The TRIB3 Q84R polymorphism, insulin resistance and related metabolic alterations.

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Abstract
Insulin resistance is pathogenic for many prevalent disorders including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), polycystic ovary syndrome, nonalcoholic fatty liver disease, Alzheimer's and Parkinson's diseases and several cancers. Unraveling molecular abnormalities of insulin resistance may therefore pave the way for tackling such heavy weight on healthcare systems.
This review will be focused on studies addressing the role of genetic variability of TRIB3, an inhibitor of insulin signaling at the AKT level on insulin resistance and several related abnormalities.
Studies carried out in several cultured cells clearly report that the TRIB3 Q84R missense polymorphism, is a gain-of-function aminoacid substitution, with the R84 variant being a stronger inhibitor of insulin mediated AKT activation as compared to the more frequent Q84 variant. Given the key role of AKT in modulating not only insulin signaling but also insulin secretion, it was not surprising that beta cells and human pancreatic islets carrying the R84 variant showed also impaired insulin secretion. Also of note is that in human vein endothelial cells carrying the R84 variant showed a reduced insulin-induced nitric oxide release, an established early atherosclerotic step.
Accordingly with in vitro studies, in vivo studies indicate that TRIB3 R84 is associated with insulin resistance, T2DM and several aspects of atherosclerosis, including overt CVD. In all, several data indicate that the TRIB3 R84 variant plays a role on several aspects of glucose homeostasis and atherosclerotic processes, thus unraveling new molecular pathogenic mechanisms of highly prevalent disorders such as T2DM and CVD.

Introduction

Insulin resistance is pathogenic for many prevalent disorders including abnormal insulin secretion, [1, 2], dyslipidemia [3], hypertension [3, 4], and endothelial dysfunction [5]. As a consequence, people with insulin resistance are at high risk of developing future type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [6-8]. In addition, insulin resistance represents a common pathogenic soil of other highly prevalent diseases, such as polycystic ovary syndrome [9], nonalcoholic fatty liver disease [10], Alzheimer's and Parkinson's diseases [11] as well as several cancers [12]. In all, insulin resistance not only causes suffering of many patients and their relatives but also imposes a heavy weight on healthcare systems. Unraveling molecular abnormalities of insulin resistance is an essential prerequisite for tackling this burden and is, therefore, timely needed.

Several animal studies have clarified that in rodents defects in the insulin signaling pathway are pathogenic for abnormalities of glucose homeostasis, endothelial dysfunction and atherosclerotic processes [13]. This makes reasonable hypothesize that also in humans insulin signaling plays a similar instrumental role on several, if not all, diseases that recognize insulin resistance as a common pathogenic soil.

Binding and phosphorylation of the insulin receptor, is the first step of insulin action [14, 15]. The activated receptor, in turns, stimulates the phosphorylation of other proteins, including insulin receptor substrates, phosphatidyl-inositol-3-kinase and the serine/threonine protein kinase AKT, a central mediator of insulin's effects on glucose and lipid metabolism [14, 15]. AKT activity is modulated by various mechanisms, including a direct interaction with TRIB3 (which acts as an inhibitor) and APPL1 (which, conversely is a positive modulator) [16]. Accordingly, the role of the AKT node on human insulin resistance has been addressed by several and diverse approaches. This review will be focused on studies addressing the role of genetic variability of TRIB3 on insulin resistance and several related abnormalities.

TRIB3 gene

Human tribbles pseudokinase 3 gene (i.e. *TRIB3*, NM_021158.4, NP_066981.2, also known as NIPK; SINK; TRB3; SKIP3; C20orf97) is located on chromosome 20p13-p12.2 and belongs to the "tribbles" pseudokinases family, which in mammals includes 3 different genes (namely, TRIB1,TRIB2 and TRIB3) with some degree of similarity among them [17], and well conserved among species.

TRIB3 gene, is organized in 4 exons and encodes for a 358-amino-acid protein which, by acting as adaptor in several signaling pathways, is able to regulate important cellular processes [18]. Among these, is of particular importance the modulation of insulin signaling, as firstly demonstrated by Du and colleagues in 2003 [19].

Of the several single nucleotide polymorphisms (SNPs) reported in this gene [20], the prevalent missense Q84R SNP (rs2295490) has been extensively studied in the last years, both *in vivo* and *in vitro.*

TRIB3 Q84R – *In vitro* studies

Functional studies carried out in human liver cells firstly showed that the TRIB3 Q84R missense polymorphism, where the charged aminoacid arginine replaces the polar and uncharged aminoacid glutamine at position 84, leads to a gain-of-function substitution [20], with the R84 variant being a stronger inhibitor of insulin mediated AKT activation as compared to the more frequent Q84 variant. This effect, which has been confirmed in several insulin target cells [20, 21, 22], is likely to be due to an altered intramolecular salt bridge formation, a direct consequence of replacing the polar and uncharged aminoacid glutamine at position 84 by the charged aminoacid arginine [22].

Of note, the stronger inhibitory activity of insulin stimulated AKT2 activation of the R84 variant,

has been also reported in human islet cells, as well as in rat MIN6 cultured beta cells [21]. Given the key role of AKT in modulating not only insulin signaling but also insulin secretion [23], it was not surprising that beta cells transfected with the R84 variant did show impaired insulin secretion [21]. Along the same line, it has been reported that human pancreatic islets isolated from TRIB3 R84-carrying donors, show defective glucose-induced insulin secretion as compared to those obtained from QQ donors [22].

Finally, it is of note that in human vein endothelial cells (HUVEC) as obtained from umbilical cords of healthy women [22] the stronger inhibition of insulin-induced AKT phosphorylation in HUVEC naturally carrying the TRIB3 Q84R or R84R genotype as compared to those carrying the Q84Q genotype was paralleled by a reduction in insulin-induced endothelial nitric oxide synthase (eNOS) Ser1177 phosphorylation and Thr495 dephosphorylation as well as a consequent reduction of nitric oxide (NO) release [22], an established early step in the development of atherosclerosis [24].

In addition in wild type HUVEC, insulin stimulation after 24 h increased monocyte adhesion, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression and finally mitogen-activated protein kinase (MAPK) kinase (MEK)–MAPK activation. Conversely, cells carrying R84 variant - either in heterozygous or in homozygous state - had increased unstimulated monocyte adhesion, VCAM-1 and ICAM-1 expression and MEK–MAPK activation which did not increase further upon insulin stimulation [25]. These changes, mainly due to a R84 variant-induced selective impairment of the PI3K/AKT axis, are able to switch insulin signaling toward the MAPK-dependent pathway. Given that in the vessel wall the imbalance between PI3K/AKT and MAPK signaling pathways may be responsible for several potentially atherogenic events (including increased endothelial expression of leucocytes adhesion molecules) and eventually to vessel wall thickening and plaque formation, it is conceivable that the observed abnormalities in cells carrying the TRIB3 R84 variant may well contribute in shaping the individual risk of atherosclerosis.

TRIB3 Q84R – *In vivo* association studies

Insulin resistance

Several studies indicate that TRIB3 R84 is associated with insulin resistance. The first evidence was obtained by the euglycemic-hyperinsulinemic glucose clamp technique, showing that insulin-mediated glucose disposal was progressively lower from QQ to QR and RR individuals [26]. Along the same line were data from a second cohort of 791 individuals, in whom an insulin sensitivity index was obtained at oral glucose tolerance test (OGTT) [26]. Similar evidences were later obtained in two Chinese cohorts; in the first one, comprising 513 individuals, carriers of the TRIB3 R84 variant showed a higher risk of being insulin resistant [27], while in the second one of 422 subjects, those carrying the TRIB3 R84 variant had higher fasting insulin levels, a proxy of insulin resistance [28].

These association data are perfectly coherent with those from cultured cell models showing that the R84 variant is a stronger inhibitor of insulin signaling [10, 16, 22], taken together with previous reports indicating that the expression of TRIB3 is inversely related to insulin sensitivity [29, 30], make it reasonable to suggest that TRIB3 over-activity, either due to increased expression or to the gain-of-function R84 variant is a determinant of human insulin resistance.

Insulin secretion

In a meta-analysis of two independent studies (comprising a total of 1,436 individuals) a clear and significant reduction of insulin secretion as derived from OGTT data was observed from QQ, QR and RR individuals [26]. In addition, in 766 Poland patients with T2DM those who were homozygous for the R84 variant had a 30% lower plasma C peptide level as compared to Q84Q counterparts [21]. These data are coherent with the reduced insulin release observed in human islets isolated from individuals carrying the R84 variant [22] as well as with the deleterious role of overexpressing the R84 variant in both human islets and rat cultured beta cells [21].

Glucose homeostasis

In a study comprising 6,634 Europeans, the TRIB3 R84 variant turned out to be significantly associated with a 19% increase (95% CI equal to 6-34%) risk of abnormal glucose homeostasis (including impaired fasting glucose, impaired glucose tolerance and frank T2DM). Of note, a stronger association (i.e. 31% increased risk) was observed with individuals whose abnormal glucose homeostasis was diagnosed before age 45 years [26], thus indicating that the R84 variant anticipates the appearance of abnormal glucose homeostasis in predisposed individuals. Analysis of OGTT data from approximately 800 individuals, clearly suggested that the association with abnormal glucose homeostasis was mainly driven by reduced insulin secretion, rather than insulin resistance. Similar associations with glucose homeostasis have been reported in a mixed population of European descents form USA and Poland [21] but not in a small sample of Chinese individuals [24]. Overall, data from studies on the association with reduced insulin sensitivity and secretion (the two major pathogenic factors influencing glucose metabolism) and most of those here presented on abnormalities of glucose homeostasis are coherent in pointing to a deleterious role of the R84 variant in shaping the risk of developing T2DM and related traits. Unfortunately, neither the TRIB3 Q84R polymorphism (rs2295490) nor other SNPs that are good proxies of it have been examined by large genome-wide association studies on T2DM; thus, further and larger studies are definitively needed to say the last word about the real role of this polymorphism on glucose metabolism in human beings.

In a broader perspective, the idea that abnormal insulin signaling is pathogenic for abnormal glucose homeostasis is also derived by recent observations showing a combined effect of several missense function polymorphisms affecting insulin signaling genes, including TRIB3 Q84R, on the risk of T2DM and related traits [32].

Vascular disease

As said before, in addition to being associated with insulin resistance the TRIB3 R84 induces endothelial cell insulin resistance [22] and pro-atherogenic profile [22]. It is, therefore, conceivable that the TRIB3 R84 variant plays a significant role in increasing the risk of vascular disease. As a matter of fact, we have reported that age at myocardial infarction is progressively reduced from QQ to QR and RR individuals who survived this event [20]. More recently, data from both Chinese [27] and Italian individuals [25] individuals have consistently reported that the Q84R polymorphism is associated with increased carotid intima-media thickness with such increase being equal to 0.051 mm per each copy of the R84 variant and a quite robust level of statistical significance (p value equal to 3 x 10-5).

Renal dysfunction, as indicated by reduced glomerular filtration rate (GFR) is an additional possible consequence of insulin resistance [33] and related abnormalities [34]. In 1,012 diabetic patients from Italy RR individuals showed an approximately 5-fold increased risk of GFR less than 60 ml/min x 1.73 m2 [35] as compared to QQ and QR individuals, considered as a single group according to a recessive model of inheritance. In an independent, smaller study in 583 diabetic patients from the US, a smaller tendency toward the same association was reported which, however, did not reach statistical significance (i.e. 44% increased risk of having low GFR for RR vs. QQ plus QR individuals) [35]. In a more recent study carried out in 812 Chinese patients with T2DM, the TRIB3 Q84R polymorphism was independently associated, according to an additive genetic model, with diabetic nephropathy as indicated by the presence of micro-/macro-albuminuria and reduced GFR [36]. Of note, GFR was reduced in patients with as compared to those without albuminuria [36]. In all, though only preliminary, the notion that the Q84R polymorphism may play a role on kidney dysfunction in patients with type 2 diabetesT2DM is well coherent with a more general role on atherosclerosis and pro-atherogenic phenotypes.

Taken together, these data strongly support the idea that the Q84 R polymorphism contributes to atherosclerosis. If this is a consequence of systemic insulin resistance [26-28], endothelial dysfunction [22, 25] or both need to be further addressed and elucidated.

Of note, the hypothesis that abnormal insulin signaling exerts a deleterious effect on vascular

disease is also supported by recent reports showing a combined effect of several missense function polymorphisms affecting insulin signaling genes, including TRIB3 Q84R, on the risk of endothelial dysfunction [37], major cardiovascular events [37] and all-cause mortality [38].

Conclusions

Though further studies are needed to definitively unravel the role of TRIB3 on human metabolic and vascular traits, some preliminary conclusions can be drawn. In fact, both in vitro and in vivo data so far obtained suggest that TRIB3 over-activity (either because of overexpression of because of the R84 gain-of-function variant) plays a role on several aspects of glucose homeostasis and atherosclerotic processes. In all, these studies have played the important function to unravel new molecular pathogenic mechanisms of highly prevalent disorders such as type 2 diabetes and cardiovascular disease.

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