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Regulatory systems for hypoxia-inducible gene expression in ischemic heart disease gene therapy ☆

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ABSTRACT

Ischemic heart diseases are caused by narrowed coronary arteries that decrease the blood supply to the myocardium. In the ischemic myocardium, hypoxia-responsive genes are up-regulated by hypoxia-inducible factor-1 (HIF-1). Gene therapy for ischemic heart diseases uses genes encoding angiogenic growth factors and anti-apoptotic proteins as therapeutic genes. These genes increase blood supply into the myocardium by angiogenesis and protect cardiomyocytes from cell death. However, non-specific expression of these genes in normal tissues may be harmful, since growth factors and anti-apoptotic proteins may induce tumor growth. Therefore, tight gene regulation is required to limit gene expression to ischemic tissues, to avoid unwanted side effects. For this purpose, various gene expression strategies have been developed for ischemic-specific gene expression. Transcriptional, post-transcriptional, and post-translational regulatory strategies have been developed and evaluated in ischemic heart disease animal models. The regulatory systems can limit therapeutic gene expression to ischemic tissues and increase the efficiency of gene therapy. In this review, recent progresses in ischemic-specific gene expression systems are presented, and their applications to ischemic heart diseases are discussed.

Contents

1. Introduction

Ischemia is the lack of oxygen from inadequate blood supply. Ischemic heart disease is a coronary disease characterized by ischemia to the heart muscle, usually due to coronary artery disease. Ischemic heart disease is one of the leading causes of death, with approximately

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7.2 million deaths worldwide caused by heart disease in 2004, which represented 12.2% of all deaths [1]. It is the most common cause of death in most western countries, and a major cause of hospital admissions. Few therapeutic options are available to treat ischemic heart diseases, although angioplasty is commonly used [2]. Although blood flow to ischemic tissue is recovered by the treatment, ischemia–reperfusion causes damage to the treated regions, suggesting that the tissue ischemia is not completely resolved [3,4]. Furthermore, blood vessels are often re-blocked by restenosis [5]. Thus, new therapeutic methods must be developed for the effective treatment of ischemic heart disease.

Gene therapy has been suggested as an efficient treatment of ischemic heart disease, ever since the first clinical trial [2,6]. However, for gene therapy of ischemic heart diseases, two important issues must be resolved. First, an effective gene delivery carrier must be developed. Naked DNA and adenoviral vectors have been used as gene carriers for ischemic heart gene therapy [6–9], but the intrinsic immunogenicity of adenoviral vectors has limited their clinical application to ischemic heart diseases [10]. Many studies have tried naked DNA in ischemic heart gene therapy. However, naked DNA does not seem to achieve the gene expression levels sufficient to produce a therapeutic effect [6]. Therefore, more efficient and safer gene carriers need to be developed. Non-viral carriers such as TerplexDNA or cholesterol-conjugated polyethylenimine (PEI) have been evaluated as gene carriers to the ischemic myocardium [11–13]. The advantages of non-viral carriers are lack of immunogenicity and moderate gene delivery efficiency [10,14]. Second, an effective therapeutic gene must be developed that provides a high therapeutic effect. For ischemic heart disease gene therapies, two kinds of therapeutic genes have been used. One is the angiogenic growth factor genes. Growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) have been widely used for ischemic heart disease gene therapy [15–18]. Growth factors increase the blood capillary density in the ischemic region and protect cells from cell death. In ischemic tissues, endogenous VEGF is induced by hypoxia-inducible transcription factors [19]. However, the expression levels are not sufficient to recover blood supply to ischemic tissue [20]. Therefore, the delivery of therapeutic genes can increase the expression of growth factors and augment the effect of the endogenous growth factors. The second group of therapeutic genes are anti-apoptotic genes such as Bcl-2 and heme oxygenase-1 (HO-1) [21–23]. Anti-apoptotic proteins protect cells in the ischemic myocardium from cell death caused by hypoxic insult or ischemia–reperfusion injury. Recently, anti-apoptotic small interfering RNAs (siRNAs) or micro-RNAs (miRNAs) such as Fas siRNA have been suggested to protect cells in the myocardium from cell death [24,25].

Gene therapy with the therapeutic genes, however, may cause deleterious side effects. Genes injected into the ischemic sites may leak into the bloodstream and be non-specifically transfected into unwanted cells. Non-specific expression of the therapeutic genes may cause uncontrolled cell proliferation and tumor formation at the expression sites. Indeed, non-specific expression of VEGF was shown to cause endothelial cell derived intramural vascular tumors [26]. VEGF receptors were suggested to be over-expressed in ischemic tissues and therefore, one solution could be maintaining the concentration of the VEGF receptors at minimal levels in normal tissue [27,28]. In that case, infusion of VEGF might induce angiogenesis only in ischemic tissues, because of the low level of the VEGF receptors in normal tissues. However, this was shown to not be the case, when VEGF function in normal tissues caused over-proliferation of endothelial cells [29]. Therefore, to avoid deleterious effects, delivery and expression of growth factors and anti-apoptotic genes must be specific to ischemic tissues.

Gene expression can be regulated at transcriptional, translational or post-translational levels. Because of extensive knowledge about gene transcription, most studies on hypoxia-specific gene expression have investigated transcriptional regulation using hypoxia-inducible promoters. However, with increasing information about translational and post-translation gene regulation, more studies have focused on post-transcriptional gene regulation. In this review, gene regulation systems at the transcriptional, translational, and post-translational levels are described for ischemic heart disease gene therapy.

2. Physiology of ischemic heart disease

Coronary circulation is responsible for the circulation in the blood vessels of myocardium. Two major coronary arteries branch off from the aorta near the point where the aorta and the left ventricle meet. Although anatomically, the left and right coronary arteries are considered arteries, they are functionally end arteries that supply all parts of the heart muscle with blood. The left coronary artery is divided into the left anterior descending artery and the circumflex artery. The left anterior descending artery supplies blood to the front side, the bottom of the left ventricle, and the front of the septum. The circumflex branch supplies blood to the left atrium and the left and backsides of the left ventricle. The right coronary artery supplies blood to the right atrium, right ventricle, the lower part of the left ventricle and the back of the septum. The septum is the tissue that separates the left and right sides of the heart. Therefore, closure of the right coronary artery causes damage to the right atrium, right ventricle, the lower part of the left ventricle, and the back of the septum.

The disease process underlying most ischemic heart disease is atherosclerosis of the coronary arteries. Atherosclerosis is a chronic multi-factorial disease triggered by a combination of genetic and environmental factors [30]. There are several reports on a role for genetic factors in the pathogenesis of stroke. These include studies of twins [31] and familial aggregation [32]. Sacco et al. had reported both environmental and genetic risk factors for ischemic stroke [33]. Risk factors for ischemic stroke include hypertension, cardiac disease, sickle cell disease, and hyperhomocysteinemia. Age is the most important modifiable risk factor of stroke [33].

Clinically, atherosclerosis is the buildup of fats in and on artery walls (plaques) that can restrict blood flow. It is followed by the infiltration of activated inflammatory cells from the coronary circulation into the arterial wall [34]. This may lead to stable angina pectoris (narrowed arteries), or acute coronary syndrome (ACS) which is usually one of three diseases involving the coronary arteries: ST elevation myocardial infarction (30%), non-ST elevation myocardial infarction (25%), or unstable angina (38%) [35].

2.1. Angina pectoris

Angina pectoris, commonly known as angina, is severe chest pain caused by ischemia of the heart muscle, generally caused by obstruction or spasm of the coronary arteries. Three major variants of angina pectoris are recognized: typical (stable) angina pectoris, Prinzmetal's (variant) angina pectoris, and unstable angina pectoris. Typical angina pectoris is the most common form and is characterized by electrocardiographic ST segment depression, which appears as left ventricular subendocardial hemorrhage. Stable angina pectoris occurs when there is regional myocardial ischemia caused by inadequate coronary perfusion and is usually but not always induced by increases in myocardial oxygen requirements [36]. Angina due to spasm, also called Prinzmetal's variant angina, is defined as ST segment elevation accompanying angina at rest caused by transmural myocardial ischemia [37]. Unstable or crescendo angina is a pattern of chest pain that occurs with progressively increasing frequency, is precipitated with progressively less effort, often occurs at rest, and tends to be of a prolonged duration. Unstable angina as a premonitory symptom of myocardial infarction is usually caused by atherosclerotic plaque rupture and vascular contraction [38].