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Review

Implication of a novel Gla-containing protein, Gas6 in the pathogenesis of insulin resistance, impaired glucose homeostasis, and inflammation: A review

Anjum Dihingia, Jatin Kalita, Prasenjit Manna *

Academy of Scientific and Innovative Research, Chennai 600113, India
Biological Science and Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam 785006, India

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ABSTRACT

Growth arrest specific 6 (Gas6), a vitamin K-dependent protein plays a significant role in the regulation of cellular homeostasis via binding with TAM-receptor tyrosine kinases. Several studies reported the role of Gas6 in cancer, glomerular injury, obesity, and inflammation, however, very little is known about its role in insulin resistance (IR) and impaired glucose metabolism. Majority of the studies reported an inverse correlation of Gas6 protein levels or gene polymorphism with plasma glucose, HbA1c, IR, and inflammatory cytokines among type 2 diabetes (T2D) and obese subjects. However, few studies reported a positive correlation of Gas6 protein levels or gene polymorphism with IR and inflammation among obese subjects. This review for the first time provides an overview of the association of Gas6 protein levels or gene polymorphism with IR, glucose intolerance, and inflammation among T2D and obese subjects. This review also depicts the probable mechanism underlying the association of Gas6 with glucose intolerance and inflammation. The outcome of this review will increase the understanding about the role of Gas6 in the pathogenesis of IR, glucose intolerance and inflammation and that should in turn lead to the design of clinical interventions to improve glucose metabolism and the lives of the T2D patients.

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* Corresponding author at: Biological Science and Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, Assam 785006, India. Fax: +91 376 2370011.
E-mail address: pmanna2012@gmail.com (P. Manna).
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1. Introduction

Gas6 (encoded by growth arrest specific gene 6) is a novel member of the protein family possessing γ-carboxyglutamic acid (Gla) residue. It is identified as the first Gla containing receptor ligand having the potentiality to regulate diverse cellular activities [1]. In 1988 Gas6 was discovered by the group of Dr. Schneider in a screening for genes whose expression was increased under growth arrest condition in embryonic mouse NIH 3T3 fibroblast cells [2,3]. Gas6 is a multimodular protein molecule composed of a Gla domain at N terminus followed by a disulphide-bridged short loop domain, four tandem repeats of epidermal growth factor (EDF)-like domains, and lastly at C-terminal two lamin G domains which are related to those of the sex hormone binding globulin (SHBG) [4]. Although Gas6 has approximately 44% amino acid sequence identity and structural similarity with protein S, the function of Gas6 and protein S are different [4]. Protein S is an abundant serum protein and a negative regulator of blood coagulation, no such role has been found for Gas6 and the concentration of Gas6 in human plasma is in the subnanomolar range [4]. The functional activities of Gas6 includes regulation of cell proliferation, differentiation, migration, and adhesion, release of pro-inflammatory cytokines, platelet aggregation, differentiation of natural killer cells, adipocyte development, etc., which suggests the role of this novel signaling molecule in various health disorders, like atherosclerosis, metabolic disorders, autoimmune disorders, and cancer [5].

Type 2 Diabetes (T2D) is gaining acceptance as one of the most serious and growing health problem worldwide [6]. Over time, T2D can lead to the pathogenesis of serious health disorders ranging from cardiovascular diseases, renal dysfunction, amputations, nerve damage, erectile dysfunction, etc [7]. In addition to several factors, low grade chronic inflammation and activation of innate immune system have been found to play an important role in the development of T2D [7]. Recent studies in the literature reported the role of Gas6 in the pathogenesis of insulin resistance, T2D, and inflammation [8–11]. This review provides an overview of the currently available evidences about the role of Gas6 in the pathogenesis of insulin resistance, glucose intolerance, and inflammation. The possible molecular mechanism underlying the association between Gas6 signaling and the pathogenesis of impaired glucose metabolism, inflammation, and insulin resistance has also been discussed in this review.

2. Gas6 and the pathogenesis of insulin resistance and impaired glucose homeostasis among different subject population

Shieh and colleagues studied the association of plasma Gas6 concentration with altered glucose tolerance and insulin sensitivity among type 2 diabetic patient population [8]. This study included a total of 278 Taiwanese adults (aged 20–75 y) consisting 96 with normal glucose tolerance (NGT), 82 with impaired glucose tolerance (IGT), and 100 with T2D. Interestingly, it has been observed that T2D patients had significantly low levels of plasma Gas6 concentrations (11.5 ± 0.42 ng/mL) compared to subjects with NGT (14.3 ± 0.66 ng/mL) ($p < 0.001$). A multivariate regression analyses have shown that individuals with a higher level of plasma Gas6 are at lower risk of T2D after adjustment of sex, BMI, waist to hip ratio, blood pressure, smoking, and alcohol consumption. The authors mentioned that plasma Gas6/TAM signaling may serve as an important risk factor for T2D and Gas6 may be considered as a potential marker for impaired glucose metabolism and insulin resistance.

In another study Shieh and colleagues also examined the association between Gas6 gene polymorphism (c.834+7G>A) with the pathogenesis of T2D using the same Taiwanese subject population as mentioned above (96 NGT, 82 IGT, and 100 T2D) [9]. The studied subjects were further classified into different subgroups based upon their Gas6 c.834+7G>A genotypes, like AA ($n = 27$), GA ($n = 90$), and GG ($n = 161$). Results showed that subjects with AA genotype had higher Gas6 levels and lower glucose, HbA1c, HOMA-IR, and triglyceride levels compared to subjects with GG genotype. It has also been observed that the AA genotype was less frequent in patients with T2D compared to NGT group but not in the IGT group. An unadjusted logistic analysis indicated a protective role of AA genotype of c.834+7G>A Gas6 polymorphism against T2D.

Hsiao et al. studied the function of circulating Gas6 and soluble Axl (sAxl) in the development of adiposity and insulin resistance among overweight and obese adolescents in Taiwanese population [10]. A cross sectional analyses was performed among 832 adolescents (420 boys and 412 girls, average age 13.3 y) and the subjects were categorized into three groups, such as lean, overweight, and obese. Results demonstrated that circulating levels of Gas6 and sAxl were significantly higher in overweight and obese adolescents compared to lean group ($P < 0.05$). Among overweight and obese adolescents, a strong positive correlation has been observed between the Gas6 levels and HOMA-IR ($r = 0.268$, $P < 0.01$) after adjustment for age, gender, Tanner stages, drinking status, and smoking status. Interestingly, every 1 ng/mL increase in plasma Gas6 concentrations was associated with 15–19% increased risk of developing insulin resistance among overweight and obese adolescents. This study provided an important clinical evidence regarding the association between Gas6 and insulin resistance. There are certain limitations of this study. Firstly, since this is a cross-

sectional study, thus interpretation of the results is limited and further longitudinal studies are required to confirm the findings. Secondly, Tanner's stage data was derived from a self-reported questionnaire instead of examination and this may prone to have confounding effects on the interpretation. Finally, data regarding smoking/drinking status may be selectively underreported due to punishment from their teachers or parents.

An association between plasma Gas6 concentrations with glucose tolerance has also been examined by Lee et al. among 300 Taiwanese subjects consisting 100 NGT, 96 IGT, and 104 with T2D [12]. In line with their previous study [8], the authors reported that plasma Gas6 concentrations were significantly lower in T2D patients compared to NGT. Among T2D subjects ($n = 104$) plasma Gas6 level was significantly and inversely correlated with corresponding glucose levels during acute glucose challenge under oral glucose tolerance test (OGTT). Age adjusted Spearman correlation studies have again shown that among all the population ($n = 300$) plasma Gas6 concentrations were significantly and negatively associated with OGTT glucose (both fasting and 2-h glucose) and glycated haemoglobin (HbA1c). This study also suggests a role of Gas6 in the development of impaired glucose metabolism among diabetic patient population.

Kuo et al. investigated the association between plasma Gas6 level with obesity and insulin sensitivity among 278 Taiwanese adults consisting 126 men (average age 49.14 y) and 152 women (average age 55.8 y) [11]. Stepwise multiple regression analyses showed that plasma Gas6 concentrations in women were negatively correlated with BMI ($r = -0.186$, $P = 0.022$), waist ($r = -0.187$, $P = 0.022$), waist to hip ratio ($r = -0.189$, $P = 0.022$), 2-h post load insulin ($r = -0.171$, $P = 0.035$), and HOMA-IR ($r = -0.171$, $P = 0.035$) and positively correlated with insulin sensitivity (QUICKI) ($r = 0.168$, $P = 0.039$). However, no such correlations were found among men population. The authors mentioned that the female hormone estrogen can activate the downstream PI3K/Akt signaling pathway and regulate insulin sensitivity and insulin resistance via binding with functional estrogen responsive element present in Gas6 promoter. This study suggests that understanding the relationship between estrogen and Gas6 may give rise to potential therapeutic applications against diabetes and its associated complications in women population. The limitation of this study includes the lack of information about the menstrual cycle or the measurement of estradiol levels. Thus, further study between pre- and postmenopausal women will be helpful in dissecting the association between Gas6 and sex hormones.

In another study Hsiao et al. investigated the effects of Gas6 and Axl gene polymorphisms on adiposity and insulin resistance among Taiwanese adolescents [13]. A cross sectional study was performed in 727 Taiwanese adolescents (358 boys and 369 girls, average age 13.3 y) excluding the subjects with cancers, autoimmune diseases, active infection, and those under medication for insulin or glucose metabolism. The polymorphism study was carried out on four selected genes found in young Taiwanese population, such as Gas6 rs8191973, Gas6 rs8191974, Axl rs4802113, and Axl rs2304232. The studied adolescents were classified into different subgroups based upon their Gas6 rs8191973 genotype (CC, CG, and GG), Gas6 rs8191974 genotype (GG, GA, and AA), Axl rs4802113 genotype (CC, CT, and TT), and Axl rs2304232 genotype (AA, AG, and GG) with gender specification. Boys with the GG genotype of Gas6 rs8191974 exhibited significantly higher BMI and WC compared to the A allele carriers even after adjusting for age, Tanner stage, smoking/drinking status, and physical activity. Boys with the GG genotype of Axl rs2304232 gene polymorphism had significantly higher insulin levels and showed an increase in HOMA-IR than those with the A allele carriers. Logistic regression analyses showed that boys with both GG genotype of Gas6 rs8191973 and GG genotype of Gas6 rs8191974 also exhibited higher BMI and WC compared to the individuals with both C allele of Gas6 rs81973 and A allele of the Gas6 rs8191974. The combined effect of both Gas6 and Axl gene polymorphisms did not show any association with the adiposity and insulin resistance among boys and girls population. In this study the authors did not observe any significant correlation between Gas6 polymorphism and adiposity as well as insulin resistance in the female group and the authors hypothesized that a disparity in sex hormone distribution may regulate the gender specific effect of Gas6 polymorphism. The major limitation of this study is that this is a cross-sectional study, thus further longitudinal studies are required to confirm the findings. Secondly, because of the limitations of questionnaire the authors were unable to estimate every adolescent's dietary energy intake, which have an important impact on genetic susceptibility.

Recently another study has been conducted by Fouad et al. to investigate whether the concentrations of plasma Gas6 protein and the genetic variation in the Gas6 gene are associated with glucose tolerance among Egyptian T2D patients ($n = 50$) [14]. Results showed that the serum concentrations of circulating Gas6 levels were significantly lower in T2D patients (12.2 ± 0.6 ng/mL) compared to age-matched control subjects (14.8 ± 0.9 ng/mL) ($P < 0.001$). The GG genotype of Gas6 c.843+7G>A was most prevalent in the diabetic patient population and the frequency of A allele was lower in the diabetic group compared to those seen in the control group ($P < 0.05$). Subjects in the AA genotype group had higher Gas6 levels (12.7 ± 0.8 ng/mL) compared to GG genotype group (11.8 ± 0.5 ng/mL). From this study the authors concluded a protective role of AA genotype of Gas6 variant (c.843+7G>A) in T2D patients.

Hsieh et al. performed a follow up study to explore the genetic effects of Gas6 variants in the development of insulin resistance, obesity, and T2D among Asian population [15]. The study was conducted under SAPPHIRe (The Stanford Asia-Pacific Program for Hypertension and Insulin Resistance) study cohort which included over 1300 sibling pairs from Stanford, Hawaii, and Taiwan. Subjects with pre-existing malignancies or major systemic diseases were excluded. For the follow-up study 750 participants from the Taiwanese study cohort were recalled. Two tag SNPs, such as rs7323932 and rs8191973 were selected from the HapMap CHB data base. In addition, rs8191974 and rs7331124 were also selected based upon the previous studies for validation. Results showed that none of the SNPs was associated with obesity in Model1

Table 1 – Effect of plasma Gas6 level or Gas6 gene polymorphism on the measures of glycemia, insulin resistance, and the reduced risk of T2D among human subjects.

Type of study/subjects	Plasma Gas6 (ng/mL)	Gas6 gene polymorphism	Outcomes	Ref.
Taiwanese adults, *n* = 278, aged 20–75 y; Normal Glucose Tolerance, NGT = 96, Impaired Glucose Tolerance, IGT = 82, and type 2 diabetes, T2D = 100	T2D (11.5 ± 0.42) NGT (14.3 ± 0.66) IGT (13.3 ± 0.63)		Higher plasma Gas6 was associated with lower fasting glucose and reduced risk of T2D	Hung et al. [8]
Taiwanese adults, *n* = 278, aged 20–75 y; NGT = 96, IGT = 82, and T2D = 100	T2D (11.5 ± 0.42) NGT (14.3 ± 0.66) IGT (13.3 ± 0.63)	Gas6 c.843+7G>A gene polymorphism, genotype: AA, GA, and GG	AA genotype of Gas6 c.843+7G>A had higher Gas6 levels and were less frequent in patients with T2D	Lee et al. [9]
Taiwanese overweight, obese, and lean adolescent, *n* = 832, average age 13.3 y, Boy = 420 and Girl = 412	Obese (13.9 ± 3.9) Overweight (13.1 ± 3.6) Lean (12.3 ± 4.4)		Plasma Gas6 levels were significantly higher in overweight and obese adolescents compared to lean group and it was positively correlated with the insulin resistance.	Hsiao et al. [10]
Taiwanese adults, *n* = 300, NGT = 100, IGT = 96, and T2D = 104	T2D (11.2 ± 0.31) NGT (15.2 ± 0.42) IGT (13.5 ± 0.48)		Lower plasma Gas6 in T2D compared to NGT and higher Gas6 was associated with lower fasting glucose.	Lee et al. [12]
Taiwanese adults, *n* = 278; Men *n* = 126, average age 49.14 y; Women *n* = 152, average-55.8y	Women (13.39 ± 0.45) Men (12.62 ± 0.51)		Higher Gas6 level was negatively correlated with insulin resistance in women and no effects on the markers of glucose metabolism in men.	Kuo et al. [11]
Taiwanese adolescents, *n* = 727, average age 13.3 y, Boy = 358 and Girl = 369		Gas6 rs8191973 gene polymorphism, genotype: CC, CG, and GG Gas6 rs8191974 gene polymorphism genotype: GG, GA, and AA	AA genotype is protective over GG genotype for Gas6 rs8191974 polymorphism in case of adiposity and insulin resistance.	Hsiao et al. [13]
Egyptian adults, *n* = 100, aged 35–62 years; T2D = 50 and Control = 40	T2D (12.2 ± 0.6) Control (14.8 ± 0.9)	Gas6 c.843+7G>A gene polymorphism, genotype: AA, GA, and GG	GG genotype of Gas6 c.843+7G>A had lower Gas6 levels and it was prevalent in T2D.	Fouad et al. [14]
SAPPHIRe study cohort included 1300 sibling pairs from Stanford, Hawaii, and Taiwan		Gas6 rs7323932, rs8191973, rs8191974, and rs7331124 gene polymorphism	Gas6 variant rs8191973 was associated with the development of insulin resistance.	Hsieh et al. [15]

(excluding lifestyle factors in the covariates). However three SNPs (rs8191973, rs8191974, and rs7323932) were associated with steady state plasma glucose (SSPG). It was seen that haplotype AACG with polymorphisms in genes, rs8191974, rs8191973, rs7323932, and rs7331124 are positively associated with SSPG levels ($p = 0.0098$). The authors suggested that Gas6 genetic variant rs8191973 was associated with the development of HOMA-IR which is in contrast with previous studies [9,13]. In addition, the authors also made a follow up study among 522 participants to investigate the role of Gas6 polymorphism in the development of T2D. Results suggested that none of the polymorphisms in the genes, such as rs8191973, rs8191974, rs7323932, and rs7331124 were significantly associated with the development of T2D after the adjustment of age, gender, BMI, region, lifestyle factors, and ethnic factors. This study explored the association between different Gas6 variants and HOMA-IR and their role in the pathogenesis of T2D. Table 1 represents a review about the effect of plasma Gas6 protein level or gene polymorphism on the measures of glycemia, insulin resistance, and the reduced risk of T2D among human subjects.

2.1. *Gas6 and the pathogenesis of inflammation among different subject population*

Hung et al. studied the association of plasma Gas6 concentration with inflammatory markers among T2D patient population [8]. This study included a total of 278 adults, consisting 96 with normal glucose tolerance (NGT), 82 with impaired glucose tolerance (IGT), and 100 with T2D, aged 20–75 years. Interestingly, it has been observed that T2D patients had significantly low levels of plasma Gas6 concentrations (11.5 ± 0.42 ng/mL) compared to subjects with NGT (14.3 ± 0.66 ng/mL) ($p < 0.001$). In contrast to the above findings, it has been observed that among all subjects plasma Gas6 concentrations were significantly and negatively correlated with TNF-α and IL-6 concentrations after adjustment for age. The authors mentioned that plasma Gas6 concentration may serve as a potential surrogate marker of inflammation in T2D.

Hsiao et al. investigated the role of soluble Axl (sAxl) and its cognate ligand Gas6 in obesity-associated inflammation among Taiwanese adolescents [10]. In this study a cross-sectional analysis was performed among 832 adolescents (420 boys and 412 girls, average age 13.3 y) and the subjects were categorized into 3 groups, such as lean, overweight, and obese. Results showed that circulating plasma Gas6 and sAxl levels were significantly higher in overweight and obese adolescents compared to those seen in lean group. Interestingly, among overweight and obese adolescents circulating Gas6 levels was significantly and positively correlated with serum TNF-α and hsCRP even after adjustment of age, gender, Tanner stage, and smoking/drinking status. Although no direct or adjusted correlations were observed between sAxl and serum TNF-α and hsCRP levels, however, a significant and positive correlations were observed between Gas6/sAxl ratio and serum levels of TNF-α and hsCRP among overweight and obese adolescents, which was independent of age, gender, Tanner stage, and smoking/drinking status. This study provides the potential clinical evidence about the association between Gas6 and chronic inflammation among overweight and child adolescents. The limitations of this study have been discussed above.

Kuo et al. also studied the relation between plasma Gas6 protein and inflammatory cytokines among men and women populations (126 men, average age 49.14 y and 152 women, average age 55.8 y) [11]. Stepwise multiple regression analyses showed that plasma Gas6 concentrations were significantly and negatively correlated with TNF-α and IL-6 levels among women population, however, only TNF-α showed significant association with plasma Gas6 levels in the men population. In line with the study done by Hsiao et al. [13] Kuo et al. also concluded that understanding the nature of interaction between estrogen and Gas6 signaling will be helpful to find the potential therapeutic in women with inflammatory diseases.

A similar study on the impact of circulating Gas6 and AXL gene polymorphism on obesity-induced inflammation in adolescents was also studied by Hsiao *et al.* [13]. The researchers carried out a cross sectional study among 727 Taiwanese adolescents (358 boys and 369 girls, average age 13.3 y) excluding the subjects with cancers, autoimmune diseases, active infection, and those under medication for insulin or glucose metabolism. The polymorphism analyses were carried out on four selected genes, such as Gas6 rs8191973, Gas6 rs8191974, Axl rs4802113, and Axl rs2304232. The studied subjects were classified into different subgroups based upon their Gas6 rs8191973 genotype (CC, CG, and GG), Gas6 rs8191974 genotype (GG, GA, and AA), Axl rs4802113 genotype (CC, CT, and TT), and Axl rs2304232 genotype (AA, AG, and GG) with gender specification. Results demonstrated that boys with the GG genotype of Gas6 rs8191974 had significantly higher hsCRP levels compared to individuals carrying the A allele of the Gas6 rs8191974 and GG genotype of Gas6 rs8191973 had significantly higher IL6 levels compared to the C allele carrier of Gas6 rs8191973 even after adjusting for age, Tanner stage, smoking/drinking status, and physical activity. Logistic regression analyses also evaluated the combined effect of two Gas6 gene polymorphisms on the serum inflammatory markers and results showed increased levels of hsCRP and IL-6 in the combined group than the reference group. Boys with the GG genotype of Axl rs2304232 had significantly higher IL-6 levels compared to individuals carrying A allele. In this study the authors did not observe any significant correlation between Gas6 polymorphism and systemic inflammation in the female group and the authors hypothesized that a disparity in sex hormone distribution may regulate the gender specific effect of Gas6 polymorphism. The major limitation of this study is that this is a cross-sectional study, thus further longitudinal studies are required to confirm the findings. Secondly, because of the limitations of questionnaire the authors were unable to estimate every adolescent's dietary energy intake, which have an important impact on genetic susceptibility. Table 2 represents a review about the effect of plasma Gas6 protein level or gene polymorphism on the markers of vascular inflammation among human subjects.

Table 2 – Effect of plasma Gas6 level or Gas6 gene polymorphism on the markers of vascular inflammation among human subjects.

Type of study/subjects	Plasma Gas6 (ng/mL)	Gas6 gene polymorphism	Outcomes	Ref.
Taiwanese adults, $n = 278$, aged 20–75 y; Normal Glucose Tolerance, NGT = 96, Impaired Glucose Tolerance, IGT = 82, and type 2 diabetes, T2D = 100	T2D (11.5 ± 0.42) NGT (14.3 ± 0.66) IGT (13.3 ± 0.63)		Plasma Gas6 concentration was negatively correlated with TNF-α and IL-6	Hung et al. [8]
Taiwanese overweight, obese, and lean adolescent, $n = 832$, average age 13.3 y, Boy = 420 and Girl = 412	Obese (13.9 ± 3.9) Overweight (13.1 ± 3.6) Lean (12.3 ± 4.4)		Circulating Gas6 levels was significantly and positively correlated with serum TNF-α and hsCRP	Hsiao et al. [10]
Taiwanese adults, $n = 278$; Men $n = 126$, average age 49.14 y; Women $n = 152$, average-55.8y	Women (13.39 ± 0.45) Men (12.62 ± 0.51)		Gas6 concentration was negatively correlated with TNF-α and IL-6 levels in women	Kuo et al. [11]
Taiwanese adolescents, $n = 727$, average age 13.3 y, Boy = 358 and Girl = 369		Gas6 rs8191973 gene polymorphism, genotype: CC, CG, and GG Gas6 rs8191974 gene polymorphism genotype: GG, GA, and AA	GG genotype of both Gas6 rs8191974 and rs8191973 had significantly higher hsCRP and IL6 compared to individuals carrying the A allele of the Gas6 rs8191974 and C allele of Gas6 rs8191973	Hsiao et al. [13]

3. Molecular mechanism underlying the association of Gas6 with glucose metabolism and inflammation

3.1. *Effect of Gas6 on the signaling cascade of glucose metabolism*

The functions of Gas6 have been found to be mediated via interaction with the TAM receptor tyrosine kinase protein family, namely Tyro3, Axl, and MerTK through binding with its SHBG domain [16,17]. The binding of Gas6 with TAM receptors is a highly regulated process and it shows highest affinity with Axl followed by Tyro3 and Mer [17]. The interaction of Gas6 with Axl causes auto phosphorylation of the three tyrosine residues, namely Tyr779, Tyr821, and Tyr866 on the intracellular tyrosine kinase domain of Axl. The Gas6-mediated tyrosine phosphorylation of Axl induces the activation of several downstream signaling molecules, like PLCγ (phospholipase C), PI3K (phosphoinositide-3-kinase), Grb2 (growth factor receptor bound protein 2), C1-TEN, Nck2 (NCK adaptor protein 2), Ran BPM (Ran-binding protein in the microtubule organizing centre), and SOCS1 (suppressor of cytokine signaling 1) leading to a variety of cellular responses (Sasaki et al. and Axelrod et al.). Activation of PI3K and its downstream target, the protein kinase B or Akt have been found to play a central role in Gas6/Axl-dependent signal transduction [17,18]. PI3K activation increases the production of PtdIns(3,4,5)P_3, which induces various cellular events via activating its effector molecules [18]. Stimulation of Akt induces the glucose uptake mediated via translocation of glucose transporter from the intracellular pool to the plasma membrane [19], glycogen synthesis by activating glycogen synthase [20], and also causes transcription of several genes involved in insulin secretion and action mediated by the regulation of FoxO transcription factor [21]. These studies suggest that the effect of Gas6 on glucose metabolism may be mediated via regulating the activation of PI3K/AKT pathway.

3.2. *Effect of Gas6 on inflammatory signaling molecules*

Gas6 has also been reported to be actively engaged in the regulation of inflammation among various cell types, such as adipocytes, endothelial cells, vascular smooth muscle cells, and bone marrow cells [5]. Gas6/TAM signaling has been known to play a significant role in triggering systematic inflammation in diverse human diseases, such as infection, acute stroke, acute coronary syndrome, and obesity [22,23]. Activation of Toll-like receptors (TLR), especially TLR2 and TLR4 play an important role in the pathogenesis of inflammation [24]. Recent studies in the literature demonstrate the role of Gas6/Axl pathway in the inhibition of inflammatory pathophysiology via downregulating the activation of TLRs and cytokine receptor signaling molecules [25,26]. TLRs are generally activated by pathogen-associated molecules, such as LPS and induce inflammation via the activation of NF-κB (nuclear factor-κB) signaling pathway. In response to TLR/cytokine signal, Axl expression is upregulated followed by the interaction with the interferon α and β receptor

(IFNAR1). The Axl/IFNAR1 complex causes the sequestration and activation of signal transduction and activators of transcription factors (STAT1) to induce the expression of cytokine and TLR inhibitors from the family of suppressors of cytokine signaling (SOCS) molecules, such as SOCS1, SOCS3, and Twist1 [17,27]. Proteins of the SOCS family are acting as the negative regulators of cytokines and TLR signaling. Combining all, it has been suggested that Gas6/Axl plays an important role in regulating the cytokine signaling, which may prevent vascular inflammation, insulin resistance, and impaired glucose metabolism.

3.3. *Effect of Gas6 on pancreatic beta cell survival*

The mitogenic and anti-apoptotic effects of Gas6 have been reported on several cell types, such as NIH 3T3L1 fibroblasts, cardiac fibroblasts, and hepatic cells [28–30]. Gas6 mRNA has been found to present in the pancreas [31] and is synthesized by the alpha cells of the islets of Langerhans [32]. A recent study by Haase et al. [33] reported the effect of Gas6 on the proliferation and functional activity of perinatal beta cells. In addition, using isolated islets Haase et al. [33] also demonstrated a mitogenic activity of Gas6 on neonatal rodent beta cells. Destruction of pancreatic beta cells has been considered as an important etiological factor in the development and progress of T2D [34]. Thus the mitogenic and anti-apoptotic effects of Gas6 on pancreatic beta cells will be helpful in preventing the pathogenesis of T2D.

3.4. *Effect of varying glucose concentrations on the Gas6 signaling*

Lee et al. studied the effects of varying glucose concentrations on the Gas6/Axl signaling in human micro vascular endothelial cells (HMEC-1) [12]. The authors reported that high glucose (HG) exposure significantly decreased the cell viability due to a decrease in Gas6/Axl signaling and an increase in the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1(VCAM-1). Results suggest that hyperglycaemia-induced down-regulation of Gas6/Axl signaling is mediated through Akt pathway but not via MAPK signaling, which causes endothelial dysfunction followed by decrease in cell viability. It was also reported that over expression of Axl receptor in HMEC-1 cells reversed the HG-induced Gas6/Axl/Akt-dependent down regulation of various signaling molecules, like VEGF and VEGFR2, which may play a significant role in endothelial dysfunction and vascular complications in T2D.

Another study by Cavet et al. demonstrated a significant effect of varying glucose concentration on Gas6/Axl signaling in vascular smooth muscle cells (VSMC) [35]. The authors observed that different concentrations of glucose exert its effects on the Gas6/Axl signaling via the Akt and ERK1/2 downstream signaling cascades. It was noticed that different glucose concentrations did not alter the plasma membrane distribution and tyrosine phosphorylation of Axl. However, varying glucose concentrations have significant effects on the interaction of Axl with its downstream binding partners, like Akt and ERK 1/2. High glucose-induced stimulation of Gas6/Axl increased the association of SHP-2 with Axl followed by the activation of ERK1/2 pathway. The interaction of 140 kDa subunit of Axl had been found to activate the ERK1/2 pathway and caused an increase in cell migration in presence of high glucose. However this effect was reversed under low glucose concentration where the stimulation of Gas6/Axl activated the PI3K/Akt/mTOR pathway via the interaction of 114 kDa subunit of Axl followed by the inhibition of caspase 3 expression and an increase in cell viability suggesting the anti-apoptotic role of Gas6/Axl. This study demonstrated a significant role of glucose in the regulation of Gas6/Axl signaling, which may play an important role in the dissection of the cardiovascular pathogenesis in diabetic.

3.5. *Effect of vitamin K on the activation of Gas6 signaling*

Investigating the function of each domain in receptor-binding and biological activities of Gas6, it has been observed that receptor-binding and growth potentiating activities of Gas6 were markedly attenuated via inhibiting the γ-carboxylation of its Gla domain, however, a Gas6 mutant composed of only an SHBG-like domain retained both of these activities. Thus, the SHBG-like domain is apparently an entity indispensable for Gas6 activities, and a carboxylation of the Gla domain has a regulatory role in retaining the activity of native Gas6 [1,36,37]. Vitamin K, a fat soluble vitamin is well-known for its beneficial function not only in activating blood coagulating factors, but also various vitamin K-dependent proteins, such as osteocalcin (OC), matrix Gla protein (MGP), and Gas6 are activated via post-translational modification of protein-bound glutamate residues into gamma carboxy glutamate (Gla) [38]. Various studies reported a beneficial role of either vitamin K supplementation or dietary intake of vitamin K on greater insulin sensitivity, glucose metabolism, and the reduced risk of T2D [39]. Carboxylation of vitamin K-dependent proteins has been found to play an important role in mediating the effect of vitamin K on insulin sensitivity and glucose metabolism [39]. Thus a lower level of plasma vitamin K is associated with a decrease in the γ-carboxylation of vitamin K dependent proteins, such as Gas6, which may reduce the functional activity of Gas6 against inflammation, impaired glucose metabolism, and insulin resistance. Fig. 1 represents a schematic diagram of proposed mechanism for Gas6 effects on insulin sensitivity, glucose homeostasis, and the reduced risk of T2D.

4. Conclusion

From the above discussion it has been observed that some studies reported a higher Gas6 levels is associated with lower plasma glucose, glycated haemoglobin, insulin resistance, and inflammatory cytokines among T2D and obese subjects. However, a few studies reported a strong positive correlation of Gas6 with insulin resistance and inflammation among obese subjects. These studies implicated a distinguished role of Gas6 signaling in the pathogenesis of vascular inflammation, insulin resistance, and impaired glucose metabolism. The ligand-receptor interaction of Gas6 and Axl is a complex phenomenon and is different in various pathological and physiological circumstances. It can also be hypothesized that

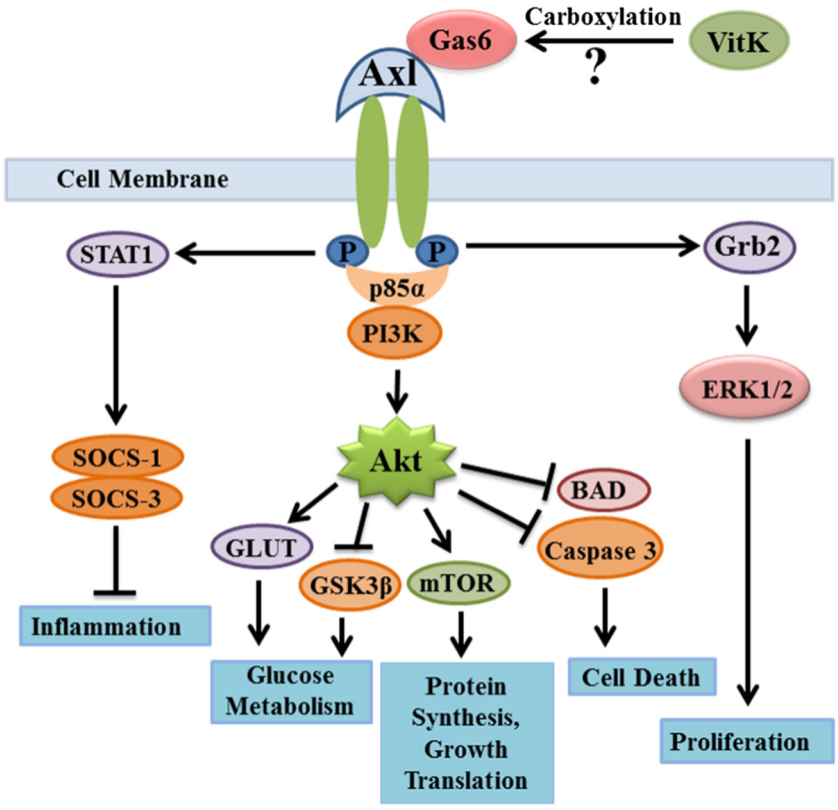

Fig. 1 – Schematic diagram of proposed mechanism for Gas6 effects on insulin sensitivity, glucose homeostasis, and the reduced risk of T2D.

high glucose may trigger the specific post translational modifications of Gas6 which may cause a reduced affinity of Gas6 to its Axl receptor. Therefore, an impaired Gas6-Axl interaction may mediate the pathological mechanism of insulin resistance, impaired glucose metabolism, and vascular inflammation. Gas6 is a vitamin K dependent protein and is activated by the carboxylation of its glutamic residues. As such we can hypothesize a significant role of vitamin K in the activation of Gas6 signaling pathway and a deficiency of vitamin K may therefor down-regulate the Gas6 activation and its associated signaling pathways. However, there is no study in the literature investigating the role of carboxylated and undercarboxylated form of Gas6 in glucose metabolism and inflammatory pathophysiology.

The interpretation of this review is limited by several factors. Firstly, this study did not analyse the effect of Gas6 gene polymorphism on plasma Gas6 levels. Secondly, this study did not compare the effect of either Gas6 gene polymorphism or plasma Gas6 levels with insulin resistance, glucose metabolism, and inflammation. Thirdly, this study did not analyse the association of Gas6 and glucose tolerance between T2D and obese subjects. Excessive plasma lipid may affect the plasma Gas6 levels in obese subjects. This review provides an overview of the association of either Gas6 protein level or gene polymorphism with insulin resistance, glucose intolerance, and inflammation among T2D and obese subjects. The probable molecular mechanism underlying the association of Gas6 with glucose metabolism and inflammation has also been discussed. Although regulation of plasma glucose level reduces the devastating complications in diabetes, however, for many patients it is very difficult to achieve tight glucose control with current regimens. Thus, understanding of new mechanisms is needed for the development of novel therapeutics to achieve better control of glycemia. The outcome of this review will increase the understanding about the role of Gas6 in the pathogenesis of insulin resistance, glucose intolerance, and inflammation, which should in turn lead to the design of clinical interventions to improve glucose metabolism and the lives of the diabetic patient population.

Conflict of interest

The authors have declared that no conflict of interest exists.

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