The Review of DIABETIC STUDIES

EDITORIAL

Is a New Immune Response Mediator in the NF-κB pathway - SUMO-4 - Related to Type 1 Diabetes?

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Introduction

Type 1 diabetes mellitus (T1DM) is hallmarked by a complete loss of insulin secretion capacity caused by T cell-mediated destruction of pancreatic β-cells [1, 2]. The disorder has a complex pathogenesis involving genetic and environmental factors, it appears impossible hitherto to explain sufficiently how islet abnormalities arise and which mechanisms trigger immune cells to become diabetogenic. However, we already know that a series of immune-responsive mediators are involved in the occurrence of autoimmunity and the damage of β-cells; the most critical mediators are MHC class I and II molecules, cytotoxic enzymes and cytokines, such as interleukin-1β (IL-1β) and interferon-γ (IFN-γ) [3]. IL-1β and IFN-γ, which are potential mediators of islet inflammation and contributors of β-cell death [4], modify the expression of a number of genes in β-cells by themselves. But indirectly these mechanisms are regulated by transcriptional modifiers through pathways involving the nuclear transcription factor NF-κB [5], the activation of which is regarded as pro-apoptotic in pancreatic β-cells [6, 7]. Elevated activation of NF-κB has been shown to implicate the destruction of β-cells and the development of T1DM [8]. NF-κB was further demonstrated to be associated with T1DM [9, 10] and deficiency in this transcriptional regulator even prevents mice from streptozotocin-induced diabetes [11, 12]. One of the modifiers of NF-κB is a newly discovered post-transcriptional protein modifier which is, due to its functional and structural similarity with ubiquitin, called the small ubiquitin-like modifier (SUMO).

SUMO proteins control and modify a wide range of processes in eukaryotic cells including protein selection and stabilization. In particular, SUMO transfers ubiquitin-like proteins to target proteins by enzymatic cascades. This process of post-translational protein modification is termed sumoylation [13]. Sumoylation is a regulatory mechanism of protein function that involves signal transduction, glucose transport, tumor suppression and genome surveillance [14, 15]. The proteins that can be sumoylated also include IκBα, the NF-κB inhibitor, and the heat shock transcription factors HSF1 and HSF2. Four members of the SUMO family (SUMO1-4) have been identified to date. While SUMO1-3 have a wide tissue distribution, SUMO4 expression is restricted to immune tissues and kidney, making it a candidate mediator of autoimmune disorders such as T1DM [16, 17].

From sumoylation to NF-κB alteration

How is sumoylation associated with T1DM etiology? The expression of cytokine and other target genes that participate in host immune responses is controlled

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by transcriptional regulators of the NF-κB family [7]. NF-κB is activated upon phosphorylation and ubiquitylation of the NF-κB inhibitor protein, IκBα, which keeps the regulator inactive. Sumoylation with SUMO1 and SUMO4 is able to modify IκBα to become resistant to proteasome-mediated ubiquitylation and thus to inhibit NF-κB activity. In contrast, once NF-κB is activated it triggers the transcription of several immune responsive genes including SUMO4 for the control and negative regulation of immune responses [17]. An impaired SUMO4 function induced by nucleotide polymorphism or mutation is suspected of inducing abnormal alterations in NF-κB activity and thus contributing to the onset of T1DM [17, 18].

Two research groups have identified a SUMO4 variant, SUMO4*M55V, carrying a valine (V) substitution to replace methionine (M) at the 55th amino acid position of its native counterpart (SUM4*M) [16, 19]. The Met variant carries a change at the protein kinase phosphorylation site at position 55, which is assumed to be critical for the functional activity of SUMO4. *In vitro* studies showed that the expression of the M55 variant is associated with elevated levels of activated HSF transcription factor. No such effect was observed with the expression of the SUMO4*M55V variant. HSF1 and HSF2 are known to regulate the expression of heat shock protein (HSP) genes [20, 21], and enhanced production of HSPs has been observed in pancreatic β-cells exposed to proinflammatory cytokines [6]. It is therefore possible that SUMO4*M conjugated HSF1 and HSF2 are ineffective in mediating down-regulation of HSP generation during insulitis to protect pancreatic islet cells from undergoing apoptosis. In contrast, no clear effects have been reported for the Val55 allele. While Bohren *et al.* detected no differences in NF-κB reporter gene expression between both SUMO4 alleles, Gou *et al.* observed a 5.5-fold increase in NF-κB transcriptional activity associated with the M55V substitution [16, 19]. The SUMO4*M55V gene product binds to one of the NF-κB inhibitors, IκBα and renders the sumoylated IκBα to lose its positive role in shutting down NF-κB activity following its activation. This condition is thought to drive prolonged transcription of all the genes regulated via NF-κB activation. Indeed, significant enhancement of IL-12 production by peripheral monocytes/dendritic cells is found in diabetic patients carrying the SUMO4*M55V variant, and increased IL-12 gene transcription is implicated to contribute to pancreatic β-cell destruction in the NOD mice [16].

Association of SUMO4 with T1DM development

The linkage between NF-κB and T1DM development has prompted two independent groups, one led by Bohren and the other by Gou and Wang, to seek for polymorphisms of genes within the IDDM5 locus on chromosome 6q25 linked to NF-κB regulation [16, 19]. Gou *et al.* have found the new polymorphic M55V allele to be associated with T1DM in an ethnically heterogeneous population, including European American and Asian patients and controls, with higher frequency in the G (Val) allele among children of European ancestry in the US as judged by observations made from multiplex and simplex family studies [16]. Interestingly, the same group found a positive association of the A (Met55) allele with T1DM in a British cohort, as did the group of Bohren with the patients and controls they have studied [16, 19]. Two other studies failed to prove the association [22, 23], but another study has confirmed the positive association in a Korean population [24]. The discrepancies in the results of these association studies may be due to several factors, such as sample size, genotypic errors, selection biases, genetic heterogeneity and population differences [16]. In order to rule out genotypic errors Wang *et al.* have extended their study to include 195 more patients and additionally 1,060 control subjects of European ancestry in Florida to confirm the positive association of SUMO4*M55V with T1DM. A further test of independence with the combined Florida data set has consolidated their findings on a significant level ($p = 0.0001$) [25]. Therefore, the authors attribute the discrepancies of the results to genetic heterogeneity due to the different populations analyzed [16].

In conclusion, the identification of the conserved form SUMO4*M and its variant SUMO4*M55V contributes to our understanding of the cause(s) of T1DM. In this regard, further studies are suggestive to unravel the complex mechanism of unbalanced NF-κB activation that may trigger the destruction of β-cells. Association studies with enlarged sample sizes and heterogeneous populations could reduce the biases and may lead to more consistent results.

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