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Hypoxia-specific gene expression for ischemic disease gene therapy ☆

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ABSTRACT

Gene therapy for ischemic diseases has been developed with various growth factors and anti-apoptotic genes. However, non-specific expression of therapeutic genes may induce deleterious side effects such as tumor formation. Hypoxia-specific regulatory systems can be used to regulate transgene expression in hypoxic tissues, in which gene expression is induced in ischemic tissues, but reduced in normal tissues by transcriptional, translational or post-translational regulation. Since hypoxia-inducible factor 1 (HIF-1) activates transcription of genes in hypoxic tissues, it can play an important role in the prevention of myocardial and cerebral ischemia. Hypoxia-specific promoters including HIF-1 binding sites have been used for transcriptional regulation of therapeutic genes. Also, hypoxia-specific untranslated regions (UTRs) and oxygen dependent degradation (ODD) domains have been investigated for translational and post-translational regulations, respectively. Hypoxia-specific gene expression systems have been applied to various ischemic disease models, including ischemic myocardium, stroke, and injured spinal cord. This review examines the current status and future challenges of hypoxia-specific systems for safe and effective gene therapy of ischemic diseases.

Contents

1. Introduction

Hypoxia is a pathological condition that deprives adequate oxygen supply to organs or tissues and thus it regulates many cellular processes. Hypoxia has been implicated in many disease processes, including ischemic brain, ischemic myocardium, and injured spinal cord. In ischemic heart disease, the coronary arteries are narrowed and the blood supply to the myocardium is decreased. Due to low blood supply, oxygen and nutrients concentrations are not enough to maintain normal heart function. Decreased oxygen concentration activates hypoxia-inducible genes such as vascular endothelial growth factor (VEGF) [1–8]. Many gene products induced under hypoxia can protect cells from apoptosis and recover blood supply through neovascularization [9,10].

Hypoxia is a physiological signal for specific types of growth factors, anti-apoptotic and angiogenic genes. In stroke, hypoxia is an important

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hallmark of disease state. Blocking of cerebral artery causes low oxygen concentration in the brain. The ischemic brain undergoes physiological responses, which are similar to ischemic myocardium such as induction of protective factors and angiogenic factors [11–13]. In the case of stroke, blood supply must be recovered immediately. After reperfusion, blood supply is not fully recovered and brain cells are still under hypoxia, since microvessels are damaged during ischemia and reperfusion [14,15]. Due to reperfusion injury, the brain undergoes secondary injury by excitotoxic neurotransmitters and infarction area increases with time [16]. In spinal cord injury, the spinal cord also undergoes secondary injury from excitotoxicity and hypoxia [17].

Tumor hypoxia is a common feature to most solid tumors due to malformed vasculature and inadequate perfusion [18–20]. Tumor growth relies on the formation of new blood vessels, and in this process, several angiogenic factors including VEGF are induced [21,22]. In addition, severe hypoxia in the core of tumor often induces necrosis of the cells. Recently, it was suggested that high mobility group box-1 (HMGB-1) was released from the necrotic tissues [23]. Inside cells, HMGB-1 is a nuclear protein involved in gene regulation [24], while outside the cells, it is a cytokine, which binds to tall like receptors of infiltrating immune cells and induces nuclear factor-kappa B (NF-κB) [23,25–27]. This process increases VEGF gene expression in the tumor and promotes angiogenesis.

Hypoxia is an important factor determining overall efficiency of tissue transplantation. Isolated islets from a donor are subjected to hypoxia due to the disruption of islet microvasculature during isolation from the pancreas. The hypoxic condition causes the islets to undergo apoptosis [28-30]. In addition, after transplantation, the blood supply to the islets is not sufficient for a significant period until new blood vessels are formed and oxygen supply is recovered. For this reason, islets after transplantation in diabetic patients suffer from high rates of cell death due to hypoxic damage [28-30].

Non-specific gene expression may induce deleterious effects and thus gene expression should be regulated. There are several reports on severe side effects when VEGF gene therapy is used for treating ischemic diseases [31]. VEGF is a potent angiogenic factor [4,32-39], and endogenous VEGF and its receptors are induced in the ischemic tissues [10]. However, this endogenous response to ischemic condition is usually not enough to recover normal state. Therefore, exogenous VEGF gene delivery may be beneficial for treating ischemic diseases. Since VEGF receptor is up-regulated in ischemic tissue, but not in normal tissue [10], non-specific VEGF expression may have minimal effect on normal tissue. Since unregulated VEGF-mediated angiogenesis has the potential to promote tumor growth, accelerate diabetic proliferative retinopathy, and promote rupture of atherosclerotic plaque. [31,40], VEGF gene expression should be regulated.

Targeted gene therapy can be achieved by two approaches. One approach is site-specific gene delivery by conjugating targeting ligands to gene carriers. The other approach to achieve targeted gene therapy is to regulate transcription with a cell-specific promoter. Several endogenous genes are regulated by hypoxia-specific transcription factors. Among them, hypoxia-inducible factor-1 (HIF-1) [7,41] is the key transcription factor, which binds to hypoxia response elements (HREs) and induces transcription in a hypoxic environment [1,42]. In addition to transcriptional regulation, post-transcriptional regulation or post-translational regulation strategies have been employed for hypoxia-inducible gene therapy. However, promoters specific to certain cell types or dependent on certain physiological condition usually have low promoter activity. Also, there are leaky expressions by hypoxia-specific promoters in normal tissue [43,44], possibly due to the basal promoter activity of these promoters. Various approaches have been taken to overcome these obstacles.

In this review, basic regulatory mechanisms of hypoxia-specific gene expression are introduced and their applications to ischemic gene therapy are discussed.

2. Hypoxia-regulated gene expression

2.1. Transcriptional regulation

Several genes are induced in response to hypoxia at the transcriptional level. The most important transcription factor for hypoxia responsive gene expression is HIF-1 [1,6,7,12,45,46], which binds to specific cis-regulatory elements in promoters and facilitates transcription under hypoxic condition [1,7,47,48]. HIF-1 specific cis-regulatory elements are hypoxia response elements (HREs) and have a consensus sequence of (G/C/T)ACGTGC(G/C). Most hypoxia-inducible genes have multiple copies of HREs at their 5′-regulatory regions. HIF-1 is a heterodimeric protein composed of two subunits, HIF-1α, which is accumulated under hypoxic conditions, and HIF-1β, which is a constitutive subunit [45]. The level of HIF-1β is stable regardless of tissue oxygen concentration. However, HIF-1α levels change rapidly in response to oxygen concentration. The regulation of HIF-1α levels is an important regulatory step for hypoxia-specific gene expression. HIF-1α is specifically stabilized under hypoxic conditions and degraded rapidly under normoxia [49], a behavior that is mediated by the ubiquitin–proteasome pathway [40,49–52].

HIF-1α is hydroxylated by proline hydroxylases (PHDs), which are specifically activated under normoxia [49,50,53-56]. PHDs recognize specific proline residues in the oxygen dependent degradation (ODD) domain of HIF-1α. These hydroxylated prolines in the ODD domain are then recognized by the von Hippel Lindau tumor suppressor protein (pVHL). pVHL contains E3 ubiquitin ligase activity and polyubiquitnates the ODD domain. The proteasome-mediated pathway degrades the polyubiquitinated HIF-1α. However, PHDs are rapidly deactivated under hypoxia, which in turn stabilizes HIF-1α. Therefore, the accumulation of HIF-1α activates the transcription of specific genes under hypoxia. The HIF-1α level is also up-regulated by the stabilization of HIF-1α mRNA under hypoxia [57]. The half-life of the HIF-1α mRNA is higher under hypoxia than normoxia, which increases the translational level of the protein. As a result, the increased level of HIF-1α activates the transcription of hypoxia-inducible protein.

Other transcription factors are also involved in hypoxia-inducible gene expression. For an example, stimulating protein-1 (SP-1) is a sequence-specific DNA-binding protein and is up-regulated under hypoxia condition [58–62]. Thus, Sp1 has been implicated in the regulation of various genes, since its binding sites are a recurrent motif in regulatory sequences of the hypoxia-inducible genes. Cyclooxygenease-2 (COX-2) or RTP801 was induced by Sp1 under hypoxia [58]. However, it is likely that Sp1 activates gene expression in cooperation with HIF-1. For example, the RTP801 and endoglin promoters have Sp1 and HIF-1 binding sites. It was suggested that Sp1 forms multiprotein complex with Smad3 and HIF-1, in which Smad3 is a coactivator and adaptor protein. Sp1–Smad3–HIF-1 complex on the promoter may cooperate to induce gene expression [59]. Hypoxic regulation of genes by transcription factors other than HIF-1 is not fully understood. However, advances in molecular biology for hypoxia-inducible gene expression may make it possible to develop more sophisticated regulatory systems for hypoxia-specific gene therapy.

2.2. Post-transcriptional regulation

Post-transcriptional regulation can be achieved by controlling mRNA stability. For example, the erythropoietin (Epo) mRNA has prolonged half-life in hypoxic cells [8,63]. The up-regulation of Epo expression is mediated mainly by transcriptional regulation [64]. The Epo enhancer has HREs and the transcription level was induced by HIF-1 under hypoxia. An Epo mRNA binding peptide (ERBP) binds to the AT-rich region of the Epo 3′-UTR under hypoxia, which increases the stability and steady-state level of the Epo mRNA [8]. As a result, the translation level of the mRNA increases, producing more protein. This hypoxia-specific stabilization is found in various genes. For example,