



Hypoxia targeting gene expression for breast cancer gene therapy[☆]

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ARTICLE INFO

Article history:

Received 24 August 2008

Accepted 28 April 2009

Available online 6 May 2009

Keywords:

Breast cancer

Hypoxia

Gene regulation

Targeting

ABSTRACT

Gene therapy is a promising strategy to treat various inherited and acquired diseases. However, targeting gene expression to specific tissue is required to minimize side effects of gene therapy. Hypoxia is present in the microenvironment of solid tumors such as breast tumors. A hypoxic tumor targeting gene expression system has been developed for cancer gene therapy. In hypoxic tissues, hypoxia inducible factor (HIF)-1 α is accumulated and stimulates transcription of the genes that have hypoxia response elements (HREs) in their promoters. Therefore, transcriptional regulation with a hypoxia inducible promoter is the most widely used strategy for hypoxic tumors targeting gene therapy. In breast cancer gene therapy, breast tumor specific promoters in combination with HREs have been used to induce gene expression in hypoxic breast tumors. Post-transcriptional regulation using an untranslated region (UTR) is also a useful strategy to increase gene expression in hypoxic tumor tissue. In addition, post-translational regulation with the oxygen-dependent degradation (ODD) domain is effective to eliminate therapeutic gene products and reduce side effects in normal tissue. In combination with the breast tumor specific promoters, hypoxic tumor targeting strategies will be useful for the development of a safe breast cancer gene therapy.

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1. Introduction

Gene therapy is one of the promising strategies to treat breast cancer. Breast cancer develops as a result of accumulation of genetic alterations. Therefore, intervention of these genetic alterations with gene therapy may successfully treat the disease. Various gene therapy

approaches such as proapoptosis, immune modulation, antiangiogenesis, and oncogene suppression have been developed [1–7]. Clinical trials with the gene therapy strategies are ongoing to evaluate their toxicities and efficiencies [8]. In addition, new technologies have been developed for breast cancer gene therapy. However, to move this laboratory technology to clinics, there are some problems that should be addressed. First, an efficient gene delivery system should be developed to increase efficiency and reduce toxicity. For this purpose, targeting delivery methods have been developed. Active targeting with specific ligands or passive targeting with enhanced permeability and retention (EPR) effect has been investigated for tumor targeting gene therapy [9–15]. Another important strategy is to target gene

[☆] This review is part of the *Advanced Drug Delivery Reviews* theme issue on “The Role of Gene- and Drug Delivery in Women's Health – Unmet Clinical Needs and Future Opportunities”.

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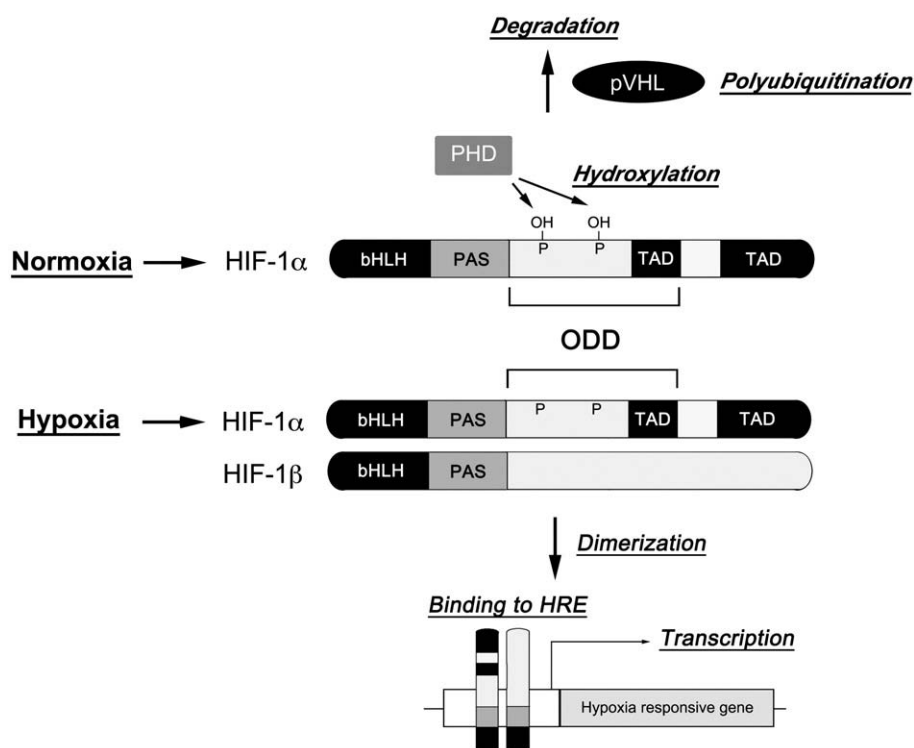


Fig. 1. Oxygen-dependent gene regulation by hypoxia inducible factor-1 α . Hypoxia inducible factor-1 α (HIF-1 α) is regulated by oxygen concentration. Under normoxia, HIF-1 α is degraded via pVHL mediated ubiquitin–proteasome pathway. Under hypoxia, HIF-1 α is dimerized with HIF-1 β and activates transcription. bHLH, basic helix–loop–helix domain; ODD, oxygen-dependent degradation domain; TAD, transactivation domain; PHD, prolyl hydroxylases.

expression. For breast cancer, tissue-specific promoters can be used to restrict gene expression to breast tumor tissue [16–19]. Various specific promoters have been evaluated for this purpose. For example, the whey acidic protein (WAP) promoter was suggested as a breast cancer specific promoter [16].

Another breast tumor specific targeting strategy is to use hypoxia. Solid tumors such as breast tumor have a hypoxic environment. In many solid tumors, the oxygen level is lower than normal tissues, due to poor vascularization in the rapidly growing tumor tissues. Angiogenesis is a process of the formation of new blood vessels from pre-existing microvessels. Especially, tumor growth and metastasis are dependent on angiogenesis. In hypoxia tumor tissues, hypoxia responsive genes are up-regulated to increase the local blood supply [20]. Therefore, tumor angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are up-regulated and promote neovascularization into the tumor tissue for more oxygen and nutrients supply [21]. This hypoxic condition can be utilized to induce therapeutic gene expression specifically in the tumor tissue. There are various possible strategies for induction of gene expression in hypoxic tissue. In this review, tumor hypoxia targeting strategies are discussed and their applications to breast cancer gene therapy are introduced. In combination with targeting gene delivery and tissue-specific promoters, hypoxia targeting gene expression will be useful for breast cancer specific gene therapy.

2. Gene expression regulation under hypoxia

The regulatory mechanism for hypoxia inducible gene expression is well conserved across the species [22]. The key regulator of gene expression under hypoxia is hypoxia inducible factor-1 (HIF-1). Under hypoxia, HIF-1 is accumulated and binds to hypoxia response elements (HREs) in the promoters of hypoxia inducible genes in a

sequence-specific manner (Fig. 1) [22–30]. HIF-1 is composed of two subunits [26]. HIF-1 β , also called aryl hydrocarbon receptor nuclear translocator (ARNT), is a constitutive subunit and exists at a stable level independent of oxygen concentration. HIF-1 α is the other subunit of HIF-1, whose concentration changes in response to oxygen concentration. HIF-1 α has three domains, which are a DNA binding domain, a transactivation domain, and an oxygen-dependent degradation (ODD) domain [24,31–33]. The ODD domain plays a key role in regulation of HIF-1 α stabilization. The ODD domain has proline residues, which are recognized by prolyl hydroxylases (PHDs) [33–35]. Under normoxia, PHDs are activated and attached to hydroxyl groups to specific prolyl groups of the ODD domain. Recent studies with anti-PHD siRNAs showed that knock-down of PHDs stabilized HIF-1 α , suggesting that prolyl hydroxylation is one of the key steps for the HIF-1 α degradation under normoxia [36]. The hydroxylated prolines are then recognized by von Hippel Lindau protein (pVHL), a ubiquitin ligase [33]. The ODD domain is multi-ubiquitinated by pVHL and the protein is degraded by the proteasome mediated pathway. However, the activities of PHDs are down-regulated under hypoxia, resulting in stabilizing HIF-1 α . Recent studies also suggested that there might be a HIF-1 independent regulatory pathway [37]. For example, some transcription factors such as Sp1 increase in their amounts under hypoxia and promote gene expression [38,39].

Post-transcriptional regulations are also suggested as a regulatory step for the production of proteins under hypoxia. Untranslated regions (UTRs) in the mRNA of hypoxia responsive genes have sequences that bind to specific proteins. For example, the erythropoietin (Epo) mRNA 3′-UTR binds to Epo RNA binding proteins (ERBPs) under hypoxia [40,41]. Due to the protein binding, the Epo 3′-UTR stabilizes the Epo mRNA under hypoxia [41–43]. In the previous report, the Epo 3′-UTR sequence was inserted into a vector downstream of chloramphenicol acetyl transferase (CAT) gene [41]. After transcription, the CAT-Epo 3′-UTR fusion mRNA was specifically stabilized and had a

longer half-life than the CAT mRNA without the UTR under hypoxia. Therefore, the Epo 3'-UTR stabilizes the linked mRNA, which is independent of the sequence of the linked mRNA. When ERBP binds to the Epo 3'-UTR, the half-life of mRNA increased approximately 40–50% depending on the exposure time to hypoxia. The VEGF mRNA also has a similar sequence in the 3'-UTR. Protein binding experiments showed that common proteins might bind to the Epo 3'-UTR and the VEGF 3'-UTR, suggesting a same RNA stabilization mechanism [42]. However, in the VEGF 3'-UTR, there are AT-rich elements that have destabilizing effects [44]. The mRNA stability with the VEGF 3'-UTR indicated that the 3'-UTR was ineffective in the mRNA stabilization. Stabilization of the VEGF mRNA may be due to cooperation of 5'-UTR, coding region and 3'-UTR [45].

3. Transcriptional regulation for tumor hypoxia targeting

3.1. Tumor hypoxia targeting transcriptional regulation systems

Transcriptional regulation of gene expression is the most widely used strategy for tumor hypoxia targeting. Hypoxia inducible promoters have been used for transcriptional regulation. The hypoxia inducible promoters originate from the regulatory regions of hypoxia responsive genes. Pioneering work in tumor hypoxia targeting was performed by Dachs et al. using HRE from the phosphoglycerate kinase-1 (PGK-1) promoter [46]. PGK-1 was induced by HIF-1 in various human cancers, suggesting that the PGK-1 promoter has the HIF-1 binding sites [27]. Indeed, the HRE of the PGK-1 gene is located in the 5'-flanking sequence of the gene. For tumor hypoxia targeting gene therapy, the HRE from the PGK-1 gene was inserted into the PGK-1, thymidine kinase (TK), or 9–3C promoter for the expression of cytosine deaminase (CD) toxin [46]. The HRE in the promoter confers hypoxia responsiveness to the promoter in gene expression. The *in vitro* study with the PGK-1 HRE showed that CD was induced in human fibrosarcoma cells under hypoxia. Therefore, sensitivity to prodrug, 5-fluorocytosine (5-FC), increased in the cells. This suggests that the HRE from the hypoxia inducible promoter can effectively induce gene expression in hypoxic tumor. The HRE from the PGK-1 promoter was also used for hypoxia induction of herpes simplex virus thymidine kinase (HSVtk) in tumor stroma [47]. In the study, 6 repeats of the HRE were inserted in the LTR of retrovirus at the place of the viral enhancer. The results showed that the PGK-1 HRE induced HSVtk expression in response to acute hypoxia in the stroma.

A similar approach was also performed using the VEGF promoter. VEGF expression is induced in hypoxic tissue for angiogenesis, which is regulated at transcriptional and post-transcriptional levels [45,48–50]. Previously, the fragment of the 5'-flanking sequence of the VEGF gene was evaluated for hypoxia inducible gene expression in human tumor cells under hypoxia [51]. Multiple copies of the HREs from the VEGF promoter were applied to the HSVtk gene therapy. *In vitro* application of the HRE from the VEGF gene increased HSVtk expression in hypoxic Lewis lung carcinoma cells, which made the cells more sensitive to prodrug, ganciclovir. In addition, *in vivo* animal experiment resulted in tumor regression, indicating the hypoxia targeting gene therapy, using the HRE from the VEGF gene, is properly working in hypoxic tumor site.

3.2. Application of hypoxia targeting transcriptional regulation systems to breast cancer gene therapy

Like the above examples in other types of cancers, the hypoxia targeting transcriptional systems have been applied to breast tumor gene therapy. Various HREs were tested in terms of specificity of gene expression [52]. Hypoxia targeting transcriptional systems for breast tumor gene therapy are summarized in Table 1. HREs from various hypoxia inducible genes were inserted upstream of the SV40 minimal promoter and the hypoxia responsiveness of the HRE–SV40 promoters

Table 1

Transcriptional targeting systems for breast tumor gene therapy.

Regulatory elements	Target gene	Reference
<i>Tumor hypoxia targeting gene expression</i>		
HRE from the PGK-1 gene	CYP2B6	[53]
HRE from the Epo gene	HSV-tk	[54]
HRE from the GAPDH gene	CAT	[55]
<i>Oncolytic virus</i>		
HRE from the VEGF gene	Ribonucleotide reductase	[56]
HRE from the VEGF gene	E1A	[57]
<i>Dual targeting gene expression</i>		
Estrogen response element + HRE from the PGK-1 gene	Harakiri	[58]
Survivin promoter + HRE from the VEGF gene	Caspase-3	[59]
The Epo E9 promoter + the WAP gene	EGFP	[60]

was tested in breast tumor cells. The most effective promoter combination was the HRE from the PGK-1 gene and the SV40 promoter. This optimized HRE was referred to as Oxford Biomedica HRE (OBHRE) and applied to human cytochrome p450 isoform 2B6 (CYP2B6) expression for breast tumor hypoxia targeting [53]. Intratumoral injection of adenoviral vector with the OBHRE regulated CYP2B6 showed tumor specific gene expression with therapeutic effect in combination with the prodrug, cyclophosphamide (CPA). OBHRE mediated gene expression reduced tumor volume and the efficiency of OBHRE in tumor was comparable to a strong viral promoter such as the CMV promoter. This confirmed that the hypoxia inducible promoter system was strong enough to have therapeutic efficacy for cancer gene therapy.

The HRE from the Epo gene was also used in the Cre/loxP 'molecular switch' for tumor gene therapy [54]. In human breast cancer cells, hypoxia activated the Epo HRE promoter and increased the Cre recombinase expression. Then, the expressed Cre recombinase eliminated 'the stop cassette', which was located between the CMV promoter and the HSVtk coding sequence for the inhibition of gene expression. As a result, the active HSVtk expression cassette was produced by recombination in an oxygen concentration dependent way. The HREs from the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were also evaluated for breast cancer specific gene expression [55]. In combination with the CMV promoter/enhancer, the GAPDH promoter/enhancer was highly active in breast cancer cells. In addition, gene expression by GAPDH promoter/enhancer was oxygen concentration dependent due to the HREs in the promoter. Therefore, the GAPDH promoter/enhancer had high specificity to hypoxic breast tumor.

Hypoxia inducible promoters are also useful for oncolytic viral vectors. The hypoxia response transcriptional regulatory system was constructed with the HREs from the VEGF gene [56]. The multimerized HREs increased gene expression in human breast cancer MCF7 cells under hypoxia, confirming hypoxia inducible gene expression. The HRE was then applied to regulation of ribonucleotide reductase (RR). RR is a rate-limiting enzyme for viral replication. Therefore, hypoxia inducible RR expression is required for viral replication specifically in hypoxic tumor cells. The application of the HREs to RR expression increased viral titer specifically in hypoxic MCF7 cells with a significant enhancement of oncolytic viral therapy. In another application, the HREs from the VEGF gene were applied to regulation of adenoviral E1A expression, which is essential for adenoviral replication in the E1B55 gene deleted adenovirus mutant [57]. It was suggested that the E1B55 gene deleted adenovirus mutant replicates specifically in tumor cells without p53 activity and lyses the tumor cells [57]. Therefore, hypoxia regulated E1A expression enables adenoviral vectors to replicate in hypoxic tumor cells. Intratumoral injection of the hypoxia regulated E1A adenoviral vector showed more tumor regression and a higher survival rate in the MDA-MB-435 breast cancer model, compared with the control vector injection.

3.3. Dual targeting transcriptional regulation systems

Dual targeting transcriptional regulation systems have been developed by combining hypoxia specific promoters with other types of transcription regulatory elements. For example, tissue-specific promoters can be combined with HREs for hypoxia and tissue-specific gene expression. A promoter system was constructed with the estrogen response element (ERE) and the HRE from the PGK-1 gene [58]. The ERE/HRE promoter enhanced gene expression in human breast cancer cells by estrogen and hypoxia. For breast cancer gene therapy, a therapeutic vector was constructed with a proapoptotic protein, harakiri and the ERE/HRE promoter. The transfection of the vector induced apoptotic cell death in an estrogen and hypoxia dependent manner.

Another example of the dual targeting system is the survivin promoter with the HRE from the VEGF promoter [59]. Survivin is an antiapoptotic protein and overexpressed in various human cancer cells, suggesting that the survivin promoter acts selectively in human cancer cells. In combination with HREs, the survivin promoter showed specific gene expression in tumor cells under hypoxia. Caspase-3 expression by the HRE-survivin promoter increased apoptotic cell death in human tumor cells.

Hypoxia- and radiation-inducible promoter is another example of dual targeting vector [60]. The Epo E9 enhancer has HRE and radiation response element, which are repeated 9 times in the enhancer. Therefore, the enhancer could induce gene expression by hypoxia and radiation. This dual functional promoter may be useful especially for the combination of radiation therapy with gene therapy. The Epo E9 enhancer elements were coupled to the breast cancer specific promoter from the whey acidic protein (WAP) gene [60]. The WAP promoter is active especially in breast tumors and the previous report showed that murine leukemia virus with the WAP promoter selectively expressed its target gene in mammary tumor cells [16]. The combination of the Epo enhancer with the WAP promoter confined gene expression in hypoxic breast tumor cells [60].

Various hypoxia inducible promoters have been identified and the promoters have their own characteristics. In addition, multimerization of HRE increases promoter activity and specificity. The combination with various types of promoters may improve the function and specificity of the transcriptional regulatory systems. Therefore, optimization of hypoxia inducible systems will be beneficial to gene therapy systems for breast cancer.

4. Tumor hypoxia targeting with a hypoxia specific transcription factor

Tumor specific or hypoxia specific promoters have relatively low transcription activity compared with the strong viral promoters such as cytomegalovirus (CMV) promoter and simian virus 40 (SV40) promoter. To increase gene therapy efficiency, two-step transcription amplification (TSTA) systems have been developed for higher promoter activity of hypoxia specific promoters. TSTA systems are composed of two expression units (Fig. 2). The first unit expresses an artificial transcription factor under the control of a hypoxia specific promoter. Therefore, the artificial transcription factor is induced under hypoxia condition. The artificial transcription factor has the Gal4 DNA binding domain (Gal4DBD) and the p65 transactivation domain (p65TAD). The expressed transcription factor then binds to the Gal4 binding sites of the upstream activating sequence (UAS) of the second expression unit. The therapeutic gene in the second expression unit is expressed under the control of UAS. Therefore, the therapeutic gene expression is induced by the artificial transcription factor under hypoxia condition. Previously, TSTA system with HRE and the SV40 basal promoter was evaluated for hypoxia specific gene expression amplification. Gene expression under hypoxia was induced by more than 100 times compared with that under normoxia [61–63].

HiF-1 α has the ODD domain, which promotes degradation of the protein under normoxia. A TSTA system with the ODD domain was developed for hypoxia targeting gene expression [64–66]. In this TSTA system, the ODD domain coding sequence was inserted between Gal4DBD and p65TAD (Fig. 3). This artificial transcription factor is expressed under the control of tissue-specific or hypoxic inducible promoters. The expressed transcription factor is stable under hypoxia while it is degraded rapidly under normoxia through ubiquitin-proteasome pathway. Under hypoxia, the transcription factor is accumulated and binds to its target sequence of the therapeutic gene expression vector, Gal4 upstream activating sequence (UAS). As a result, the target gene expression is induced specifically in the tumor hypoxic region. For the expression of artificial transcription factor, breast cancer specific promoter can be integrated in the transcription factor expression vector, which confers dual specificity to the gene expression system.

The TSTA system has been used to amplify transcription activity of the tissue-specific promoter [61–63]. Usually, the tissue-specific promoters have weak activity compared with the conventional viral

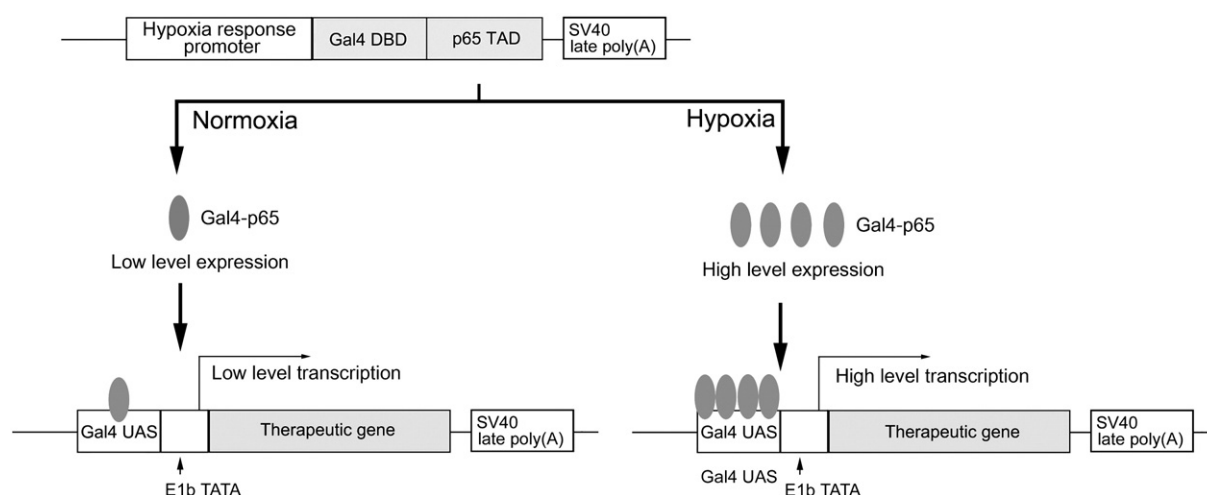


Fig. 2. Two-step transcription amplification system with the hypoxia response promoter. Two-step transcription amplification (TSTA) system has been developed to enhance the transcriptional activity of the hypoxia response promoter. An artificial transcription factor, Gal4–p65 is produced under the control of hypoxia response promoter. Under hypoxia, Gal4–p65 is induced and binds to Gal4 UAS region of the second expression unit, which increases the expression of therapeutic gene.

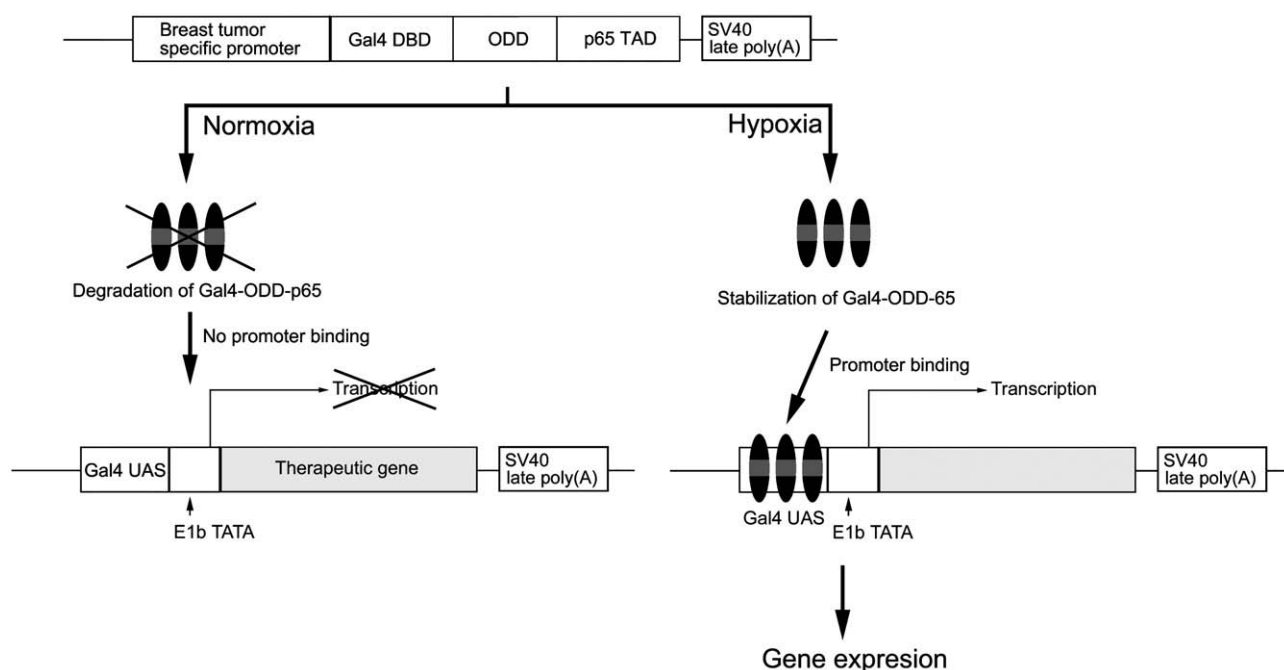


Fig. 3. Two-step transcription amplification system with the ODD domain for breast cancer targeting gene therapy. A hypoxia specific artificial transcription factor, Gal4-ODD-p65 is expressed by a breast tumor specific promoter. The Gal4-ODD-p65 is degraded under normoxia via ubiquitin–proteasome pathway. Under hypoxia, Gal4-ODD-p65 is accumulated and activates the transcription of a target therapeutic gene.

promoters such as the CMV or SV40 promoters. The weak activity of the tissue-specific promoters should be improved, since gene expression by the tissue-specific promoter is usually not enough for therapeutic effect. Gal4 is a yeast transcription factor and highly specific to Gal4 binding site in mammalian cells. Also, the p65 transactivation domain originates from NF- κ B and has strong transactivation effects. Therefore, target gene expression by the artificial transcription factor in the TSTA system is much higher than the direct gene expression system from the tissue-specific promoter. The previous report showed that the TSTA system increased gene expression level by several hundred times compared with the direct expression vector [61]. Therefore, the TSTA system with the ODD domain has the advantage of strong gene expression as well as hypoxia and tissue-specific expression. The TSTA system with the ODD domain has been evaluated for ischemic disease such as ischemic myocardium and the results showed highly specific gene expression [64–66]. By combining the breast tumor specific promoter in the Gal4-ODD-p65 expression vector, the TSTA system will be useful for hypoxia breast cancer targeting gene therapy.

5. Post-transcriptional regulation for tumor hypoxia targeting

Post-transcriptional regulation is mainly achieved by regulation of the mRNA stability. For the hypoxia targeting gene expression, mRNA stability can be regulated by specific UTRs. It was previously reported that the Epo 3'-UTR bound to the stabilizing proteins such as ERBP under hypoxia and increased steady-state level of the mRNA [40,41]. The increase of the mRNA level resulted in up-regulation of translation. In our study, the luciferase or VEGF gene linked to the Epo 3'-UTR expressed approximately 10 times more proteins under hypoxia than normoxia *in vitro* [67]. Also, the Epo 3'-UTR increased the gene expression level in the ischemic spine *in vivo* [68]. Although direct application of the Epo 3'-UTR to breast tumor targeting was not reported, the Epo 3'-UTR in combination with the breast cancer specific promoters will be useful to increase gene expression in hypoxic breast cancer.

The VEGF 5'- or 3'-UTR also has potential to be used as a regulator of the mRNA stability [42]. It was previously reported that the VEGF

3'-UTR bound common proteins to the Epo 3'-UTR under hypoxia [42]. However, the VEGF 3'-UTR did not increase the VEGF mRNA stability under hypoxia [44]. The stability of the VEGF mRNA seems to be regulated by interaction of 5'-, 3'-UTR and coding region [45]. The HIF-1 α mRNA 5'-UTR was also found to increase the mRNA level under hypoxia [69]. This stabilization of the HIF-1 α mRNA may partly contribute to rapid increase of HIF-1 α under hypoxia. Iron responsive element (IRE) was also reported to be involved in the stabilization of mRNA under hypoxia [70]. The transferrin receptor 3'-UTR, the ferritin 5'-UTR and the mitochondrial aconitase 5'-UTR have IRE and may be involved in the mRNA stability under hypoxia. However, further elucidation of the mRNA stabilization mechanism by UTR is required for application of the various UTRs to hypoxic tumor targeting gene therapy.

It has been reported that some microRNAs (miRNAs) are involved in gene expression regulation under hypoxia. miRNAs silenced gene expression by the degradation of target mRNAs. Hypoxia inducible miRNAs may be involved in tumor progress, since the hypoxic inducible miRNAs are overexpressed in tumor tissues [71,72]. Therefore, hypoxia inducible miRNAs may be targets of cancer gene therapy. Antisense oligonucleotide technology has been used to inhibit the function of miRNAs [73–75]. The antisense oligonucleotides will bind to complementary miRNAs to reduce the binding of miRNAs to their target mRNAs. For efficient inhibition of miRNAs, antisense oligonucleotides should have higher affinity to its complementary miRNAs than the target mRNAs. Locked nucleic acids (LNAs) have been developed, which are bicyclic RNA analogs. LNAs have high affinity to their complementary mRNAs. However, further investigations are required to identify the gene regulatory network of the hypoxia inducible miRNAs and their expression patterns in specific cancers.

6. Post-translational regulation for tumor hypoxia targeting

Post-translational regulation is related to protein stability. The therapeutic protein stability in response to hypoxia can be regulated by using the HIF-1 α ODD domain. In the previous report, the ODD domain coding sequence was inserted upstream or downstream of luciferase gene [76,77]. The ODD domain linked to luciferase decreased

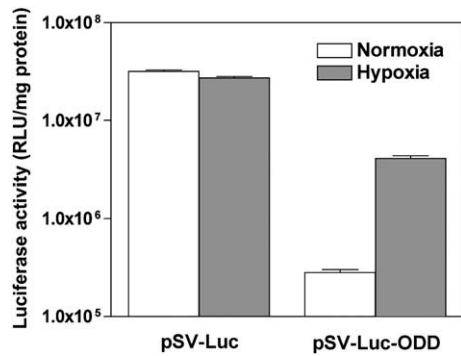


Fig. 4. Post-translational regulation of gene expression using the oxygen-dependent degradation domain. pSV-Luc is a luciferase plasmid, in which luciferase expression is controlled by the SV40 promoter. The oxygen-dependent degradation (ODD) domain was inserted downstream of the luciferase cDNA of pSV-Luc for construction of pSV-Luc-ODD. pSV-Luc and pSV-Luc-ODD were transfected into 293 cells and the cells were incubated under normoxia or hypoxia. The results showed that the ODD domain facilitates degradation of the luciferase protein under normoxia condition.

the steady-state level of luciferase significantly under normoxia, compared with hypoxia (Fig. 4). This suggests that the ODD fusion protein was degraded through ubiquitin–proteasome pathway under normoxia. When post-translational regulation with the ODD domain was combined with transcriptional regulation with HREs, gene expression was highly specific in hypoxic cells, showing more than 1,000 times induction in Neuro2A cells under hypoxia [76]. The combination of the HREs and the ODD domain enables real-time imaging of the HIF-1 activity in tumor [77].

For cancer therapy, a model fusion protein was produced with HIV-TAT, the ODD domain and caspase-3 [78]. The TAT-ODD-caspase-3 fusion protein was stabilized in hypoxic human pancreatic tumor cells, while it was rapidly degraded in normoxic cells. The fusion protein effectively reduced tumor size when injected into tumor-bearing mice. In other research, the ODD domain was fused to diphtheria toxin A (DT-A) [79]. After gene transfer into Lewis lung carcinoma cells, the expressed DT-A/ODD protein was rapidly degraded under normoxia through ubiquitin–proteasome pathway, minimizing cytotoxicity of the protein. However, the stability of the protein increased under hypoxia and induced apoptosis of the cells. Fusion with the ODD domain may alter the property of the therapeutic protein. Therefore,

the fusion protein should be carefully evaluated in terms of safety as well as efficiency. However, as proved in previous reports, the post-translational regulation with the ODD domain will be a useful strategy for hypoxic breast cancer specific gene therapy.

7. Conclusion

Hypoxia targeting gene regulation is one of the useful strategies for gene therapies of solid tumors including breast cancer. As discussed above, hypoxia targeting can be achieved by various methods. Hypoxia itself is an excellent target, but combination of various regulatory strategies will be more useful to minimize side effects and maximize efficacy (Fig. 5). First, hypoxia inducible systems are positive regulatory systems under hypoxia. However, hypoxia inducible systems have leaky basal level expression in normal tissue, since hypoxia inducible promoters usually have basal level transcription activity under normoxia. Therefore, a negative regulatory system under normoxia will be beneficial to reduce gene expression safely. A post-translational regulatory system such as the ODD fusion protein is a useful strategy to reduce the basal level expression. The combination of positive and negative regulatory systems will increase safety of the gene therapy system. Second, a hypoxia regulatory system should be combined with a tissue-specific regulatory system for higher specificity. The combination hypoxia specific expression system with the breast tissue-specific promoters will be effective to confine gene expression to a target region by dual targeting. In conclusion, the combination of transcriptional, post-transcriptional, and post-translational regulations will be an effective strategy for safe and efficient breast cancer gene therapy. The application of the various hypoxia targeting approaches to breast cancer gene therapy was not fully evaluated. Further investigation is required for full assessment of the regulatory systems and their combination. However, considering efficiency, specificity, and self-regulating property of the systems, the hypoxia targeting expression systems in combination with the breast cancer specific promoters will be useful to eventually treat breast cancer with gene therapy.

Acknowledgment

This work was supported by grants from World Class University Project, Ministry of Education, Science and Technology, Republic of Korea (grant no. 200900000000024).

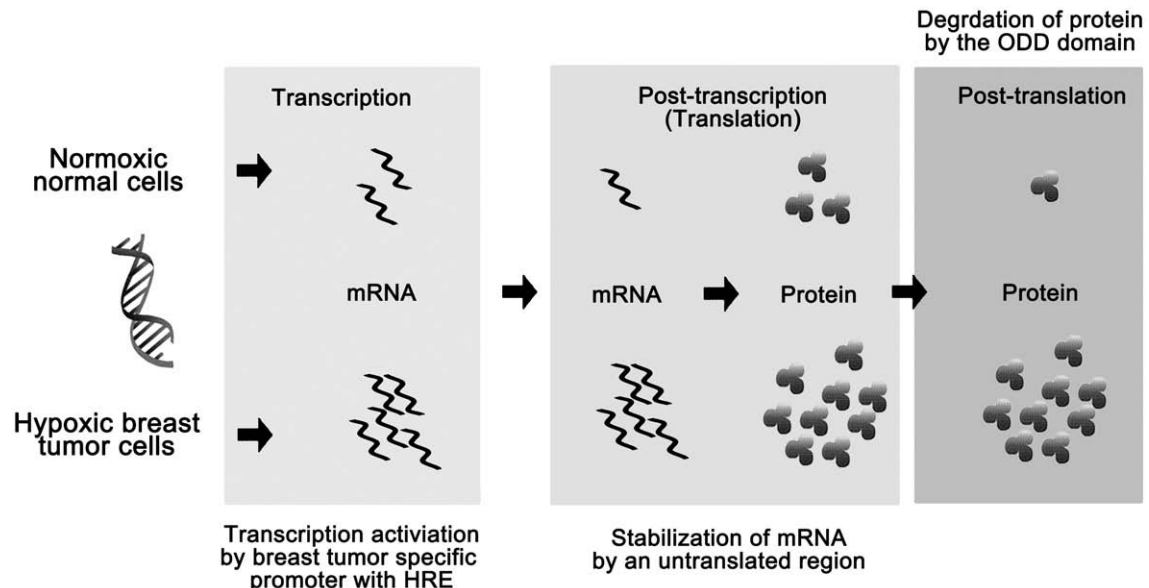


Fig. 5. Hypoxic breast cancer targeting gene expression system. Gene expression is regulated at the transcriptional, post-transcriptional and post-translational steps.

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