A M55V Polymorphism in a Novel SUMO Gene (SUMO-4) Differentially Activates Heat Shock Transcription Factors and Is Associated with Susceptibility to Type I Diabetes Mellitus*

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Three SUMO (small ubiquitin-related modifier) genes have been identified in humans, which tag proteins to modulate subcellular localization and/or enhance protein stability and activity. We report the identification of a novel intronless SUMO gene, SUMO-4, that encodes a 95-amino acid protein having an 86% amino acid homology with SUMO-2. In contrast to SUMO-2, which is highly expressed in all of the tissues examined, SUMO-4 mRNA was detected mainly in the kidney. A single nucleotide polymorphism was detected in SUMO-4, substituting a highly conserved methionine with a valine residue (M55V). In HepG2 (liver carcinoma) cells transiently transfected with SUMO-4 expression vectors, Met-55 was associated with the elevated levels of activated heat shock factor transcription factors as compared with Val-55, whereas the levels of NF-κB were suppressed to an identical degree. The SUMO-4M (Met) variant is associated with type I diabetes mellitus susceptibility in families (p = 4.0 × 10⁻⁴), suggesting that it may be involved in the pathogenesis of type I diabetes.

SUMO-1[1] is involved in the post-translational modification of cellular proteins and regulates various cellular processes such as nuclear transport, oncogenesis, stress response, inflammation, and the response to viral infection (1). SUMO-1 is related to ubiquitin, and although both share only an 18% sequence homology, they have a similar three-dimensional structure (1). In contrast to ubiquitin, which tags proteins for degradation, SUMO-1 seems to enhance protein stability and modulate the subcellular localization. The list of mammalian cellular proteins and regulates various cellular processes.

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EXPERIMENTAL PROCEDURES

RT-PCR—Total RNA was purchased from Ambion (Austin, TX), and RT-PCR performed using the Omniscript RT-PCR kit from Qiagen (Valencia, CA). Total RNA (0.5 µg) was analyzed in the first strand reverse transcriptase reaction using a primer that includes poly(dT)₃-5'-CATTTTAAAC found in SUMO-2 mRNA and deduced from the poly(A) site in SUMO-4 genomic DNA by following directions from Qiagen. The primer was removed from the reaction mixture by purification using a Cycle-Pure kit from Omega Bio-Tek (Doraville, GA). The purified cDNA (10 µl per one-third of the total) was amplified in 25 µl of PCR reactions containing dNTPs, 1.25 mM betaine (Sigma), and primers for SUMO-4F1 forward (5'-GCAATATGCTTGTGACATCAC-3') and either SUMO-4R1 (reverse) (5'-CACAGAAGAGTCAAGACTGAG-3') or SUMO-2R1 (5'-CAAGGAAGGTCAGACTGAG-3'). The PCR reaction was "hot-started" by adding Taq polymerase (PGC Scientific, Gaithersburg, MD) at 95 °C. The SUMO-2R1 and SUMO-4R1 primers have different roles in cellular functions.

Here, we identify and characterize a novel fourth SUMO gene (SUMO-4) whose protein product is 87% homologous with SUMO-2 and report a unique polymorphism not encoded in any of the other SUMO genes (M55V). The SUMO-4M (Met variant) activates HSFs to a larger extent and is associated with susceptibility to type I diabetes (p = 4.0 × 10⁻⁴). SUMO-4M may be involved in the pathogenesis of type I diabetes.

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[2] The abbreviations used are: SUMO, small ubiquitin-like modifier; 4M, Met variant; 4V, Val variant; NF-κB, nuclear factor κB; HSF, heat shock factor; HSE, heat shock element; HSFs, heat shock proteins; HepG2, hepatoma carcinoma cell line; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein isopeptide ligase; RT, reverse transcriptase; SBE, single base extension; HEK, human embryonic kidney; CMV, cytomegalovirus; DTT, dithiothreitol; HMW, high molecular weight; IDDM, insulin-dependent diabetes mellitus; HepG2, heat shock protein of 60 kDa; Luc, luciferase; MES, 4-morpholineethanesulfonic acid; DBD, DNA binding domain; WCE, whole cell extract.
to confirm the specificities of the SUMO-2 and SUMO-4 RT-PCR products were analyzed by ion-pair reverse-phase high pressure liquid chromatography with the WAYVE System (Transgenic Inc., Omaha, NE).

**Recombinant Clones—Vectors**
PHE-Luc, pNF-eB-Luc, pTAL-Luc, and pCMV-Myc were purchased from Clontech (Palo Alto, CA). Renilla luciferase reporter phRL-null vector was purchased from Promega (Madison, WI), and HSF2 was purchased from the American Type Culture Collection (Manassas, VA). pET-15b vector was purchased from Pierce protocol and was tested for specificity against recombinant proteins for SUMO-1, SUMO-2, SUMO-3, SUMO-4M, and SUMO-4V by Western blotting. The IgG was fractionated over the SUMO-2 peptide column using the Pierce protocol and was tested for specificity against recombinant proteins containing a His tag at the N terminus of the recombinant protein. The recombinant proteins were propagated in BL21(DE3)-transformed Escherichia coli. The recombinant proteins were purified utilizing the His-bind resin (Ni²⁺) as described by the manufacturer (Novagen). Purified proteins were dialyzed against 25 mM MES, pH 6.0, and 75 mM NaCl. All of the recombinant proteins were expressed in pET-15b vector. This vector allows the expression of recombinant proteins containing a His tag at the N terminus of the recombinant protein.

The recombinant proteins were dialyzed against 50 mM Tris-Cl, pH 7.5, with the exception of SUMO-4, which was dialyzed against 25 mM MES, pH 6.0, and 75 mM NaCl. All of the recombinant proteins were expressed in pET-15b vector. This vector allows the expression of recombinant proteins containing a His tag at the N terminus of the recombinant protein.

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**Western Blotting**—Nuclear, cytoplasmic, or WCEs were resolved on 10% SDS-PAGE (NuPAGE) and transferred onto nitrocellulose. Blots were blocked in 5% nonfat dry milk and probed with primary antibodies followed by anti-rabbit horseradish peroxidase or anti-mouse horseradish peroxidase conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized using ECL chemiluminescent kit (Amerham Biosciences) and autoradiography (Kodak X-Omat AR film). The IgG fraction of the antiserum (4 μl/μl) was purified by the Ag antibody purification kit from Pierce. SUMO-2 peptide-CERQGLSMRQFRFD was detected only SUMO-4M and SUMO-4V at approximately 27 kDa. The supernatant (cytoplasmic extract) was recovered from the pellets, the cells were washed with ice-cold buffered saline and scraped and the cells were concentrated by centrifugation. Nuclear or cytoplasmic extracts were prepared as described previously (14). The pellet was suspended in buffer 1 (200 μl of 10 mM Tris-HCl, pH 7.6, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM EDTA, 0.5 mM DTT) for 60 min. The nuclear extract was recovered from the supernatant after centrifugation and stored at −20 °C. The nuclei were suspended in buffer 2 (50 μl of 20 mM Tris-HCl, pH 7.6, 25% sucrose, 400 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT) for 60 min. The nuclear extract was recovered from the supernatant after centrifugation and stored at −20 °C. Protein concentration was measured using the Bradford assay as described by the manufacturer (Bio-Rad). The concentration of nuclear extracts was determined by Western blotting using c-Myc primary antibody. For co-transfections of pCMV-Myc-SUMO-4(p)/CMV-Myc-DBD-HSF2 constructs, 1 μg of each plasmid was transfected in 24-well plates as described above and whole cell extracts (WCEs) were made by dissolving the cells directly into 100 μl of 1 × NuPAGE lithium dodecyl sulfate sample buffer containing reducing agent (Invitrogen). 20 μl of WCE was analyzed by Western blotting using the c-Myc primary antibody.

Luciferase Assay—Transfections for luciferase reporter assays were done as described above using 2.2 μg of transfection DNA in 24-well plates. The amount of DNA transfected was 1 μg of test plasmid (either SUMO-4M or SUMO-4V construct or the control vector) and 0.2 μg of expression vector containing the SUMO-4 sequences), 1 μg of either firefly luciferase reporter vector (pHSE-Luc or pNF-eB-Luc) or the pTAL-Luc control vector lacking any response element, and 0.2 μg of Renilla vector. The Renilla vector (phRL-null) served as an internal control for transfection efficiency. Dual-luciferase assays were done using a kit from Promega (Madison, WI). The amount of firefly luciferase activity was normalized to that of the control Renilla luciferase activity to determine the specific effect of the heat shock element (HSE) and NF-eB response elements without SUMO-4 overexpression in HepG2 cells, the combination of pCMV-Myc-control/pTAL-Luc/phRL-null vector.
tor was transfected to determine background luciferase activity. The combination of pCMV-Myc control/pHSE-Luc/phRL-null or pCMV-Myc control/pNF-x-B-Luc/phRL-null were transfected next into HepG2 cells, and the ratio of firefly to Renilla luciferase was compared with the background levels determined above. This comparison provided a relative amount of active transcription factors in the resting HepG2 cells that could bind to the HSE or NF-xB response elements, respectively. SUMO-4M or SUMO-4V/pHSE-Luc or pNF-x-B-Luc/phRL-null combinations were transfected into HepG2 cells to determine the effect of SUMO-4M or SUMO-4V overexpression on the specific response elements. The effect of overexpression of SUMO-4 on reporter gene expression was compared with the levels of reporter expression lacking SUMO-4 calculated above. To study the effect of oxidative stress, H2O2 (Sigma) was added to media to a final concentration of 2 or 4 mM during the final 18 h of transfection. 4–8 transfections were done for each combination of constructs, and the results were plotted as the mean ± S.E. The student's t test was used to determine the significance of the findings.

In Vitro Sumoylation Assay—HepG2 cells (3 × 10^5) were concentrated by centrifugation and suspended in 100 μl of solution 3 (50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 12.5 mM MgCl2, 20% glycerol, 0.1 mM KCl, 1% Triton X-100) (14). After 5 min of incubation on ice, the lysed cells were centrifuged for 5 min at 10,000 rpm and the supernatant was collected. The whole cell extract was used in the in vitro sumoylation assay. Formation of the thioester adduct between the recombinant E2 enzyme UBC9 and SUMO-4M or SUMO-4V was initiated essentially as described previously (15). Reactions contained whole cell extracts from HepG2 (7 μg), and recombinant SUMO-4M or SUMO-4V (2 μg) and UBC9 (5 μg) were incubated in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 5 mM ATP, and 2 mM DTT (30 μl of total reaction volume). Control reactions lacking recombinant UBC9 or including 100 mM DTT also were prepared. Reactions were incubated for 90 min at 30 °C and then stopped by adding 5 μl of NaPAGE lithium dodecyl sulfate buffer and heated to 100 °C for 4 min. Samples (15 μl) were resolved on 10% SDS-PAGE, transferred onto nitrocellulose, and analyzed by Western blotting using SUMO-4-specific primary and anti-rabbit-horseradish peroxidase secondary antibodies as described above.

Genetic Analysis—The chromosome 6q25 region has been shown to be associated with type I diabetes susceptibility (IDDm5) by linkage studies (16). SUMO-4 is located in this region. The Tsp test (17) was used to access linkage disequilibrium between the SUMO-4 marker alleles and disease, because this test provides a valid χ2 test for linkage disequilibrium in the presence of linkage. Top examines whether a heterozygous parent transmits the same or different marker alleles to each of their affected children (sibling pairs).

RESULTS

Tissue Specificity of an mRNA Encoded by a Novel SUMO Gene on Chromosome 6q25—A SUMO-related nucleotide sequence was located within intron 6 of the TAB2 (mitogen-activating protein kinase kinase kinase 7-interacting protein-2, MAPK7IP2) gene on chromosome 6q25 (TAB2 DNA NCBI accession number AL031133 and smt3-like protein NCBI accession number CAAA20019). In contrast to SUMO-1, SUMO-2, and SUMO-3, the SUMO-related DNA sequence does not contain any introns; however, it contains an open reading frame of 95 amino acids, a putative polyadenylation site, and a consen-sus ribosome-binding site (Kozak sequence) for correct translation initiation. Moreover, the predicted amino acid sequence is similar to that of SUMO-1, SUMO-2, and SUMO-3 with the largest sequence homology to SUMO-2 (Fig. 1, 87% amino acid homology). We have named this "putative" protein SUMO-4 based on the strong sequence homologies.

To determine whether an mRNA specific to SUMO-4 is expressed, we performed RT-PCR from total RNA of various tissues. In contrast to SUMO-2, which is highly expressed in all of the tissues examined, SUMO-4 mRNA was detected mainly in kidney and HEK cells (Fig. 2). A nucleotide sequence analysis of the RT-PCR products confirmed their identities as SUMO-2 and SUMO-4, respectively.

Amino Acid Polymorphism in SUMO-4—A SNP was identified previously in this gene (rs237025, chromosome 6 position 149656820; www.ncbi.nlm.nih.gov). The C/T polymorphism is in the antisense strand and encodes methionine 55 (ATG) and valine 55 (GTG), respectively. Indeed, DNA isolated from HEK cells is heterozygous for the AG DNA polymorphism. To determine the frequency of the SNP, we typed 115 unrelated Caucasian individuals. The allele frequencies of A and G were 0.49 and 0.51, respectively. Met-55 is conserved in SUMO-1, SUMO-2, and SUMO-3 proteins in humans (Fig. 1) as well as in SUMO-related sequences in the following organisms: mouse, Xenopus; Drosophila, and yeast (18). The high degree of conservation of Met-55 between SUMO proteins within humans and among different species suggests that the M55V substitution may affect the structure and function of SUMO proteins.

In Vitro Sumoylation—To determine whether SUMO-4 utilizes the E2 enzyme, UBC9, we tested recombinant SUMO-4M and SUMO-4V proteins in an in vitro sumoylation assay containing whole cell extract from HepG2 cells containing or lacking recombinant UBC9 (Fig. 3, lanes 1–6). High molecular weight (HMW) bands with molecular masses greater than 60 kDa were identified in Western blots for both SUMO-4M and SUMO-4V proteins (Fig. 3, lanes 1 and 4, respectively) but were absent when recombinant UBC9 was omitted from the reaction (Fig. 3, lanes 2 and 5). The HMW bands were also absent when recombinant UBC9 plus 100 mM DTT was present (Fig. 3, lanes 3 and 6). DTT (100 mM) inhibits the formation of the thioester bond and therefore provides additional evidence that the HMW bands are the result of sumoylation (Fig. 3, lanes 1 and very weak in lane 4). These complexes are of the correct size for a SUMO-4 dimer and a SUMO-4-UBC9 complex, respectively (Fig. 3).

Transfection Studies in HepG2 Cells—To test SUMO-4 overexpression in vivo, we used SUMO-4M and SUMO-4V constructs tagged with c-Myc in mammalian expression vectors

![Fig. 1. Sequence homologies of SUMO proteins.](image-url)
FIG. 2. RT-PCR analysis of SUMO-2 and SUMO-4 in various tissues/cells. RT-PCR was done on total RNA of the following sources: lane 1, kidney; lane 2, liver; lane 3, pancreas; lane 4, testes; lane 5, smooth muscle; and lane 6, HEK cells. The RT-PCR products were separated on an agarose gel, and SUMO-2 and SUMO-4 were detected by staining with ethidium bromide.

FIG. 3. In vitro and in vivo sumoylation with SUMO-4M and SUMO-4V. Lanes 1–6, in vitro sumoylation studies using HepG2 WCE; lanes 1–3, recombinant SUMO-4M; lanes 4–6; recombinant SUMO-4V; lanes 1 and 4, total reaction mixture; lanes 2 and 5, lack recombinant UBC9; and lanes 3 and 6, 100 mM DTT. SUMO-4 and SUMO-4-conjugated proteins are detected by Western blotting with SUMO-4 antibody. Lanes 7–10 are in vivo transfection studies of SUMO-4M (lanes 7 and 8) and SUMO-4V (lanes 9 and 10) expression vectors into HepG2 cells. Lanes 7 and 9 are nuclear extracts, and lanes 8 and 10 are cytoplasmic extracts analyzed by Western blotting using c-Myc antibody.

containing the CMV promoter that were transfected into HepG2 cells (Fig. 3, lanes 7–10). In cytoplasmic extracts prepared from SUMO-4M and SUMO-4V transfections, most of the protein detected in Western blots was unconjugated free SUMO-4 (Fig. 3, lanes 8 and 10). In contrast, nuclear extracts from these experiments demonstrated mostly multiple bands greater than 50 kDa (Fig. 3, lanes 7 and 9). These results suggest that SUMO-4 targets multiple proteins, and these are found primarily in the nucleus. Furthermore, the SUMO-4M and SUMO-4V expression products detected by the c-Myc tag appear to be equally present in the HepG2 cells (Fig. 3, lanes 7–10), demonstrating that the most abundant sumoylated proteins in HepG2 cells are not sumoylated differentially by the Met and Val variants.

To characterize a specific substrate, we tested lysine 82 located in the DBD of HSF2, which is known to be sumoylated with SUMO-1 in vitro (7), for sumoylation with SUMO-4M and SUMO-4V. SUMO-4M or SUMO-4V and either PCMV-Myc-HSF2-DBD82K (lysine-normal) or PCMV-Myc-HSF2-DBD82R (arginine-mutant) were co-transfected into HepG2 cells, and the WCE was analyzed by Western blotting with c-Myc antibody (Fig. 4). Both DBD82K (Fig. 4, lane 5) and DBD82R (Fig. 4, lane 6) were expressed as 20-kDa proteins in HepG2 cells, whereas free SUMO-4 is expressed as a 12-kDa protein (Fig. 4, lanes 3 (Met) and 4 (Val)). A small amount of SUMO-4 is conjugated to HMW (100,000) HepG2 proteins (Fig. 4, lanes 3 and 4). However, in co-transfection experiments, DBD82K (Fig. 4, lanes 1 (Met) and 2 (Val)) was sumoylated, whereas DBD82R (Fig. 4, lanes 7 (Met) and 8 (Val)) was not. These results confirm a single sumoylation site in the DBD of HSF2 at lysine 82 (7). However, the major sumoylated species was not 32 but was 44 kDa, whereas minor sumoylated products were detected at 32 and 56 kDa (Fig. 4, lanes 1 and 2). These results suggest that the DBD-SUMO-4 complexes have 1–3 copies of SUMO-4 and one copy of DBD82K. Indeed, SUMO-4 has a consensus sumoylation site, VKTE, located at amino acids 10–13 (Fig. 1), and thus, our results are consistent with SUMO-4 forming polymeric SUMO side chains. The degree of sumoylation of DBD82K with SUMO-4M and SUMO-4V was not different (Fig. 4, lane 1 versus 2 and data not shown).

To determine whether SUMO-4 overexpression had a biological effect in the HepG2 cells, luciferase reporter constructs containing or lacking multiple copies of HSE or NF-κB binding elements were utilized in co-transfection experiments. These two response elements were selected because IκBα, HSF1, and HSF2 are known targets of sumoylation by SUMO-1 (3, 6, 7). IκBα sumoylation inhibits NF-κB activity, whereas HSF1 and HSF2 sumoylation is required for the transcription factors to efficiently
bind to and activate the HSE elements. The transfection of SUMO-4M activated the expression from the HSE elements (Fig. 5, lane 4 versus 5; p = 3.6 × 10^-5) while suppressing activation from the NF-kB elements by ~32% (Fig. 5, lane 1 versus 2; p = 3.7 × 10^-4). Transfection of SUMO-4V also activated the expression from the HSE elements (Fig. 5, lane 4 versus 6; p = 0.002); however, the increase was significantly less compared with SUMO-4M (Fig. 4, 2.8- versus 4.2-fold, lane 6 versus 5; p = 0.004). Likewise, the transfection of SUMO-4V suppressed NF-kB activation (Fig. 5, lane 1 versus 3; p = 7.4 × 10^-4); however, the suppression was the same for SUMO-4M compared with SUMO-4V (Fig. 5, lane 2 versus 3; p = not significant).

In transfection studies of HepG2 cells under basal conditions (Fig. 5, lane 4), endogenous HSFs were not active (relative luciferase levels of ~1) but were activated 4.2- and 2.8-fold when transfected with SUMO-4M and SUMO-4V, respectively (Fig. 5, lanes 5 and 6). To test the effect of oxidative stress on HSF activation, HepG2 cells were treated with 2 or 4 mM H2O2 for 18 h. Endogenous HSFs were activated 2.1- and 8.5-fold, respectively (Fig. 6, lanes 1 and 4). At 2 mM H2O2, the transfection of SUMO-4M and SUMO-4V both increased the amount of HSF reporter levels (Fig. 6, lane 1 versus 2, 5.7-fold activation, p = 0.0004; lane 1 versus 3, 4.2-fold activation, p = 0.002), and the levels were higher for SUMO-4M than they were for SUMO-4V (Fig. 6, lane 2 versus 3; p = 0.0008). At 4 mM H2O2, SUMO-4M increased reporter levels (Fig. 6, lane 4 versus 5, 8.5–13.5-fold activation; p = 0.04) and SUMO-4V levels were intermediate (Fig. 6, lane 6, 10.4-fold activation); however, the SUMO-4V activation was not statistically different compared with either 4 mM H2O2 alone or SUMO-4M (Fig. 6, lanes 4 versus 5 and 6 versus 6, respectively).

Association of SUMO-4M with Susceptibility to Type I Diabetes Mellitus—Studies have shown the linkage of markers on chromosome 6q25 (IDDM5) with susceptibility to type I diabetes mellitus (16). However, the specific identity of IDDM5 has yet to be determined. Type I diabetes is characterized by selective β-cell destruction, resulting in an absolute requirement for exogenous insulin and a young albeit heterogeneous age of onset. The etiology and pathogenetic mechanisms of β-cell destruction are not understood completely, although an autoimmune process clearly is involved. The location of SUMO-4 in the IDDM5 region makes it an interesting “candidate gene” for the IDDM5 susceptibility. We typed the M55V DNA polymorphisms (A/G) in 478 families to determine whether there was a familial association with type I diabetes. The A polymorphism (encoding Met) was transmitted 57.1% of the time (467/818) to the diabetic offspring from heterozygous (A/G) parents (Tsp = 4.0 × 10^-4). For comparison, the A polymorphism was transmitted 46.2% of the time (78/169) to the unaffected offspring from the heterozygous parents. The transmission results in the unaffected siblings demonstrates that the association with type I diabetes susceptibility is not a general transmission bias.

DISCUSSION

We have characterized a novel SUMO gene that is related to SUMO-2 but whose mRNA is expressed in a limited number of tissues. We confirm that this novel protein belongs to the SUMO family, because its expression uses the SUMO-specific E2 enzyme UBC9 in vitro. This becomes the fourth member of the SUMO family (SUMO-4). We identified and characterized a polymorphism within this family, SUMO-4M55V. Although, the allelic frequency of SUMO-4V is 0.51 in the Caucasian population, the valine substitution has not been reported in any other human SUMO member (18). The highly conserved nature of this Met amino acid across various SUMO family members (Fig. 1) and across species (18) suggests that it may be biologically important for the function of the molecule.

We demonstrate an increase in HSE reporter expression due to SUMO-4M compared with SUMO-4V. However, the differences in reporter expression were specific for HSE and were not found for NF-kB (Fig. 5) or activator protein 1 (data not shown). HSF1 molecules are not normally active in resting cells but become active after cells are exposed to various stress conditions (19). In contrast, HSF2 is activated by hemin in human K562 erythroleukemia cells and is active in mouse ES cells during early embryogenesis but is not activated by the classical stress stimuli (19). The difference in HSE-reporter expression due to SUMO-4M compared with SUMO-4V also was found under mild oxidative stress conditions (2 mM H2O2), which resulted in a 2-fold activation of endogenous HSFs (Fig. 6). However, under severe oxidative stress (4 mM H2O2), the increased levels of HSF-reporter levels of Met-55 compared with Val-55 was less evident (Fig. 6) and may be attributed to endogenous SUMO-1, SUMO-2, or SUMO-3, competing with SUMO-4 for activated HSF-1 substrate. Alternatively, the HSE reporter assay may no longer be in a linear range because of saturation of HSE binding sites in the reporter. It remains to be determined whether our differential activation of HSFs by SUMO-4M compared with SUMO-4V is due to HSF1 and/or HSF2. However, the roles of HSF1 and HSF2 may not be independent in stressed cells because interactions and cooperative effects have been detected in both of them (20).

We also have demonstrated that SUMO-4M is associated with type I diabetes susceptibility. SUMO-4 sumoylation of HSF1 and/or HSF2 may be involved directly in the type I diabetes pathogenesis through abnormal HSP expression in pancreatic β-cells. HSPs are “molecular chaperons” present during the assembly of proteins and take part in the processing and presentation of antigens. In the non-obese diabetic mouse, the onset of β-cell destruction is associated with spontaneous development of T-lymphocytes reactive to members of the 60-kDa heat shock protein (Hsp60). Autoimmune non-obese diabetes may involve molecular mimicry between epitopes of Hsp60 and a β-cell-specific molecule (21). Furthermore, in humans, autoantibodies are found in multiple autoimmune diseases, including type I diabetes and rheumatoid arthritis, which react with Hsp60 (22). Type I diabetes seems to respond favorably to therapeutic vaccination with a peptide of Hsp60 (23). The SUMO-4 association with the type I diabetes may be due to altered HSF regulation of Hsp60, although the specific mechanism of susceptibility remains to be determined.

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