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The Differential Regulation of Human ACT1 Isoforms by Hsp90 in IL-17 Signaling

Ling Wu,*,†,1 Chenhui Wang,†,1 Bertrand Boisson,‡ Saurav Misra,§ Patricia Rayman,† James H. Finke,‡ Anne Puel,†,*,‡,∥ Jean-Laurent Casanova,*,†,∥,‡,2 and Xiaoxia Li†,2

IL-17 is a proinflammatory cytokine implicated in the pathogenesis of autoimmune diseases including psoriasis. ACT1 is an essential adaptor molecule in the IL-17 signaling pathway. A missense single nucleotide polymorphism (rs33980500; SNP-D10N) that resulted in the substitution of an asparagine for an aspartic acid at position 10 of ACT1 (ACT1-D10N) is associated with psoriasis susceptibility. Due to alternative splicing in humans, SNP-D10N encodes two mutated ACT1 proteins, ACT1-D10N and ACT1-D19N. Although both ACT1 isoforms are Hsp90 client proteins, the nine additional amino acids in ACT1-D19N provide an additional Hsp90 binding site that is absent in ACT1-D10N. Therefore, whereas ACT1-D10N is a dead protein that is unable to transduce IL-17 signals for gene expression, ACT1-D19N is fully responsive to IL-17. Intriguingly, the two ACT1 isoforms are differentially expressed in ACT1-D10N fibroblasts and T cells. Fibroblasts express both isoforms equally, enabling ACT1-D19N to compensate for the loss of ACT1-D10N function. ACT1-D10N/T cells, however, express predominantly ACT1-D10N. Lacking this compensatory mechanism, ACT1-D10N/T cells behave like ACT1-deficient T cells, exhibiting a dysregulated and hyperactive Th17 phenotype with overproduction of IL-22 and IL-17. The hyperactive Th17 response combined with fully responsive fibroblasts likely synergized to contribute to psoriasis susceptibility in SNP-D10N patients. The Journal of Immunology, 2014, 193: 000–000.

Psoriasis is a chronic inflammatory disease of the skin characterized by epidermal hyperplasia, aberrant differentiation and hyperproliferation of keratinocytes, and marked infiltration of leukocytes including T cells and neutrophils into the dermis (1, 2). Recent studies have highlighted a role for the proinflammatory CD4+Th17 cells and their cytokine network...
22 neutralization. More importantly, this hyperactive Th17 response in Act1-deficient mice is T cell intrinsic, as T cell–specific Act1 deficiency is sufficient for a hyperactive Th17 response (29). This suggests that Act1 in T cells provides a negative-feedback mechanism that regulates Th17 differentiation.

Based on the previous findings, we were surprised to find that fibroblasts derived from an individual who is homozygous for SNP-D10N (ACT1D10ND10N) are hyperresponsive rather than unresponsive to IL-17 stimulation (13). Human ACT1 undergoes alternative splicing so that SNP-D10N results in an amino acid substitution at two different positions of two ACT1 isoforms (variant 1 and variant 2) to generate ACT1-v2-D10N and ACT1-v1-D19N, the latter of which contains nine additional amino acids at the N terminus. In this study, we sought to determine the functional differences between ACT1-v2-D10N and ACT1-v1-D19N and to investigate the mechanisms by which SNP-D10N predisposes patients to psoriasis. In this manuscript, we demonstrated that although ACT1-v2-D10N is nonfunctional, ACT1-v1-D19N retains the ability to interact with Hsp90 and is fully responsive to IL-17A stimulation. Intriguingly, the distribution of the two isoforms varies between cell types. Although ACT1-v2-D10N and ACT1-v1-D19N are equally expressed in ACT1D10ND10N fibroblasts, ACT1D10ND10N T cells express predominantly ACT1-v2-D10N. The presence of a fully hyperactive Th17 response with elevated IL-17 and IL-22 expression, which resemble Act1-deficient T cells and exhibit a dysregulated and induced IL-17A stimulation, whereas this compensatory mechanism is absent in ACT1D10ND10N T cells. In this regard, ACT1D10ND10N T cells behave like Act1-deficient T cells and exhibit a dysregulated and hyperactive Th17 response with elevated IL-17 and IL-22 expression. Moreover, ACT1D10ND10N T cells are refractory to stimulation by IL-25 (a member of the IL-17 cytokine family, which also requires ACT1 in its signaling cascade) and demonstrate little IL-4 and IL-5 induction upon treatment. Together, these findings reveal the intricate mechanisms by which this SNP can be linked to human disease.

Materials and Methods

Sample collection

This study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from each patient. This study was approved by the local ethics committee of Necker-Enfants Malades Hospital and The Rockefeller University Hospital. Genotyping analysis was conducted as reported in Boisson et al. (13).

Cell culture and reagents

Control and patient-derived fibroblasts were grown in DMEM supplemented with 10% FCS and penicillin G (100 mg/ml) and streptomycin (100 mg/ml). HEK293 cells and HeLa cells were maintained in DMEM supplemented with 10% (v/v) FBS, penicillin G (100 mg/ml), and streptomycin (100 mg/ml). PBMCs were collected, washed, and resuspended at a density of 4 × 10^6 cells/well in 48-well plates with a final volume of 0.5 ml/well, in the presence of 10 ng/ml recombinant human IL-2 (R&D Systems) and 10 ng/ml recombinant human IL-17E (R&D Systems). After 3 d, the PBMCs were harvested with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. cDNA was synthesized with random hexamers (Applied Biosystems) and Superscript II reverse transcriptase (Promega) and followed by quantitative real-time PCR analysis.

Short hairpin RNA–mediated knockdown of HeLa cells

Human ACT1 short hairpin RNA (shRNA) lentiviral plasmids were purchased from Sigma-Aldrich. For the infection of HeLa cells, lentiviral supernatants were collected at 36 and 48 h after transfection of 293T cells with shRNA plasmids and psiPAX2 and pCMV-VSVG. HeLa cells were transduced with lentiviral supernatants for 48 h followed by puromycin selection for an additional 48 h.

Retroviral production

For the infection of mouse embryonic fibroblasts (MEFs), viral supernatants were collected 36 h after transfection of Phoenix cells with 5 μg human ACT1-v1, human ACT1-v2, human ACT1-v1-D19N, or human ACT1-v2-D10N cloned into pMx-IP.

Quantification of ACT1 isoforms using real-time PCR

Primers were designed to amplify a region in exon 2 of the human ACT1 gene (32) using the primer–annealing tool EZcrossing (http://www.ezcrossing.com). The output sequence with the best C-score (−2.0) was further processed using ModRefiner to improve the model geometry (34).

Quantitative RT-PCR

Total RNA was extracted from fibroblasts or HeLa cells with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The cDNA was synthesized with random hexamers (Applied Biosystems) and Superscript II reverse transcriptase. For the amplification of human ACT1 isoforms, 1 μg total RNA was used as a template in a 20-μl reverse transcription reaction. PCR amplification of cDNAs was conducted with 35 cycles of 30 s at 94°C, 30 s at 60°C, and 40 s at 72°C. Primers for detection of human ACT1 mRNAs were as follows: ACT1 sense (exon 1, 5′-untranslated region), 5′-AGCCGCAGGAAGCAGCTAC3′ and antisense, 5′-GCGATAGACGCGTCTGAGAAG3′ (exon 3). RT-PCR products were assayed by 1% or 1.5% agarose gel electrophoresis and ethidium bromide staining.

Immunoprecipitation and luciferase assays

Cells were lysed in lysis buffer (0.5% Triton X-100, 20 mM HEPES [pH 7.4], 150 mM NaCl, 12.5 mM β-glycerophosphate, 1.5 mM MgCl2, 10 mM NaF, 2 mM DTT, 1 mM sodium orthovanadate, 2 mM EGTA, 20 mM aprotinin, and 1 mM PMSF). Cell extracts were incubated with the appropriate Abs overnight at 4°C with 20 μl protein A Sepharose beads. After incubation, beads were washed four times with lysis buffer, resolved by SDS-PAGE, and analyzed by Western blotting. NF-κB luciferase reporter assays were performed as previously described (21).

PBMC culture and stimulation

Frozen PBMCs were cultured in DMEM supplemented with 10% (v/v) FBS, penicillin G (100 mg/ml), and streptomycin (100 mg/ml). PBMCs were treated with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. cDNA was synthesized with random hexamers (Applied Biosystems) and Superscript II reverse transcriptase (Promega) and followed by quantitative real-time PCR analysis.

Differential regulation of ACT1 isoforms in IL-17 signaling

A computational model for the N-terminal region of ACT1-v1 was generated using I-Tasser (33), using the first 42 residues of ACT1-v2 as the input sequence. The output structure with the best C-score (−2.0) was further processed using ModRefiner to improve the model geometry (34).
pression of ACT1-v1 in each of the cell types, where y = ΔCt and x = percentage of ACT1-v1 relative to ACT1-v1 + ACT1-v2.

**Results**

**SNP-D10N fibroblasts are hyporesponsive to IL-17 stimulation**

We demonstrated previously that the N terminus of Act1 is critical for its interaction with the molecular chaperone Hsp90 (29). SNP-D10N causes a single amino acid change at the N terminus of Act1 from aspartic acid to asparagine (Act1-D10N) and results in a loss of Act1 interaction with Hsp90. We and others have shown that Act1-D10N is unable to interact with known components in the IL-17 signaling cascade, including the IL-17R and TRAF molecules (TRAF2, TRAF5, and TRAF6) (29, 32, 35). Consequently, Act1-D10N is unable to mediate IL-17 signaling for gene transcription or mRNA stabilization of inflammatory cytokines and chemokines in MEFs (29, 36–39).

To investigate the IL-17 responsiveness in fibroblasts derived from a control donor or from an individual who is homozygous for SNP-D10N (ACT1_D10N/D10N), cells were stimulated with IL-17A alone, TNF-α alone, or IL-17A plus TNF-α for 3 h (Fig. 1A). IL-17A-dependent induction of IL8, IL6, and GMCSF was significantly attenuated and almost negligible in ACT1^D10N/D10N fibroblasts compared with the induction in control fibroblasts (Fig. 1A). However, in the presence of TNF-α treatment, IL-17A was able to generate a response in ACT1^D10N/D10N cells. Although this response was significantly weaker than seen in the controls, it was not absent. These findings suggest that the fibroblasts from ACT1^D10N/D10N individuals are still partially responsive to IL-17 stimulation.

To assess the ability of ACT1^D10N/D10N cells to mediate downstream signaling in response to IL-17, control and ACT1^D10N/D10N fibroblasts were stimulated with IL-17A for the indicated times and analyzed for IL-17-dependent NF-κB and MAPK activation. IL-17A treatment of control fibroblasts resulted in robust NF-κB activation, indicated by the phosphorylation of IκB-α and p65, and by the activation of MAPKs including ERK1/2, JNK, and p38 (Fig. 1B). ACT1^D10N/D10N fibroblasts remained generally quiescent to IL-17 stimulation, although there were still signs of NF-κB activation upon IL-17A treatment as indicated by IκB-α phosphorylation. IL-17A stimulation leads to posttranslational modifications on ACT1 (such as phosphorylation) by upstream kinases like IκB kinase (23). Whereas ACT1 was modified upon IL-17 treatment in control fibroblasts (indicated by the arrow pointing to the shifted Act1 band), ACT1 modifications in ACT1^D10N/D10N fibroblasts were greatly reduced (Fig. 1B).

A single polymorphic ACT1 variant encodes two mutated proteins: ACT1-v1-D19N and ACT1-v2-D10N

Human ACT1 undergoes alternative splicing to generate various isoforms (40, 41). Using 5’ RACE, we previously reported two alternatively spliced ACT1 cDNAs in breast cancer cell lines, both of which were equally active in promoting NF-κB activation as determined by luciferase reporter and NF-κB gel-shift assays (41). Variant 1 of ACT1 (ACT1-v1) is translated from an mRNA that consists of all of the exons in the ACT1 gene (exons 1–10), with the translational start site in exon 2 (Fig. 2A, Table I). A shorter ACT1 isoform (ACT1-v2) is the result of alternative splicing involving the excision of exon 2, which codes for nine amino acids at the N terminus of the protein (Fig. 2A). Its translational start site occurs in exon 3 (Fig. 2A, Supplemental Fig. 2). Thus, ACT1-v1 differs from the alternatively spliced ACT1 (ACT1-v2) only in the nine additional amino acids at the N terminus. To visualize the expression of the two isoforms, we designed RT-PCR primers to amplify a region between exon 1 and exon 3 (Fig. 2A, Supplemental Figs. 1, 2). We were able to detect two cDNAs coding for the two ACT1 isoforms in human fibroblasts, one of which is ∼100 bp longer than the other, accounting for the presence of exon 2 (111 bp) (Fig. 2B). Indeed, both ACT1 isoforms are equally expressed in these cells. SNP-D10N (G > A nucleotide change), which is located in exon 3, results in the substitution of an asparagine for an aspartic acid at position 19 of ACT1-v1 to generate ACT1-v1-D19N and at position 10 of ACT1-v2 to generate ACT1-v2-D10N (Fig. 2A, Supplemental Fig. 2). The distribution of the two ACT1 isoforms is not altered by SNP-D10N,
suggested that ACT1-v2-D10N and ACT1-v1-D19N are equally expressed in ACT1
D10N/D10N fibroblasts (Fig. 2B).

Using primers to amplify a similar region in mouse fibroblasts, we
found that although two isoforms are detected in human fibroblasts,
only one Act1 isoform is expressed in mice (Fig. 2C). The N ter-
minus of ACT1 has increased in length during the evolution from
fish to human such that the nine additional amino acids in ACT1-v1
exists only in humans but not in rodents (40). Sequence homology
analysis revealed that exon 1 of mouse Act1 is analogous to exon 1
of human ACT1 (82% identity), but exon 2 of mouse Act1 corre-
sponds to exon 3 rather than exon 2 of human ACT1 (78% identity)
(Supplemental Figs. 1, 2). Though the 111 bp of human ACT1 exon
2 do not exist in the mature mRNA sequence of mouse Act1, an
intronic region in the mouse genome between exon 1 and exon 2 of
Act1 does exhibit an ~33% sequence identity to human ACT1 exon
2 (Supplemental Fig. 3). Nevertheless, mouse Act1 lacks the nine
amino acids found at the N terminus of ACT1-v1 and is thus
analogous to ACT1-v2 (Fig. 2A, 2C) (40).

ACT1-v1-D19N is fully functional in IL-17 signaling

To evaluate the function of SNP-D10N on the two ACT1 protein
isoforms, we generated constructs expressing ACT1-v1, ACT1-v2,
ACT1-v1-D19N, and ACT1-v2-D10N. In an NF-kB–driven lu-
ciferase reporter assay in HEK293 cells, transfection of constructs
expressing ACT1-v1 or ACT1-v2 resulted in the strong induction
of luciferase activity, indicating that both protein isoforms are
capable of activating NF-kB. ACT1-v2, however, appeared to be
more potent in this regard, as the luciferase activity of ACT1-v2

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Table I. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>SNP-D10N</td>
<td>Refers to the SNP rs33980500 (G &gt; A)</td>
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<td>ACT1-v1</td>
<td>Human ACT1 variant 1</td>
</tr>
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<td>ACT1-v1-D19N</td>
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<td>ACT1-v2-D10N</td>
<td>Human ACT1 variant 2</td>
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<tr>
<td>ACT1-v1-D19N/9A</td>
<td>The aspartic acid at amino acid 19 substituted by an asparagine</td>
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<tr>
<td>Act1</td>
<td>Mouse Act1</td>
</tr>
<tr>
<td>Act1-D10N</td>
<td>Mouse Act1</td>
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FIGURE 2. An SNP resulted in two mutated ACT1 proteins. (A) Schematic diagram of ACT1 isoforms. Amino acids labeled in blue are the additional nine amino acids not present in ACT1-v2, ACT1-v2-D10N, mouse Act1, or mouse Act1-D10N. Two translational start sites are present, one in exon 2 and one in exon 3. SNP-D10N is located in exon 3. Arrows indicate the binding sites of RT-PCR primers used in (B) and (C). (B) RT-PCR analysis for the expression of ACT1 isoforms in control and ACT1D10N/D10N fibroblasts. Data are representative of two independent experiments. (C) MEFs were analyzed for Act1 expression by RT-PCR. Data are representative of two independent experiments.
cells were transfected as in (Fig. 3A). Similar to our previous report, the expression of ACT1-v2-D10N did not induce NF-κB activation. The expression of ACT1-v1-D19N, in contrast, resulted in NF-κB activation at a similar level to that of ACT1-v1 (Fig. 3A). This indicates that the amino acid change from D to N at position 19 has no effect on the function of ACT1-v1, whereas this amino acid change at position 10 in ACT1-v2 drastically diminishes protein function.

To further compare the functionality of ACT1-v1-D19N with ACT1-v2-D10N in response to IL-17, we knocked down ACT1 in HeLa cells using lentivirus expressing ACT1 shRNA, followed by the transfection of constructs expressing ACT1-v1, ACT1-v2, ACT1-v1-D19N, and ACT1-v2-D10N to restore ACT1 (Figs. 3B, 3C). IL-17A stimulation of ACT1 knockdown (ACT1-KD) cells restored with ACT1-v1 or ACT1-v2 resulted in the activation of NF-κB as indicated by the phosphorylation of IκBα (Fig. 3C). Importantly, whereas ACT1-KD cells reconstituted with ACT1-v2-D10N did not exhibit IL-17A-induced NF-κB activation, cells reconstituted with ACT1-v1-D19N resulted in NF-κB activation similar to that of cells reconstituted with ACT1-v1 or ACT1-v2. IL-17A stimulation led to ACT1 posttranslational modifications (indicated by the arrow) in cells reconstituted with ACT1-v1, ACT1-v2, and ACT1-v1-D19N, but not in cells reconstituted with ACT1-v2-D10N, emphasizing the functional differences between the two altered ACT1 isoforms. Consistent with this, transfection of ACT1-v1-D19N in the ACT1-KD cells restored IL-17A plus TNF-α-mediated induction of IL8 and IL6, whereas transfection of ACT1-v2-D10N did not elicit this response (Fig. 3D).

Unlike ACT1-v2-D10N, ACT1-v1-D19N interacts with Hsp90
Hsp90 chaperone activity is required for proper folding, stability, and conformational regulation of its client proteins (42, 43). We previously identified an Hsp90-interacting domain at the N terminus of Act1. The D > N change at position 10 of Act1 resulted in a loss of interaction with the molecular chaperone, rendering Act1 D10N completely nonfunctional (29). Because ACT1-v1 contains nine additional amino acids at the N terminus compared with ACT1-v2, we investigated whether ACT1-v1 interacts with and is regulated by Hsp90 and whether the amino acid change in ACT1-v1-D19N affects this interaction. Flag-tagged ACT1-v1 and ACT1-v2 communoprecipitated with Hsp90, suggesting that both ACT1 proteins interact with Hsp90 (Fig. 4A). Furthermore, using the Hsp90 inhibitor PU-H71 to inhibit the ATP-dependent chaperone function of Hsp90, we found a dose-dependent degradation of ACT1-v1 and ACT1-v2, indicating that both protein isoforms are under Hsp90 regulation (Fig. 4B, 4C). As previously reported, Flag-tagged ACT1-v2-D10N did not pull down Hsp90; however, ACT1-v1-D19N resulted in the communoprecipitation with Hsp90 (Fig. 4A) (29). In addition, PU-H71 treatment resulted in the dose-dependent degradation of ACT1-v1-D19N but not ACT1-v2-D10N (Fig. 4B, 4C). These results suggest that although the amino acid change in ACT1-v1-D10N affects a critical region for Hsp90 regulation of ACT1-v2, the nine additional amino acids at the N terminus of ACT1-v1-D19N may compensate for the D19N mutation, possibly by acting as an additional Hsp90 binding site.

Mutation of Arg9 to Ala inactivates ACT1-v1-D19N
Computational modeling of aa 1–42 of the N terminus of ACT1-v1 suggests that the nine additional amino acids at the N terminus allow for the formation of two helices that are connected by a loop with aspartic acid 19 at its center (Fig. 5A). Given that D19N had no effect on the interaction of ACT1-v1-D19N with Hsp90, we sought to determine whether mutating the other charged residues

![Figure 3](http://www.jimmunol.org/)
in this predicted loop region affects the function of ACT1-v1. We generated expression constructs mutating each of the three glutamic acids (at positions 17, 20, and 22) in the loop region to alanine. As assessed by NF-\(\kappa\)B–driven luciferase reporter assay, mutating these amino acids had very little effect on NF-\(\kappa\)B activation, suggesting that changes in the predicted loop alone have no effect on the function of ACT1-v1 (Fig. 5B). We next investigated whether the nine additional amino acids in ACT1-v1 and ACT1-v1-D19N contributes to the function of the protein. Each of the nine amino acids at the N terminus was mutated to alanine and assessed for NF-\(\kappa\)B–dependent luciferase activity (Fig. 5C). Although individually mutating aa 2–8 to alanine had little effect on NF-\(\kappa\)B activation, mutating arginine-9 to alanine resulted in a significant loss of luciferase activity (Fig. 5C, arrow).

To further assess the function of ACT1-v1-D19N/R9A, ACT1-KD HeLa cells were transfected with vector alone or vector encoding Flag-tagged ACT1-v1. Flag-tagged ACT1-v1, Flag-tagged ACT1-v2, Flag-tagged ACT1-v1-D19N, or Flag-tagged ACT1-v2-D10N and subjected to immunoprecipitation (IP) with anti-Flag Abs. Lysates were immunoblotted (IB) for Hsp90, TRAF6, and Flag. IB analysis of ACT1 in Act1\(^{-/-}\) MEFs transduced with retrovirus encoding ACT1-v1 or ACT1-v1-D19N and then left untreated (0) or treated for 12 h with the indicated concentrations of the Hsp90 inhibitor PU-H71. The percentage of ACT1 remaining (relative to untreated) assessed by densitometry using ImageJ (National Institutes of Health) is shown in the bottom panel. Data are representative of two independent experiments.

Asymmetrical distribution of ACT1 isoforms

Though the ratio of ACT1-v1 to ACT1-v2 approaches 1:1 in fibroblasts (Figs. 2B, 6A), we have previously observed that ACT1-v2 is the dominant ACT1 isoform expressed in breast cancer cell lines, whereas ACT1-v1 is expressed at a much lower level (41). To determine whether the distribution of the two isoforms varies between cell types, we amplified the two ACT1 isoforms from fibroblasts, keratinocytes, monocytes, B cells, and T cells. We found that the two isoforms are indeed differentially expressed between different cells (Fig. 6A, 6B, Supplemental Fig. 4). In contrast to fibroblasts, in which ACT1-v1 and ACT1-v2 are expressed at about a 60/40% ratio, B cells, monocytes, and T cells primarily express ACT1-v2. The asymmetrical distribution of the two isoforms is most dramatically evident in T cells, in which ACT1-v2 constitutes \(\sim 80\%\) of total ACT1 (Fig. 6A, 6B).

This asymmetrical distribution is also evident in ACT1\(^{D10N/D10N}\) T cells in which the nonfunctional ACT1-v2-D10N constitutes \(\sim 80\%\) of total ACT1 (Fig. 6C, 6D). Therefore, although ACT1\(^{D10N/D10N}\) fibroblasts retain the ability to respond to IL-17...
due to compensation by a fully functional ACT1-v1-D19N, the low ACT1-v1-D10N expression in ACT1

**FIGURE 5.** Mutation of Arg9 to Ala inactivates ACT1-v1-D19N. (A) Computational modeling of the human ACT1-v1 N-terminal sequence encompassing aa 1–42. (B) Luciferase reporter assay of NF-κB activity in HEK293 cells transfected with an E-selectin–luciferase reporter (100 ng) and vector DNA alone or Flag-tagged ACT1-v1, Flag-tagged ACT1-v1-E17A, Flag-tagged ACT1-v1-E20A, or Flag-tagged ACT1-v1-E22A. Bottom panel shows a Western blot analysis of transfected protein expression. Data are representative of two independent experiments. (C) Luciferase reporter assay of NF-κB activity in HEK293 cells transfected with an E-selectin–luciferase reporter (100 ng) and vector DNA alone or Flag-tagged ACT1 P2A, P3A, Q4A, L5A, Q5A, E7A, T8A, R9A, or D19N. Bottom panel shows a Western blot analysis of transfected protein expression. Data are representative of three independent experiments. (D) ACT1-KD HeLa cells were transfected with vector, Flag-tagged ACT1-v1-D19N, or Flag-tagged ACT1-v1-D19N/R9A and then left untreated (UN) or treated for 3 h with TNF-α (10 ng/ml), IL-17A (50 ng/ml), or TNF-α plus IL-17A, followed by RT-PCR analysis for IL6 and GMCSF. Gene expression is graphed as mean fold induction over untreated ± SEM. Data are representative of two independent experiments. (E) HEK293 cells were transfected with vector alone or vector encoding Flag-tagged ACT1-v1, Flag-tagged ACT1-v1-D19N, or Flag-tagged ACT1-v1-D19N/R9A followed by immunoprecipitation (IP) with anti-Flag Abs. Lysates were immuno-blotted (IB) for Hsp90 and Flag. Data are representative of two independent experiments. *p < 0.05. WCL, whole-cell lysates.
rather than unresponsive to IL-17A stimulation. In humans, ACT1 undergoes alternative splicing such that the presence or absence of exon 2—coding for nine amino acids at the N terminus—can result in two ACT1 proteins, ACT1-v1 or ACT1-v2, respectively. SNP-D10N results in the mutation of both ACT1 proteins to give rise to an ACT-D10N and an ACT1-v1-D19N. In this study, we showed that whereas ACT1-v2-D10N is a loss-of-function protein, the amino acid change in ACT1-v1-D19N has no effect on ACT1’s function. ACT1-v1-D19N retains the ability to interact with Hsp90 and is fully responsive to IL-17A stimulation for gene induction in fibroblasts. This is consistent with previous studies showing that deletion of aa 10–25 of ACT1-v1 does not impair the synergistic response to IL-17A plus TNF-α (19, 48). These findings indicate that the nine additional amino acids at the N terminus of ACT1-v1-D19N might provide an alternative Hsp90 binding site. Through mutagenesis analysis, we found that the arginine at position 9 in ACT1-v1-D19N plays a crucial role in the interaction of ACT1-v1-D19N with Hsp90, demonstrating the differential regulation ACT1 isoforms by Hsp90 in IL-17 signaling.

Intriguingly, the expression ratios of the two isoforms vary between cell types. Although the expression of ACT1-v1 and ACT1-v2 is close to a one-to-one ratio in fibroblasts, cell types such as T cells predominantly express ACT1-v2 (Fig. 6A). Accordingly, individuals who are homozygous for SNP-D10N may have a spectrum of IL-17 responsiveness, due to the differential expression ratios of the fully functional ACT1-v1-D19N to the loss of function ACT1-v2-D10N in different cells. For example, in fibroblasts, in which ACT1-v2-D10N constitutes ∼40% of total ACT1, the fully functional ACT1-v1-D19N could compensate for the loss of ACT1-v2-D10N function. However, in T cells, in which ACT1-v2-D10N constitutes ∼80% of total ACT1, compensation by ACT1-v1-D19N is greatly reduced. As such, ACT1D10N/D10N T cells behave like Act1-deficient T cells and exhibit a hyperactive Th17 phenotype, with elevated IL17A and IL22 expression, similar to what is observed in Act1-deficient mice (29).

We previously demonstrated that IL-22 is the major contributor to skin inflammation in Act1-deficient mice as IL-22 neutralization in vivo prevents the skin phenotype. Given that both IL-17 and...
IL-22 play critical roles in the pathogenesis of psoriasis (8, 9, 49–52), elevated IL17A and IL22 expression in ACT1<sup>D10N/D10N</sup>-T cells provides a further explanation for the association of SNP-D10N with psoriasis susceptibility. The fact that ACT1<sup>D10N/D10N</sup> fibroblasts remain responsive to IL-17 stimulation suggests that IL-17 continues to be a major player in the skin inflammation of psoriatic patients homozygous for SNP-D10N. Therefore, the missense SNP-D10N results in two components that pose potential risks for prolonged inflammation. First, a dysregulated and hyperactive Th17 response produces abnormally high levels of IL-22 and IL-17 to increase the propensity for inflammatory events. Second, the continual responsiveness of fibroblasts to IL-17 further perpetuates the inflammatory process in these patients. Interestingly, the PBMCs from ACT1<sup>WT/D10N</sup> individuals also exhibited an elevated IL17A and IL22 expression, although the IL17A expression was significantly lower in the ACT1<sup>WT/D10N</sup> individuals compared with the ACT1<sup>D10N/D10N</sup> individuals. However, IL22 expression was comparably high in both genotypes. Although we cannot exclude the possibility that other genetic factors may be at play, the elevated IL22 expression in PBMCs from ACT1<sup>WT/D10N</sup> individuals suggests that this hyper Th17 phenotype, under particular environmental conditions, may contribute to disease susceptibility.

The question regarding how the two ACT1 isoforms are differentially regulated in various cells, however, remains to be determined. In addition, IL17 responsiveness in other cell types, like keratinocytes from SNP-D10N patients, requires further analysis. Future studies are required to elucidate the mechanism by which the ACT1 splicing machinery is governed and how factors that influence this machinery may impact the balance between host defense and susceptibility to autoimmunity. It is also important to note that although ACT1-v2-D10N failed to interact with Hsp90, this protein seems to be quite stable in the cells. It is possible that ACT1-v2-D10N may interact with other unknown proteins that help to stabilize ACT1-v2-D10N. Although it is unclear whether ACT1-v2-D10N is misfolded, one might speculate the potential ramifications of this stabilized mutant ACT1 in causing certain cellular dysfunctions. Further studies are required to further define the biochemical characteristics of ACT1-v2-D10N and its possible pathogenic role.

Recent genetic studies have only begun to link SNP-D10N to other autoimmune diseases. For one, analysis in patients with inflammatory bowel disease indicated that SNP-D10N is associated with cutaneous extraintestinal manifestations such as pyoderma gangrenosum and erythema nodosum in Crohn disease (odds ratio [OR] = 3.03; p = 0.026) (53). Assessment of the ACT1 locus in SLE susceptibility revealed that SNP-D10N is significantly associated with the disease (OR = 1.71; p = 0.021) and can increase the risk of pericarditis in SLE patients with SNP-D10N (OR = 2.59; p = 0.002) (54). Our previous studies in Act1-deficient mice have extensively linked Act1 deficiency to Sjogrens syndrome and SLE-like disease in mice (27, 28, 55, 56). In B cells, Act1 is a negative regulator of the CD40 and BAFF receptor signaling pathway for B cell activation and maturation, respectively. Because ACT1-v2 is the dominant ACT1 isoform that is expressed in B cells (Fig. 6A, 6B), it is likely that B cells from SNP-D10N patients are hyperactive. Our preliminary analysis of autoreactive Abs in the plasma of SNP-D10N patients indicates elevated autoantibodies relative to control samples (L. Wu and X. Li, unpublished observations). These recent observations further highlight the multifactorial nature and complexity of autoimmune disease development, by which an SNP can provide the genetic susceptibility to multiple autoimmune diseases.

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Disclosures
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References


