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Functional regulation of Alu element of human angiotensin-converting enzyme gene in neuron cells

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ABSTRACT

The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) genotype and its protein activity have been widely implicated to be associated with Alzheimer's disease (AD). However, whether the insertion sequence, Alu element, in intron 16 of the human ACE gene plays a functional role remains uncertain. To investigate the influence of the I/D polymorphism on ACE promoter, we recombined the I and D form fragments with the human ACE promoter sequence before the reporter gene in pSEAP-Basic2 vector. The effect of the Alu element on regulating the transcriptional activity of ACE promoter was examined using transient transfection in SH-SY5Y cells. We found that the I form fragment upregulated the transcriptional activity of ACE promoter by approximately 70% but that the D form fragment did not. Our study first reveals that Alu sequence in human ACE gene possesses a regulatory function on the ACE promoter activity in neuron. This novel finding bridges the gap between the association of ACE I/D genotype with AD, and suggests that Alu sequence is not merely a "junk" DNA in human ACE gene.

1. Introduction

Alzheimer's disease (AD) is the most common form of degenerative dementia and has become an important worldwide health problem, with increasing costs associated with the treatment and care of affected individuals (Brunden, et al., 2009). AD involves development of a progressive neuropsychiatric disorder that is characterized by gradual memory impairment, loss of acquired skills, and emotional disturbances. Although the 2 hallmark pathological lesions of the disease, amyloid-β (Aβ)–containing plaques and neurofibrillary tangles of hyperphosphorylated tau in the cerebral cortex and limbic system, have been studied (Ittner and Gotz, 2011), the precise etiology of the lesions remain to be defined. For more than a decade, a number of other pathogenic hypotheses related to AD have also been implicated, including encompassing genetics (Adalbert, et al., 2007; Bekris, et al., 2010; Di Fede, et al., 2009; Larner and Doran, 2009), damage of DNA and RNA (Lee, et al., 2009), inflammation (Lee, et al., 2010), oxidative stress (Mondragon-Rodriguez, et al., 2010), the brain renin–angiotensin system (RAS) (Wright and Harding, 2010), secretases, metabolic dysfunction, and cell cycle re-entry. However, the precise cause is still elusive.

The angiotensin converting enzyme (ACE) gene, a gene that has been carefully studied for association with late-onset AD with inconsistent results (Alvarez, et al., 1999; Chapman, et al., 1998; Kehoe, et al., 1999; Scacchi, et al., 1998), plays a key role in the RAS, as it generates the active octapeptide angiotensin (Ang) II from the decapeptide angiotension I (Wright and Harding, 2010). The inconsistent results may come from the various diagnosed criteria for the AD and normal control (Yang, et al., 2011), racial differences (Lehmann, et al., 2005), or other factors contributing to the development of AD. ACE protein not only makes an impact on vasoconstriction but is also involved in inflammatory processes, oxidative stress, cell growth as well as matrix deposition, and may interfere with acetylcholine (Ach) release in neuron that related to cognitive processing (Bartus, et al., 1982). Interestingly, increasing ACE in

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cerebrospinal fluid (CSF) or brain tissue has been found in AD patients (He, et al., 2006; Savaskan, et al., 2001). Meanwhile, ACE inhibitors and angiotensin receptor blockers have been reported to slow the cognitive decline in AD patients with hypertension more than other classes of antihypertension drugs. The enzymatic function of ACE in degrading Aβ has been hypothesized to underpin the association between the ACE and AD (Hu, et al., 2001; Oba, et al., 2005). Nevertheless, the precise mechanism of how ACE acts in the brain to be related the neuron degeneration is still unknown.

More recently, the continually updated meta-analysis functions of the Internet-based Alzgene database (www.alzgene.org; gene id = 125) ranked ACE variation as the second most significant genetic susceptibility locus for AD after APOE e4, which applied to its dataset criteria for the assessment of growing epidemiologic evidence from genetic associations (Ioannidis, et al., 2008). The insertion (I)/deletion (D) polymorphism (indel) in intron 16 of the ACE gene, 1 of the most well-studied polymorphisms of ACE gene, was first proposed in association with Alzheimer's disease in 1999 (Kehoe, et al., 1999). The insertion sequence (~287 bp) in intron 16 of the human ACE gene is a member of the *Alu* family (Lei, et al., 2005). Alu, 1 of mammalian short interspersed elements (SINEs), is the most abundant type of mobile elements in the human genome, occupying ~10% of the human genomic DNA (Cordaux and Batzer, 2009). A meta-analysis determined a highly significant association of ACE I/D polymorphism in patients with AD (Bertram, et al., 2007; Lehmann, et al., 2005), and some studies indicated that increases in ACE expression and activity in plasma have also been associated with ACE I/D variant (Almeida, et al., 2010; Tiret, et al., 1992). Our previous work demonstrated that ACE I/D polymorphism and its corresponding plasma protein level were associated with AD (Yang, et al., 2011). These heterogeneous results highlight the necessity to clarify the association between the function of the insertion sequence (Alu) of ACE to the ACE protein, if any. A previous study indicated that the intron 16 insertion of human ACE gene had no effect on regulating transcription (Rosatto, et al., 1999); however, it was too imprudent to draw a conclusion, with their simplified reporter constructs. With emerging evidence of Alu elements functioning as gene expression regulators such as transcriptional enhancer, transcriptional silencer, and alternative splicing (Brosius, 1999), it becomes an important and indeed urgent to examine the bio-functional role of the Alu sequence in intron 16 of the human ACE gene. The present study aimed to clarify whether the Alu element regulates the transcriptional activity on its ACE promoter region in neurons.

2. Methods

2.1. Plasmid construction for luciferase reporter assay

The backbones of our reporter vectors are pSEAP-Basic2 (pSEAP-Bas) and pSEAP-control (pSEAP-con) (BD Biosciences Clontech, Palo Alto, CA) which were used in our previous report (Wu, et al., 2004). Based on previous reports (Eyries, et al., 2002; Goraya, et al., 1994; Hubert, et al., 1991), the promoter fragment of human ACE (−760 to +130), including many demonstrated binding elements, was amplified by polymerase chain reaction (PCR) and first cloned into pGEM-T Easy Vector (Promega Corporation) using the following primers: forward primer, 5′GGACTCTGTTGGGATCGT 3′; reverse primer, 5′GCCTCGTCAGCAGAAAAGTT 3′. Then the ACE promoter fragment was cut and fused into a multiple cloning site of pSEAP-Bas vector using EcoRI restriction enzyme. The strains with forward and reverse directions of ACE promoter, pACEpro(f)-SEAP-Bas and pACEpro(r)-SEAP-Bas, were obtained respectively. Structures of the vectors were illustrated in Fig. 1A.

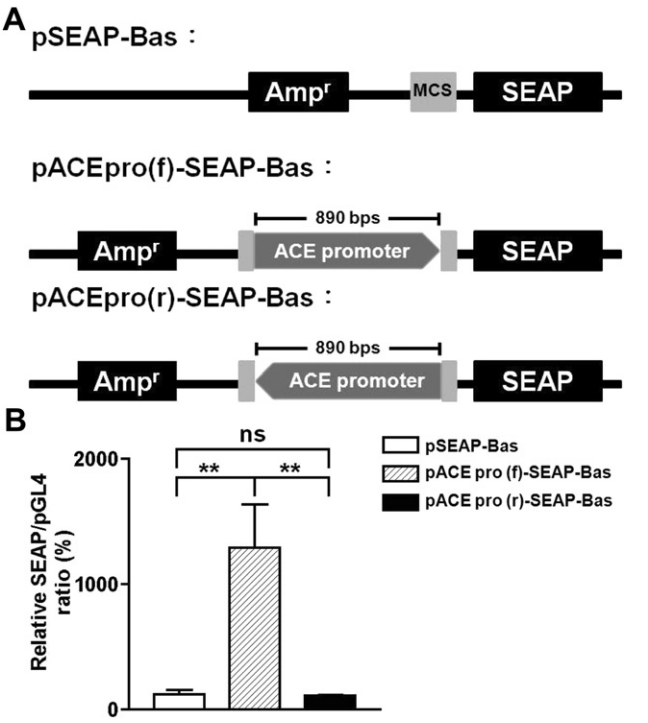

Fig. 1. Basal transcriptional activity of human angiotensin-converting enzyme (ACE) promoter in SH-SY5Y cells. (A) Schematic diagrams of reporter constructs with forward ACE promoter region (pACEpro(f) -SEAP-Bas) and reverse directions of the same ACE promoter fragment (pACEpro(r)-SEAP-Bas) in front of SEAP gene in the backbone vector (pSEAP2-Bas), respectively. (B) Mean relative SEAP/pGL-4 ratios of at least 3 independent experiments are represented in SH-SY5Y cells. Reporter construct pSEAP2-Bas is designated as 100%, and values are expressed with standard error. $^{**}p < .01$. Abbreviation: ns, not significant.

The I allele and the D allele of human ACE intron 16 were also identified by the PCR method, and the amplified fragments, 479 bp of I form fragment and 192 bp of D form fragment, were extracted and inserted between the Hind III and Xho I sites in front of ACE promoter region in pACEpro(f)-SEAP-Bas to obtain p-I-ACEpro-SEAP and p-D-ACEpro-SEAP, respectively (Fig. 2A). I form fragment and D form fragment were individually fused into the multiple cloning site of the pSEAP-Bas and the pSEAP-con using Hind III and Xho I restriction enzyme site to obtain a series of target reporter vectors (Fig. 3A and Fig. 4A). All of these reporter constructs were verified by completely sequencing. The primers used for PCR and DNA sequencing of the I and D fragment were as follows: forward primer, 5′-ACCGCTCGAGCTGGAGAGCCACTCCCATCCT TTCT-3′; reverse primer, 5′-CTAAAAGCTTGACGTGGCCATCACATTCGTCAGAT-3′.

2.2. Cell culture and transient transfection

SH-SY5Y cells, comprising a neuroblastoma cell line, were maintained in MEM/F-12 (1:1) containing 10% fetal bovine serum, 100 U/mL of penicillin and 100 μg/mL of streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. To maintain uniform conditions, all experiments were carried out within passage 12. Plasmid DNA for transfection was prepared using Qiagen columns (Qiagen GmbH, Hilden, Germany). DNA concentrations were determined by spectrophotometry and checked by gel electrophoresis and ethidium bromide staining. Transient cotransfection was performed in SH-SY5Y cells. The cells were freshly subcultured at a density of 2×10^5 cells on 6-well cassettes and transfected using a transient liposome co-transfection protocol