AC3: A Novel Gene Plays a Role in the Regulation of Body Weight

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Abstract: AC3 is one of adenylyl cyclase isoforms involved in cAMP and insulin signaling pathway. A previous study using Goto-Kakizaki rat, a non-obese type 2 diabetes model, indicates that AC3 is over-expressed in pancreatic islets. A recent genetic study has demonstrated that AC3 DNA polymorphisms are associated with body mass index (BMI) in the subjects with obesity and type 2 diabetes. Furthermore, AC3 knock out mice exhibit obese when they age mainly due to low locomoter activity, hyperphagia and leptin insensitivity. These findings suggest that AC3 plays an important role in the regulation of body weight. This review summarizes genetic and biological relevancies of AC3 in the regulation of body weight and also discusses about the potential development of anti-obesity drug using AC3 as a target.

Keywords: Adenylyl cyclase 3, obesity, type 2 diabetes.

INTRODUCTION

Obesity and type 2 diabetes are complex diseases and are influenced by genetic and non-genetic (environmental and life-style) factors. Both diseases are recognized as conditions of growing biomedical importance to societies worldwide. Moreover, type 2 diabetes and obesity are connected. Approximately, 85% of the people diagnosed with type 2 diabetes are overweight [1-5]. Scientists have put the effort to search for susceptibility and resistance genes in obesity and type 2 diabetes. Genetic and functional analyses of the genes in both diseases will provide better knowledge of their patho-mechanisms and for future therapies [6].

BIOINFORMATICS OF AC3

Adenylyl cyclases (ACs or ADCYs) are enzymes that catalyze the synthesis of cyclic 3'5'-AMP (cAMP) from ATP. There are 10 closely related isoforms including ACs 1-9 and AC activating polypeptide 1 (ADCYAP1) that have been cloned and characterized in mammals [7]. AC3 (OMIM: 600291) is the third member in the AC family, and the aliases for AC3 gene are ATP pyrophosphate-lyase 3 and adenylate cyclases III. This gene is located in chromosome 2p23.3 and spans 100,018 bases from the position of 24,895,542 to 24,995,559 bp (Reference ID numbers of g.DNA sequences are NC_000002.10 and NT_022184.14). AC3 mRNA (NM_004036.3) is 4,410 bp at length and the gene is widely expressed in various tissues including pancreatic islets, brain, heart, kidney, liver, lung and skeletal muscle. AC3 protein consists of 1,144 amino acids and the molecular weight is 128,960 Da. The protein consists of two trans-membrane regions M1 and M2, each containing six predicted membrane-spanning helices, and two cytoplasmic regions C1 and C2. This membrane-associated enzyme catalyzes the formation of the secondary messenger cyclic adenosine monophosphate (cAMP), and is involved in a number of physiological and patho-physiological metabolic processes.

AC3 EXPRESSION ANALYSIS WITH GK RAT

Goto-Kakizaki (GK) rat is a non-obese Wistar substrain and develops type 2 diabetes early in life. The model was developed by Goto and Kakizaki at Tohoku University, Sendai, Japan in 1975. The GK rat exhibits moderate hyperglycaemia and markedly reduced glucose-induced insulin release *in vivo,* and in the isolated perfused pancreas and isolated islets. In addition to reduction of beta-cell mass, beta-cell defects have been found to associate with reduced insulin secretion, which are similarly evidenced by the studies in pancreatic islets isolated from type 2 diabetes patients. Therefore, GK rat is a useful animal model of type 2 diabetes [8-12].

The Stockholm GK rat colony was generated by 5 pairs of F40 generation of the Japanese colony in August 1989. Since then, research in diabetes with this animal model has been conducted in our department. A previous study from our laboratory has demonstrated that the AC3 mRNA was over-expressed in the pancreatic islets of GK rat, which was caused by two point mutations at positions –28 A/G and –358 A/C of the promoter region. The insulinotropic effect of forskolin in GK rat islets is associated with an enhanced cAMP generation and with over-expression of AC3 mRNA [13]. Moreover, liver adenylyl cyclase activity was increased in the membranes of male ob/ob mice in comparison to the lean control mice [14]. These findings suggest a role for the AC3 gene in the pathogenesis of type 2 diabetes and obesity. Questions whether AC3 has genetic defect in type 2 diabetes patients and obese subjects are then taken into consideration.

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GENETIC STUDY OF AC3 DNA POLYMORPHISMS

In order to evaluate the genetic defect of AC3 in the development of type 2 diabetes and obesity, we have conducted a genetic association study for the AC3 gene in type 2 diabetes patients and obese subjects in a Swedish population [15]. Initially, we have carried out variation screening for the putative promoter region of human AC3 gene with a direct-sequencing analysis approach. A novel single nucleotide polymorphism (SNP) i.e. -17A/T in the AC3 gene is identified. This promoter polymorphism has a relatively low minor allele (A) frequency. The A allele frequencies of this polymorphism in control subjects and type 2 diabetes patients were 4% and 8%, respectively, which is less significant (P=0.076). Subsequently, we have genotyped and analyzed additional 13 valid SNPs. We found that two SNPs, rs2033655 and rs1968482, had significant low minor allele frequencies in control subjects compared with all type 2 diabetes patients. Furthermore, these two SNPs are found to be strongly associated with obese subjects with NGT and obese type 2 diabetes patients (body mass index, BMI $\geq$ 30 kg/m^2). The association of these two polymorphisms with non-obese type 2 diabetes patients (BMI $\leq$ 26 or <30 kg/m^2), however, is found to be non-significant or of borderline significance. Moreover, the analyses for diplotypes constructed with these two polymorphisms also predict a significant association with BMI in obese subjects. Thus, this genetic association study provides the first evidence indicating that genomic DNA polymorphisms in the AC3 gene are associated with decreased BMI in Swedish subjects with and without diabetes.

PHENOTYPES IN AC3 KNOCK OUT MICE

Evidence from the genetic study suggests the interesting possibility that AC3 may play an important role in the regulation of body weight. To test this hypothesis, Wang *et al*. have generated a mouse model of AC3 deficiency [16]. The AC3$^{-/-}$ mice right after birth are about half the size compared to wild type littermates mainly due to the lack of olfaction and pheromone responses which impair sucking. But, AC3$^{-/-}$ mice achieve similar size and weight as wild control mice after two months. Since then, AC3$^{-/-}$ mice become obese. Adult male AC3$^{-/-}$ mice are about 40% heavier than wild type male mice, while adult female AC3$^{-/-}$ mice are 70% heavier. The body weight gain is exclusively in fat mass but not in lean mass. Furthermore, Wang *et al*. have performed comparison analyses of serum leptin, insulin and triglyceride levels between AC3$^{-/-}$ and AC3$^{+/+}$ mice. Data indicate that serum leptin, insulin and triglyceride levels in AC3$^{-/-}$ are significantly increased compared to AC3$^{+/+}$ mice.

Hypothalamus in brain has a functional role in appetite and body weight control. To explore the cellular mechanism of AC3 in the regulation of body weight, Wang *et al*. have measured AC3 activity in the hypothalamus of AC3$^{-/-}$ mice and found that AC3 activity is decreased compared to AC3$^{+/+}$ mice [16]. Thus, observation of phenotypes of AC3$^{-/-}$ mice is consistent with the genetic association study and indicates that AC3 plays an important role in the regulation of body weight. However, the patho-mechanism of AC3 in increasing risk of BMI is still not fully clear.

KEY MESSAGES AND PERSPECTIVE

From the studies of Wang *et al*. and ours, the key messages that we have learnt are: I. AC3 is over-expressed in pancreatic islets of non-obese and type 2 diabetes GK rat; II. AC3 genetic polymorphisms are associated with decreased risk of BMI; III. AC3 knock out mice become obese when they are adult, and IV. AC3 activity is reduced in hypothalamus while serum leptin, insulin and triglyceride levels are increased in AC3$^{-/-}$ mice.

Evidence has indicated that AC3 plays an important in the regulation of body weight. But, we are still lack of knowledge concerning the pathway from hypothalamus to pancreatic islets and to adipose tissues. Very recently, new genetic loci including AC5, another isoform of AC family, are found to implicate in fasting glucose homeostasis. The AC5 genetic polymorphisms are associated with type 2 diabetes [17, 18]. Interestingly, both AC3 and AC5 are membrane-associated enzymes and activated/regulated by Gsα *via* forskolin, Ca^{2+}/calmodulin dependent protein kinase. Therefore, these two proteins have higher potentiality as targets for drug development. Further investigation of AC3 as well as AC5 in patho-physiology particularly in the pathway from hypothalamus to pancreatic islets and to adipose tissues has been taken into our consideration.

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