Review

Variations of Human Adrenomedullin Gene and Its Relation to Cardiovascular Diseases

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The studies concerning the structure and variations of the human adrenomedullin (AM) gene are reviewed, and their relations to the gene function and genetic predisposition to cardiovascular diseases are discussed. The genomic human AM gene is composed of four exons, and the whole nucleotide sequence corresponding to mature AM resides in the fourth exon. In chromosomal sublocalization, the AM gene is located in the distal portion of the short arm of chromosome 11 (11p15.1-3). Analysis of the promoter region of the AM gene has revealed that two transcription factors, nuclear factor for interleukin-6 expression (NF-IL6) and activator protein 2 (AP-2), participate in the regulation of AM gene expression. It is surmised that NF-IL6 mediates inflammatory stimuli and AP-2 mediates signals of phospholipase C and protein kinase C activation. In addition to these factors, hypoxia induces AM gene expression via the hypoxia inducible factor-1 (HIF-1) binding site. The 3'-end of the AM gene is flanked by a microsatellite marker of cytosine adenine (CA) repeats. In Japanese, there are four types of alleles with different CA-repeat numbers: 11, 13, 14 and 19. It is suggested that existence of the 19-repeat allele is associated with genetic predispositions to develop essential hypertension and diabetic nephropathy. (Hypertens Res 2003; 26 (Suppl): S129–S134)

Key Words: adrenomedullin, gene transcription, gene polymorphism, hypertension, diabetic nephropathy

Introduction

Adrenomedullin (AM) is a vasodilator peptide originally discovered in pheochromocytoma tissue by Kitamura et al. (1). However, the mRNA of AM has been shown to be expressed not only in the adrenal gland but also in various cardiovascular organs, such as the heart, kidney and blood vessels (2). Furthermore, it has been demonstrated that a significant level of AM exists in the circulating human plasma (3). We have also reported that plasma AM levels are increased in patients with various cardiovascular diseases, such as hypertension, heart failure and renal failure (4, 5).

In order to clarify the roles of AM in the cardiovascular system, it is essential to analyze the structure and the function of the human AM gene. In particular, analysis of the 5'-flanking region of the genomic gene should provide information on the regulatory mechanism of the gene transcription. In addition, if there is inter-individual variation in the DNA nucleotide sequences of the human AM gene, such variation may be related to the function of the AM gene and the pathogenesis of cardiovascular disorders. In this review, we survey the results of studies on the human AM gene and its relation to cardiovascular diseases, and outline the possible pathophysiological implications of AM in the cardiovascular system.

Genomic Structure of the Human AM Gene

The cDNA sequence of the human AM gene was reported by Kitamura et al. (2). We previously cloned the genomic DNA encoding the human AM gene from a human liver genomic library constructed in charon 4A λ phages by the plaque hybridization method using human AM cDNA as the probe...
The DNA nucleotide sequence was then determined by a dideoxynucleotide chain terminating method and urea-polyacrylamide gel electrophoresis. The gene is composed of four exons interposed by three introns. The whole nucleotide sequence corresponding to the 52 amino acid residues of the mature AM is included in the fourth exon, and the nucleotides of proadrenomedullin N-terminal 20 peptide (PAMP), another bioactive peptide produced from AM mRNA, are spread over the second and third exons.

We have performed Southern blot analysis of the DNA from 20 human-hamster somatic cell lines carrying certain human chromosomes using the cloned genomic DNA fragment encoding the human AM gene as a probe. The labeled probe DNA was hybridized only with the DNA from cell lines containing human chromosome 11. It was thus determined that the human AM gene is localized on chromosome 11. Then, we carried out further chromosomal sublocalization of the human AM gene using the fluorescence in situ hybridization (FISH) technique. The cultured human lymphocytes treated with 5-bromo-2-deoxyuridine (BrdU) for synchronization were fixed on slides and hybridized with a fluorescence-labeled genomic DNA fragment encoding the full-length human AM gene. The fluorescent signal from the probe was detected on the distal end of the short arm of chromosome 11. Thus, the human AM gene was localized on chromosome 11 region p15.1-3, in close proximity to several other known genes, such as sphingomyelinase (p15.1-4), parathyroid hormone (p15.1-2) and lactate dehydrogenase (p14-15.1).

### Transcriptional Regulation of the Human AM Gene

In addition to the adrenal gland, the mRNA of AM is widely expressed in cardiovascular tissues. Cultured cells such as fibroblasts, vascular endothelial cells and smooth muscle cells have been shown to express AM mRNA prominently. We examined the promoter activity of the human AM gene in cultured human aortic endothelial cells (HAEC) (15).

First, the transcription start site was determined by the primer extension method using total RNA extracted from HAEC as the template. In this way, two transcription start sites were identified, both at the cytosine nucleotides. These are 21 and 25 bases downstream from the TATA box, and the nucleotides of proadrenomedullin N-terminal 20 peptide (PAMP), another bioactive peptide produced from AM mRNA, are spread over the second and third exons.

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gene transcription, Nakayama et al. have also reported that phorbol ester induces AM gene expression in human monocytic leukemia cells, and the cis-acting region (−70 to −30) containing multiple AP-2 binding sites is necessary for this induction (16). As already mentioned, plasma AM is increased in patients with hypertension, heart failure and renal failure (4, 5, 17). In the process of cardiovascular disease development, cardiovascular neuroendocrine systems such as the sympathetic nerve system and renin-angiotensin system are activated. Stimulations of α1-adrenergic receptor and type 1 angiotensin II receptor both elicit activation of phospholipase C and protein kinase C and consequently induce AP-2. This pathway may be involved in the mechanism of increased plasma AM in various cardiovascular diseases. On the other hand, Barker et al. have suggested that a protein kinase other than protein kinase C is involved in the regulation of AM mRNA production by bovine aortic endothelial cells (18). Furthermore, Autelitano et al. have indicated that phorbol ester and protein kinase C activation inhibit, rather than promoting, AM gene expression by neonatal rat cardiomyocytes (19). Although the effect of AP-2 may differ according to the species and the cell types, AP-2 is thought to participate in the transcriptional regulation of the AM gene.

In vitro experiments using cultured cells have indicated that the production of AM is increased by the stimulation of cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) (20). In clinical settings as well, plasma AM has been shown to be markedly elevated in patients with infectious or inflammatory disorders (21, 22). Considering that NF-IL6 has been shown to be induced by stimulation with IL-1, TNF-α and IL-6 itself, NF-IL6 may partly mediate these increases in AM induced by cytokines. In the acute-phase reaction against inflammation or tissue injury, NF-IL6 is assumed to play an important role as a transcription factor. It is speculated that AM, induced by NF-IL6, dilates regional blood vessels and facilitates the delivery of blood and leukocytes to the inflamed tissue.

We and other groups have reported that plasma AM is increased in patients with hypertension (4, 17, 23), and acute pressure overload has been shown to stimulate left ventricular AM gene expression in rats (24). Conflicting results have been reported as to the effect of shear stress on AM mRNA expression in cultured endothelial cells (25, 26). However, considering that there are two consensus sequences of the shear stress responsive element in the intron 1 of human AM gene (Fig. 1), it is possible that transcription of the AM gene is affected by physical stimuli.

AM is known to stimulate cyclic adenosine monophosphate (cAMP) production in various cells, and cAMP is thought to serve as a second messenger of the biological actions of AM. On the other hand, AM gene expression has been shown to be decreased by cAMP (27, 28). Although it has not yet been proven, the consensus sequence of the cAMP responsive element existing in intron 1 may transmit this negative feedback signal.

Multiple lines of evidence indicate that hypoxia or oxidative stress induces AM mRNA expression (29–32), and Cormier-Regard et al. have reported that the hypoxia-inducible factor-1 (HIF-1) consensus binding site locating at nucleotide position −1095 of the mouse AM gene mediates this hypoxia-induced AM gene expression (29). The 5-flanking region of the human AM gene also contains this
HIF-1 consensus sequence at nucleotides - 825, - 863 and - 1203. Wang et al. have reported that AM mRNA expression is increased by focal ischemic injury in the rat brain cortex (33). The increased AM may dilate focal blood vessels and thereby serve to restore blood flow to the ischemic tissue.

It has been shown that several tumor cells express AM mRNA and produce AM (34–37). On the other hand, Wang et al. have reported that the v-Myc oncoprotein represses AM gene expression in mouse fibroblasts, and they attributed this Myc-mediated repression to the initiator element (INR) in the promoter region of the mouse AM gene (38). Although the consensus sequence for INR does not exist in the promoter region of the human AM gene, the expression of the AM gene may have some relation to oncogenesis or carcinogenesis considering that AM has been shown to inhibit cell proliferation and apoptosis (39).

Based on the various findings on AM gene expression described above, Fig. 2 shows the putative mechanism of transcriptional regulation of the AM gene.

**DNA Polymorphism of the Human AM Gene**

Nucleotide sequencing of genomic DNA adjacent to the human AM gene revealed that there is a microsatellite marker with a variable number of cytosine adenine (CA) repeats at 4 kb downstream from the 3′end of the AM gene (8, 40). We investigated the relation of this microsatellite DNA polymorphism flanking to the 3′end of the AM gene with genetic predispositions to develop various cardiovascular diseases. Genomic DNA was extracted from peripheral leukocytes of normal healthy subjects, patients with essential hypertension, patients with coronary artery disease, hemodialysis patients with type 2 diabetes mellitus, and type 2 diabetic patients without nephropathy. In the Japanese population, there existed four types of alleles with different CA-repeat numbers: 11, 13, 14 and 19. The frequencies of these alleles did not differ among normal subjects, patients with coronary artery disease, and diabetic patients without nephropathy; however, the frequency of the 19-CA-repeat allele was increased in patients with essential hypertension and diabetic patients with end-stage renal failure (41). These findings suggest that the 19-CA-repeat allele of the microsatellite DNA polymorphism adjacent to the human AM gene is associated with the genetic predispositions to essential hypertension and diabetic nephropathy in Japanese subjects. However, this gene polymorphism is not likely to be associated with predispositions to develop coronary artery diseases or type 2 diabetes mellitus itself.

In addition to the CA-repeat polymorphism downstream of the AM gene, there may exist some single nucleotide polymorphisms (SNP) in the human AM gene and the adjacent region. Numerous SNPs of this sort have already been recognized, and some of them are thought to confer a genetic predisposition to develop cardiovascular disorders (42). Therefore, it may be worthwhile to investigate such SNPs in the AM gene in relation to the gene function and predisposition to cardiovascular diseases. Considering the prominent bioactivity of AM in the cardiovascular system, such SNPs of the AM gene may also have some relation to the pathophysiology of cardiovascular disorders.

Thus far, a number of gene polymorphisms have been suggested to confer a predisposition to cardiovascular diseases (43–45). Most of them are related to genes of cardiovascular hormones and their signal transduction systems. Some of these gene polymorphisms have been shown to affect the expression of genes or the activity of gene products (46, 47). Because the plasma AM levels are not different among the genotypes, the microsatellite CA-repeat polymorphism examined in our study is unlikely to affect expression of the AM gene (40). It may be possible that this microsatellite polymorphism is associated with the function of other genes, because the number of short tandem repeats may affect the conformation of DNA and thereby may affect the transcription of nearby genes. In this context, it should be noted that several genes, such as sphingomyelinase, parathyroid hormone, and lactate dehydrogenase, are known to be located near the AM gene in the short arm of chromosome 11.

Microsatellite markers, like the one we examined, consist of a variable number of repeats of short nucleotides, and hundreds of such repeat markers are thought to be scattered throughout the genomic DNA. These microsatellite markers can be utilized to locate the genomic region responsible for hereditary diseases or traits. Until now several diseases have been shown to be associated with such microsatellite DNA polymorphism. For example, the CA-repeat polymorphism lying upstream of the aldose reductase gene affects the development of nephropathy and retinopathy in type 1 diabetes mellitus (48), and a certain number of TCAT repeats in intron 1 of the tyrosine hydroxylase gene has been associated with genetic predisposition to develop essential hypertension (49). Accumulation of information about the association of cardiovascular disorders with various microsatellite markers may serve to clarify the genetic risks of cardiovascular diseases that are attributed to multiple genomic regions.

In conclusion, together, the accumulated results from experimental and clinical studies reveal that AM is involved in the defense of the cardiovascular endocrine system against the progression of organ injuries and dysfunction. Production of AM is increased in various cardiovascular disorders, such as hypertension, heart failure and renal failure (4, 5, 17, 23). AM dilates the blood vessels and improves regional blood flow. In addition, AM inhibits growth and migration of the cells and thereby inhibits cardiovascular hypertrophy (39, 50). AM exhibits a natriuretic effect in the kidneys and inhibits bronchoconstriction in the lungs. These versatile actions of AM are thought to help prevent the vicious cycle leading to cardiovascular organ failure. Thus, it is suggested that AM plays a significant role in the pathophysiology of
the cardiovascular system. Future analyses of the structure and variation of the AM gene should further clarify the theoretical basis of AM gene function and its implication in the pathogenesis of cardiovascular diseases.

References