surgical procedure.

The progression of the disease follows multiple steps, from benign hyperplasia to hormone-independent metastatic disease. Unfortunately, approximately 90% of patients with advanced disease will develop bone metastases, which are associated with severe pain, loss of mobility and spinal cord compression. Other affected organs may include the liver, lungs and brain [2]. Despite extensive research efforts, little hope exists for patients with hormone-refractory

†Author for correspondence: The Vaccine Research Institute of San Diego, 10835 Road to the Cure, San Diego, CA 92121, USA ■ Tel.: +1 858 581 3960 ■ Fax: +1 858 581 3970 ■ vbaron@sdibr.org.

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prostate cancer that is resistant to hormone therapy, radiation and conventional chemotherapy. Discovery of new treatments for advanced prostate cancer and identification of patients at risk of progression can only be achieved through a better understanding of the molecular mechanisms underlying the progression of the disease.

Recent studies have highlighted the involvement of the transcription factor early growth response-1 (EGR1) in prostate cancer. This is paradoxical, since EGR1 had been demonstrated to act as a tumor supressor in other types of cancer (reviewed in [3]). This review offers an overview of the evidence of EGR1 contribution to prostate cancer and its molecular mechanism of action. We briefly discuss potential strategies to target EGR1 for cancer therapy, providing that the uncertainties regarding its function are eliminated.

EGR1

EGR1 is a nuclear phosphoprotein that was first identified based on its early induction by mitogens and differentiation factors [4-8]. It is also known as NGFI-A, Zif268, T1S8 and krox-24. The unstimulated EGR1 level of expression is low in most tissues, except in brain where high expression is observed [9]. EGR1 contains a highly conserved DNA-binding domain composed of three zinc fingers that bind to the prototype target GC-rich consensus sequence GCG (G/T) GGGCG [3,10]. In addition, EGR1 binds to the regulatory proteins NAB-1 and NAB-2 (NGF-I A-binding proteins) that repress its transcriptional activity [11-13].

EGR1 is induced by growth factors, cytokines and stress signals such as radiation, injury or mechanical stress [3,10,14]. Cloning of the *EGR1* promoter has revealed the presence of response elements for various transcription factors. Specifically, the promoter contains several serum response elements (SREs), an AP-1 binding site, several cAMP regulatory elements (CREs) and Sp1 consensus sequences [15-18]. Most often, increased transcription of EGR1 is mediated by the MAP-K signaling pathway (FIGURE 1). Three MAP-K families, known as ERK1/2, JNK and p38MAP-K, are most commonly involved in EGR1 activation. While ERK1/2 mediates EGR1 expression in response to growth factors [19-22], a combination of ERK1/2, p38MAP-K and/or JNK is required to induce EGR1 in response to stress [23,24]. The activated MAP-K translocates to the nucleus, phosphorylates and activates transcription factors of the Elk-1/SAP-1/2 family. Elk-1 then associates with the ternary complex factor consisting of CREB-binding protein (CBP) and serum response factor (SRF), and binds to and transactivates the *EGR1* promoter (reviewed in [25,26]). An alternative pathway for EGR1 activation was recently discovered, in which transcription factor NF-κB mediates EGR1 transcription in response to UV exposure of human skin. This study identified a canonical NF-κB binding site and demonstrated the direct binding of p65 (RelA) to the *EGR1* promoter [27]. In another study, two functional nonconsensus binding sites for the tumor suppressor p53 were identified. Binding of p53 to the *EGR1* promoter in response to DNA damage leads to sustained expression of EGR1 and efficient apoptosis [28]. Finally, EGR1 binds to its own promoter and represses transcription, thereby initiating a negative feedback loop soon after activation [29].

EGR1 activity and stability are regulated by post-translational modifications. Acetylation, mediated by the transcriptional co-activator p300/CBP, stabilizes EGR1 and may promote survival, whereas phosphorylation in response to stress may favor cell death [30]. Sumoylation is mediated by tumor suppressor p19ARF and directs EGR1 to the nucleus [31]. The short-lived EGR1 is then ubiquitinated on multiple sites and degraded by the proteasome [32]. We suspect that the full spectrum of post-translational modifications regulating EGR1 activity is far from being fully deciphered.

EGR1 knockout mice

EGR1 is involved in the development and response to stress of various organs, and consequently emerges in a number of patho logical conditions. However, suppression of EGR1 in mice produces few defects. Two strains of $EGR1^{-/-}$ mice have been generated that exhibit somewhat distinct phenotypes. The $EGR1^{-/-}$ mice generated by Milbrandt's team show a fairly normal phenotype, except for infertility in the female [33,34]. By contrast, the mice developed by Charnay's team, which are also infertile (both male and female), have reduced body size and weight resulting from the altered development of the pituitary gland. Female $EGR1^{-/-}$ mice in this study had atrophied reproductive organs with a smaller uterus and ovaries, whereas the $EGR1^{-/-}$ males had smaller testes, seminal vesicles and, notably, a smaller prostate [35].

Mouse embryo fibroblasts (MEFs) derived from Charnay's animals display a reduced rate of proliferation compared with $EGR1^{+/+}$ MEFs [35]. Importantly, $EGR1^{-/-}$ MEFs derived from Milbrandt's animals bypass senescence and display the growth properties of immortalized cells. EGR1 regulation of senescence is mediated by p53, as indicated by the observation that forced expression of EGR1 restores senescence in $EGR1^{-/-}$ cells, but not in $p53^{-/-}$ cells [36].

EGR1-null mice do not spontaneously develop tumors, although an accelerated development of tumors was observed in a two-step carcinogenesis model of skin cancer, arguing in favor of a tumor suppressor role in response to DNA damage *in vivo* [37]. Many studies have used these animals to examine the role of EGR1 in a variety of pathological situations, which is beyond the scope of this review. Findings that are the most relevant to prostate cancer come from the breeding of EGR1 knockout mice with transgenic mouse models of prostate cancer, such as the cryptdin-2-T-antigen model (CR2-TAg) and the transgenic adenocarcinoma mouse prostate (TRAMP). CR2-TAg mice develop prostate carcinoma from neuro endocrine prostate cells due to the targeted expression of SV40 large T antigen (SV40-TAg) [38]. Strikingly, CR2-TAg/$EGR1^{-/-}$ mice survive appreciably longer than CR2-TAg/$EGR1^{+/-}$ or CR2-TAg/$EGR1^{+/+}$ mice [39]. Increased tumor latency is observed in CR2-TAg/$EGR1^{-/-}$ mice that is not caused by a decreased rate of initiation or by a decreased rate of tumor growth, but is due to delayed progression from prostatic intraepithelial neoplasia lesions to adenocarcinoma. A similar delay in prostate cancer progression was observed in TRAMP mice [39], which develop tumors from the luminal epithelial cells of the prostate [40]. These observations strongly establish EGR1 as a key player in the progression of prostate cancer.

Role of EGR1 in prostate cancer progression

Many other observations support the notion that EGR1 contributes to prostate cancer progression (previously reviewed in [41,42]). In prostate adenocarcinoma, the mRNA encoding EGR1 is expressed at higher levels compared with normal tissues [43,44]. The observation that levels of mRNA and protein expression correlate with Gleason scores and inversely correlate with the degree of differentiation of carcinoma cells also suggests that EGR1 is involved in cancer progression [43]. EGR1 expression in the primary tumor correlates with radiation response in terms of complete control of the local tumor. In the postirradiated biopsies it correlates with residual tumor and treatment failure [45]. NAB2, the regu latory protein that represses the transcrip tional activity of EGR1, is downregulated in human primary prostate carcinomas [46]. Interestingly, EGR1 is also overexpressed in the tumors of CR2-TAg mice, whereas NAB2 expression is decreased [39]. Thus, both the upregulation of EGR1 and loss of its repressor, NAB2, participate in determining the high level of EGR1 activity in human prostate cancer.

There are only a few documented examples of EGR1 overexpression in cancer. EGR1 is elevated in Wilm's tumors and ectopic expression of EGR1 increases the proliferation of kidney cells. In addition, it was observed that EGR1 antagonizes the effect of the tumor suppressor

Wilm's tumor-1 (WT-1) [47]. In melanoma, EGR1 overexpression is associated with mutation of *B-RAF* [48]. Approximately 60% of human melanomas contain a mutation in the *B-RAF* gene [49]. As RAF acts upstream of the ERK pathway, activating mutations of *B-RAF* result in constitutive ERK1/2 activity. EGR1 overexpression requires both the presence of mutant *B-RAF* and the activity of ERK1/2 [48].

To understand the molecular mechanism leading to EGR1 upregulation in prostate cancer, we used a collection of transformed, immortalized or primary prostate cell lines. A stringent correlation between EGR1 and p53 expression was observed [SAUER L *ET AL*.: EGR-1 INITIATES A FEEDBACK LOOP THAT INVOLVES THE EGF-RECEPTOR/ERK1/2 pathway and is regulated by p53 in prostate cancer cells. MANUSCRIPT SUBMITTED]. Among EGR1 expressing cells, the DU145 cell line contains a p53 mutant [50]. Other cell lines in which EGR1 was overexpressed exhibit high p53 protein expression, owing to its stabilization by SV40-TAg used for immortalization. EGR1 expression was high in all cells with the p53 mutant or SV40-TAg, whether they were transformed or not, and its expression was abrogated by pharmacological inhibition or silencing of p53. Conversely, EGR1 expression was low in cancer and nontransformed cells containing low or undetectable p53 expression.

It is interesting to note, in this context, that EGR1 overexpression was observed in CR2-TAg mice that also express SV40-TAg [39], suggesting that it is a common mechanism with *in vivo* significance. EGR1 deficiency results in delayed cancer progression in these mice or in SV40-Tag-expressing TRAMP mice. One can wonder what would be the effect of EGR1 deficiency in other models of cancer that do not rely on SV40-TAg.

Whether EGR1 is involved in the etiology of prostate cancer is yet to be established. One would contend that EGR1 is not essential for tumor initiation because tumors do develop in $EGR1^{-/-}$ transgenic mouse models of prostate cancer [39]. On the other hand, transgenic expression of SV40-TAg in prostate cells is powerfully tumorigenic and overrides the need for an endogenous initiating signal. To our knowledge, forced expression of EGR1 in nontransformed prostate cells has not been achieved, with one arguable exception. Stable transfection of EGR1 in 267B nontransformed prostate cells results in increased colony formation and increased growth in soft agar, two hallmarks of transformation *in vitro* [51]. However, the 267B cell line originated from a fetal prostate epithelium and was immortalized using SV40-Tag [52], which is now known to interfere with EGR1 function [SAUER L *ET AL*.: EGR-1 INITIATES A FEEDBACK LOOP THAT INVOLVES THE EGF-RECEPTOR/ERK1/2 pathway and is regulated by p53 in prostate cancer cells. MANUSCRIPT SUBMITTED]. Therefore, the possibility is real that similar results may not be observed in nontransformed cells that do not contain SV40-TAg. The fact that increased transformation was observed in 267B clones overexpressing EGR1 suggests that the oncogenic action of EGR1 may be proportional to its level of expression.

The particular role of EGR1 in prostate cancer could be due, at least in part, to a crosstalk with the androgen receptor (AR). Specifically, EGR1 physically interacts with the AR in hormone-sensitive prostate cancer cells. Moreover, the EGR1–AR complex binds to the promoter of *PSA*, an endogenous target of AR [53]. Overexpression of an activated mutant of EGR1 in AR-positive prostate cancer cells leads to translocation of AR to the nucleus and contributes to the regulation of PSA transcription [53]. As a consequence of this crosstalk, over expression of a constitutively active mutant of EGR1 in hormone-sensitive LNCaP cancer cells leads to increased proliferation *in vitro*. In a xenograft model, it enhances tumor growth in castrated animals [54]. Since castration mimics hormone therapy in human patients, these results suggest that EGR1 may be involved in the acquisition of resistance to hormone therapy.

Several new targets of EGR1 were identified in a microarray analysis of mouse prostate cancer cells, some of which are involved in cell-cycle regulation [55]. Three targets with cell-cycle

function were formally validated: cyclin D2, MAD and $p19^{ink4d}$. It was demonstrated that MAD and $p19^{ink4d}$, which are growth inhibitors, were downregulated by EGR1, whereas cyclin D2, which contributes to cell-cycle progression through G1, was upregulated. Through altered transcription of cell-cycle regulators, EGR1 promotes the growth of prostate cancer cells, as evidenced by the fact that EGR1 silencing inhibits cell proliferation, colony formation and growth in soft agar [55,56].

EGR1 also stimulates the production of many growth factors and cytokines. A microarray analysis performed in LAPC4 prostate cancer cells, in which EGR1 overexpression was driven by adenovirus-mediated transfection, identified IGF-II, PDGF-A and TGF-β1 as EGR1 targets [57]. Interestingly, several genes identified in this array are associated with neuroendocrine differentiation, which is known to occur frequently in prostate cancer. *In vivo*, the amount of both TGF-β1 and PDGF-A produced in prostate tumors was decreased in CR2-TAg/$EGR1^{-/-}$ mice compared with CR2-TAg/$EGR1^{+/+}$ mice [39]. Accordingly, human tumor-derived DU145 cells that display high EGR1 expression secrete TGF-β1 and PDGF-A [SAUER L *ET AL.*: EGR-1 INITIATES A FEEDBACK LOOP THAT INVOLVES THE EGF-RECEPTOR/ERK1/2 pathway and is regulated by p53 in prostate cancer cells. MANUSCRIPT SUBMITTED]. In addition, three ligands of EGF-R (epiregulin, HB-EGF and amphiregulin) are induced by EGR1 in DU145 cells, resulting in autocrine activation of the EGF-R and activation of survival and proliferative signals.

Thus, the increased transcription of growth factors and cytokines leads to autocrine cell growth and to alteration of the tumor environment, and is likely to directly contribute to cancer progression. Indeed, PDGF-A promotes angiogenesis (reviewed in [58]), whereas TGF-β1 is associated with advanced prostate cancer and promotes alteration of the microenvironment and metastasis, as well as immune suppression and angiogenesis [59-61].

Finally, a role for EGR1 in metastasis has been suggested. Salah *et al.* demonstrated that EGR1 regulates the transcription of human protease-activated receptor 1 (hPAR1), which is implicated in epithelial malignancies [62]. hPAR1 expression is regulated by androgen, explaining its high and low levels in prostate cancer tissues before and after hormone ablation therapy, respectively. However, high levels of hPAR1 are found in hormone-resistant prostate carcinoma [63]. EGR1 binds the *hPAR1* promoter *in vivo* and *in vitro*. Bombesin, a neuro endocrine peptide known to activate EGR1 [64], induces hPAR1 expression and cell invasion in an androgen-independent manner [62]. A direct correlation was observed between the expression of EGR1 and PAR1, and the degree of progression in human prostate tumors [62]. Another study found that heparanase, which degrades heparan sulfate and has been implicated in invasion and metastasis, is regulated by EGR1 in prostate cancer. High heparanase expression in tumors appears to be associated with increased EGR1 expression, coupled with the hypomethylation of the heparanase promoter in an EGR1 target sequence [65]. Finally, EGR1 regulates the transcription of HYAL-1, an enzyme that degrades hyaluronic acid and is involved in tumor growth and metastasis. Interestingly, the EGR1 binding site in the *HYAL-1* promoter overlaps with an SP1 binding site. Whereas SP1 binds to the methylated promoter to repress transcription, EGR1 binds to the nonmethylated promoter and activates transcription. Levels of HYAL-1 expression are proportional to EGR1 expression in prostate cancer cell lines [66]. Taken together, these studies indicate that EGR1 regulates the expression of various extracellular proteins that function in the remodeling of the extracellular matrix and are known to participate in metastasis.

Role of EGR1 in stress-induced apoptosis

A paradoxical role of EGR1 in cancer lies in its capacity to promote apoptosis in response to stress and DNA damage, potentially acting as a tumor suppressor. In fact, in a number of other types of cancer, EGR1 has a tumor suppressive function (reviewed in [67]). EGR1 transcription

is increased following DNA damage, stress or mitogen exposure. Strong evidence for a pro-apoptotic function is provided by the observation that $EGR1^{-/-}$ MEFs are resistant to apoptosis induced by ionizing radiation [68].

EGR1 belongs to a network of tumor suppressor genes that function together in response to stress. For example, EGR1 activates the transcription of the tumor suppressor PTEN *in vitro* and *in vivo*. EGR1-deficient cells that are resistant to apoptosis also lack PTEN induction upon stress [69]. In a recent study, EGR1 and PTEN were identified as mediators of $p14^{ARF}$ function [31]. EGR1-dependent expression of PTEN is controlled by $p14^{ARF}$ through ARF-mediated sumoylation of EGR1. This requires the phosphorylation of EGR1 by the protein kinase AKT, which promotes the association of EGR1 with $p14^{ARF}$. EGR1 is not sumoylated in $ARF^{-/-}$ fibroblasts or $ARF^{-/-}$ mouse tissues, and this correlates with defective PTEN expression. Both tumor suppressors, PTEN and $p14^{ARF}$, are often downregulated in human cancer, or their function impaired owing to genetic mutation [31].

There are numerous instances of EGR1 activity in response to stress in prostate cancer cells. For example, EGR1 is activated by hypoxia in DU145 prostate cancer cells and binds to the promoter of *HIF-1α*, increasing its transcription [70]. In prostate cancer cells lacking p53, apoptosis caused by ionizing irradiation is mediated by EGR1-induced secretion of TNF-α, a cytokine with known pro-apoptotic properties [71]. In cells that do contain it, p53 is a target of EGR1 and a major mediator of EGR1 apoptotic function [36,37,72]. Another study has demonstrated that EGR1 increases the expression of Rb, which prevents Mdm2-mediated degradation of p53, and thus also upregulates p53 [68]. In addition to p53, EGR1 also induces the expression of p73 upon stress. p73 is an isoform of p53 that also functions as a transcription factor and is involved in stress-induced apoptosis. The three proteins EGR1, p73 and p53 are required for the full apoptotic response to take place. In addition, both p73 and p53 induce EGR1 expression in a feedback loop that promotes sustained expression of the three proteins and persistence of the apoptotic signal [28].

A recent study from Ahmed's laboratory shows that sensitivity of prostate cancer cells to irradiation-induced apoptosis is increased upon forced expression of EGR1, but is decreased when EGR1 is knocked down [73]. In response to DNA damage, EGR1 directly interacts with the YAP-1 protein, and the EGR1–YAP-1 complex induces the transcription of the pro-apoptotic protein Bax. Overall, these results indicate that direct transactivation of Bax by EGR1–YAP-1 sensitizes the cells to irradiation-induced apoptosis [73].

Arora *et al.* found that EGF-R in M12 prostate cancer cells (that contain SV40-TAg) mediates UV-induced apoptosis through activation of EGR1 [74]. The fact that UV exposure activates the EGF-R has been known for some time [75]. In this recent study, UV irradiation activated the EGF-R, induced EGR1 expression and led to apoptosis. Novel targets of EGR1 were identified by ChIP-on-chip analysis, 24 of which belong to the EGF-R signaling pathway. The validated targets include FasL, MAX and RRAS2, which are involved in apoptosis or growth arrest [74].

How do we resolve the paradox of an oncogenic function of EGR1 (when overexpressed in prostate cancer) with its tumor suppressor function? We have previously proposed that in prostate cancer, the tumor suppressor function of EGR1 becomes deficient in many ways [76]. For example, two major mediators of EGR1 apoptotic function are inactivated in the majority of prostate tumors. The first, p53, is inactivated in 25–50% of prostate cancer cases [77]. The second, PTEN, is not normally regulated by EGR1 in prostate cancer cells that contain wild-type *PTEN* ([56] and [BARON VT *ET AL.*, THE VACCINE RESEARCH INSTITUTE OF SAN DIEGO. UNPUBLISHED DATA]). One hypothesis is that inactivation of the *PTEN* gene owing to hypermethylation, which occurs in approximately 50% of prostate cancer cases [78], may prevent the binding of EGR1 to the

promoter. In addition, *PTEN* is the most commonly altered gene in prostate cancer [79]. Lack of PTEN correlates with high Gleason score and advanced stage cancer [80]. Up to 60–80% of prostate tumors contain a hemizygous deletion of *PTEN*, and haplo-insufficiency is enough for the progression of prostate cancer [79,81,82].

Another tumor suppressor that is regulated by EGR1 in prostate cancer is TGF-β1 [55,57,83]. Forced expression of EGR1 in HT1080 fibrosarcoma suppresses transformation, owing, in part, to upregulation of TGF-β1, which inhibits growth by an autocrine mechanism [83]. However, many prostate cancer cells become resistant to TGF-β1 [59,84]. In addition, a switch of function occurs, since TGF-β1 also promotes cancer progression *in vivo* by acting as an angiogenesis factor and by suppressing the anti-tumor immune response [60,61].

Targeting EGR1 for prostate cancer therapy

Despite the role that EGR1 plays in prostate cancer, very little has been done towards its validation as a target for therapy. This is likely to be owing to the fact that EGR1 function in cancer remains paradoxical and is not fully understood. These paradoxes must be addressed before we can target EGR1 for cancer therapy. In particular, the observation that EGR1 can be a tumor suppressor warns us of the possible danger of long-term inhibition. EGR1-null mice do not spontaneously develop cancer, which argues against the hypothesis that long-term treatment with EGR1 inhibitors will cause the formation of tumors. On the other hand, whereas loss of EGR1 delays prostate cancer progression in transgenic mouse models [39], it accelerates tumor growth in a two-step skin carcinogenesis model [37]. This suggests that systemic inhibition of EGR1 could increase the incidence of DNA damage-induced cancers caused by, for example, smoking or excessive sun exposure. This may be addressed by using local delivery (such as intratumoral injection), or through molecular targeting of the drug to the tumor.

Strategies to antagonize EGR1 function

RNA interference (RNAi; antisense oligonucleotides, vector-based small interfering RNA or DNAzyme) is currently the best available strategy to specifically inhibit EGR1. Discussing the limitations of the RNAi approach for clinical use is not within the scope of this review. However, the lack of other effective and specific small-molecule EGR1 inhibitors does limit our ability to investigate EGR1 function and pursue other therapeutic options.

Antisense oligonucleotides that block EGR1 expression have demonstrated efficacy in a mouse model of prostate cancer. In a test experiment performed in TRAMP mice, a dose of 25 mg/kg of EGR1 antisense oligonucleotide nearly completely inhibited EGR1 expression in the prostate tumor [51]. Male TRAMP mice were treated with saline, a three-base mismatch control oligonucleotide or EGR1 antisense oligonucleotide. The mice were 22 weeks old at the start of treatment and received intraperitoneal injections for 10 weeks [56]. The incidence of tumor was lower in antisense-treated mice, and inhibition of EGR1 significantly delayed tumor growth [56]. Importantly, at a dose of antisense oligonucleotide that is commonly used in mice, no apparent toxicity was observed. Thus, inhibition of EGR1 delays the development of prostate cancer and does not seem to be associated with significant short-term side effects.

Overexpression of the EGR1 repressor NAB2 has been examined as a way to decrease EGR1 activity. Forced NAB2 expression in vascular cells decreases the transcription of EGR1 target genes, and inhibits angiogenesis *in vitro* [85]. To our knowledge, this approach has not been used in cancer cells. Potential problems may arise from the fact that NAB2 binds to all EGR family members. In addition, a co-activator function of NAB2 has been recently discovered. Thus, NAB2 represses or activates EGR1-mediated transcription depending on each target gene [86], adding a complexity that may preclude its use for therapeutic purposes.

The use of natural compounds that have no toxic side effects, such as curcumin, may be an interesting alternative. Curcumin, found in turmeric, exhibits antioxidant, anti-inflammatory and anticarcinogenic effects [87]. Curcumin inhibits the activity of EGR1 in monocytes and colon cancer cells through inhibition of ERK-1/2 and Elk-1 phosphorylation [88,89]. It prevents EGR1 transcription in response to IL-1β in lung epithelial cells, which decreases the expression of the inflammation enzyme mPGES-1 [90]. On the other hand, curcumin activates EGR1 in human glioma cells, leading to EGR1-mediated transcription of cell-cycle inhibitor p21 and inhibition of cell proliferation [91]. It should be noted that curcumin exerts its anticancer effects through a variety of signaling molecules, and is by no means specific to EGR1 [92].

Other strategies that may be developed include single or double-stranded DNA mole cules that mimic the EGR1 cognate DNA-binding sequence. These small DNA analogs could compete with cellular DNA for EGR1 binding and would capture EGR1 and prevent its binding on target genes. Such an approach has been used, for example, against the STAT1 and STAT3 transcription factors [93,94]. Finally, a high-throughput platform could be used to screen chemical libraries in order to isolate small-molecule drugs that decrease EGR1 transcriptional activity. A small-molecule drug may have the advantage of solubility and good pharmacological properties.

Ectopic expression of EGR1

Another strategy to target EGR1 for the therapy of prostate cancer could take advantage of its proapoptotic function in response to DNA damage. Indeed, a very recent study has demonstrated that forcing EGR1 expression in PC3 prostate cancer cells using an adenovirus vector (adEGR1), combined with irradiation, decreased the growth of xenograft tumors in nude mice more efficiently than irradiation alone [73]. This result is especially relevant to the fact that prostate cancer is often treated with radiotherapy, and indicates that forced expression of EGR1 can increase the sensitivity of cancer cells to DNA damage. Prostate tumors are notoriously resistant to chemotherapy, and therefore it would be interesting to determine whether the combination with ectopic expression of EGR1 would also improve the efficacy of current chemotherapies. Systemic treatment, rather than targeted treatment, may be considered, since ectopic expression of EGR1 in endothelial cells was found have a potent anti-angiogenic function and to inhibit cell invasion in a xenograft model; it also inhibited tumor growth in a mouse fibrosarcoma model [95]. It remains to be determined if the anti-angiogenic function of EGR1 would be maintained in combination with radio- or chemo-therapy. Finally, the potential danger of increasing EGR1 oncogenic function will have to be carefully evaluated.

Other therapeutic uses of EGR1

A different approach has been developed that does not directly target EGR1, but takes advantage of its responsiveness to DNA damage and stress, such as that caused by irradiation. In this approach, the *EGR1* promoter is placed upstream of the *TNF*-α gene into a replication-deficient adenovirus, termed Ad.Egr.TNF. This vector is currently delivered by intratumoral injection and shows little toxicity. In principle, local radiation therapy allows for temporal (and spatial) control of TNF-α release, which induces apoptosis of the tumor cells. *In vivo* studies have demonstrated that injection of tumors with Ad.Egr.TNF potentiates the cytotoxic effect of irradiation. Ad.Egr.TNF is currently under clinical investigation for the treatment of various solid tumors (reviewed in [96]). A new alternative for patients who are intolerant to radiation comes from resveratrol, a natural compound that induces the expression of EGR1 [97]. A recent study demonstrated that resveratrol activated Ad.Egr.TNF in tumor xenografts, and increased the anti-tumor response in both human and rat models. Thus, the activation of TNF-α expression by resveratrol may extend the use of Ad.Egr.TNF [98].

Conclusion & future perspective

The observation that EGR1 is overexpressed in human prostate tumors and promotes prostate cancer progression undeniably challenged the view that EGR1 is purely a tumor suppressor. Our knowledge of EGR1 function in prostate cancer has improved considerably since these discoveries were made. However, given its paradoxical function in cancer, further elucidation of its mechanism of action remains essential and many uncertainties must be resolved before EGR1 can safely be considered a target for prostate cancer therapy.

The paucity of EGR1 antagonists greatly restricts our ability to study its function, especially *in vivo*. The availability of a cell permeable, small-molecule inhibitor of EGR1 DNA binding or transcriptional activity would definitely advance the field. In addition, more research is required to delineate the best approach to treatment. Larger preclinical studies are needed to evaluate various approaches to block EGR1 function and to study the combination of ectopic expression with radiotherapy or chemotherapy in various animal models. Finally, further understanding of EGR1-related signaling networks in human tumors may eventually allow the definition of populations of patients who would be the most likely to benefit from EGR1-targeted therapies.

Executive summary

EGR1

- Early growth response-1 (*EGR1*) is an early response gene involved in cellular responses to stress and growth factors.
- EGR1 regulated mainly at the transcriptional level, following the activation of transcription factors of the Elk family by the MAP-kinase pathway.

Knockout mice

- Two laboratories have generated $EGR1^{-/-}$ mice with somewhat distinct phenotypes.
- Breeding of $EGR1^{-/-}$ mice with transgenic prostate cancer mouse models has indicated that a lack of EGR1 delays the progression of prostate cancer.

Role in prostate cancer progression

- EGR1 is overexpressed in human prostate tumors. However, its involvement in the etiology of prostate cancer has not been formally demonstrated.
- EGR1 may play a specific role in prostate cancer owing to its interaction with the androgen receptor.
- Microarray analyses and other studies have identified EGR1-responsive genes in prostate cancer cells. These include various growth factors, cell-cycle regulators and proteins with a role in metastasis.

Role in stress-induced apoptosis

- EGR1 induces the transcription of several tumor suppressor genes in response to stress and belongs to a tumor suppressor network.
- These tumor suppressors are often altered in human cancer, either genetically or epigenetically.

Targeting EGR1 for prostate cancer therapy

■ Available strategies to antagonize EGR1 function are still very limited. Silencing of EGR1 using antisense oligonucleotides delays the growth of tumors *in vivo*, validating this transcription factor as a potential target for prostate cancer therapy.

■ On the other hand, ectopic expression of EGR1 through gene therapy may increase cancer cell sensitivity to chemotherapy or irradiation.

■ Another therapeutic use takes advantage of EGR1 responsiveness to stress and DNA damage. A gene therapy vector was engineered with the EGR1 promoter controlling the cytotoxic TNF-α cytokine.

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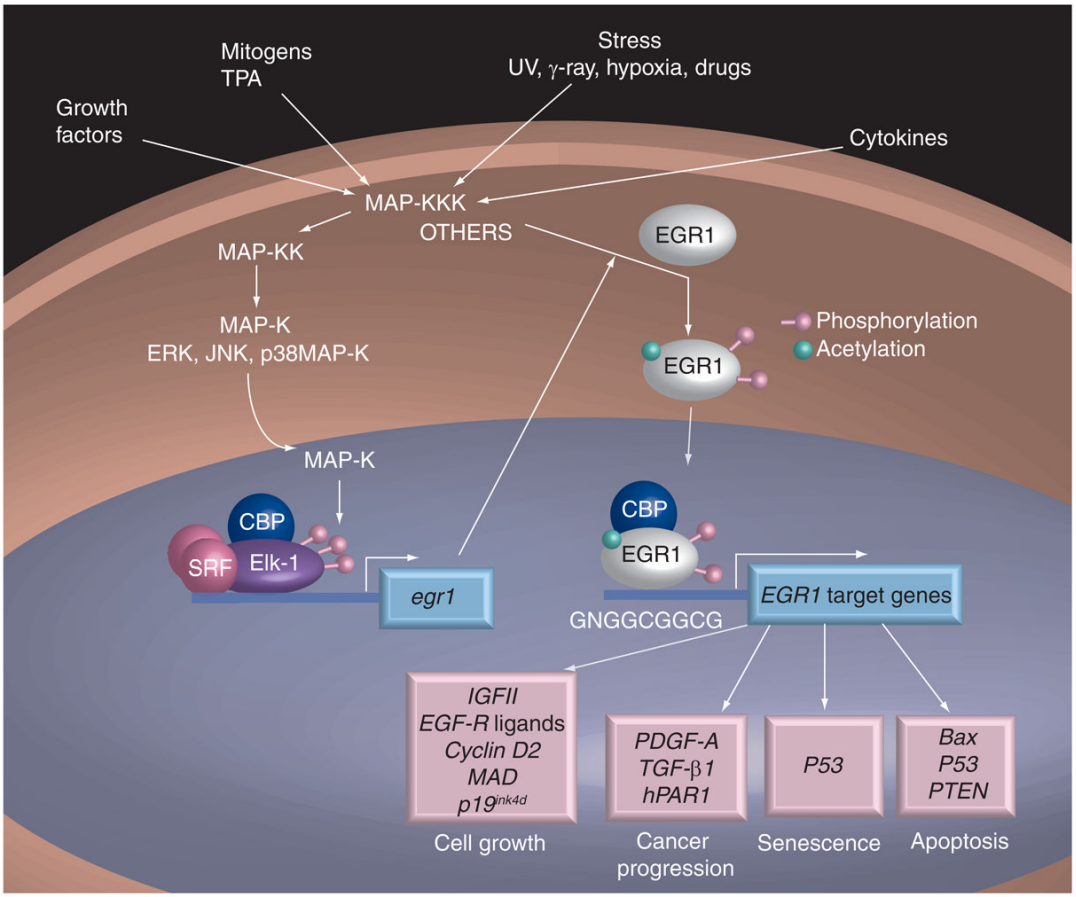

Figure 1.
EGR1 mechanism of activation.