A Novel Splice Variant of the Fas Gene in Patients with Cutaneous T-Cell Lymphoma

Remco van Doorn,1,2 Remco Dijkman,1 Maarten H. Vermeer, Theo M. Starink, Rein Willemze, and Cornelis P. Tensen3

Department of Dermatology, Vrije Universiteit Medical Center, 1081 HV Amsterdam, the Netherlands, and Department of Dermatology, Leiden University Medical Center, 2333 AL Leiden, the Netherlands

Abstract

Defective apoptosis signaling has been implicated in the pathogenesis of primary cutaneous T-cell lymphomas (CTCLs), a group of malignancies derived from skin-homing T cells. An important mediator of apoptosis in T cells is the Fas receptor. We identified a novel splice variant of the Fas gene that displaces retention of intron 5 and encodes a dysfunctional Fas protein in 13 of 22 patients (59%) in both early and advanced CTCL. Impairment of Fas-induced apoptosis resulting from aberrant splicing potentially contributes to the development and progression of CTCL by allowing continued clonal expansion of activated T cells and by reducing susceptibility to antitumor immune responses.

Introduction

CTCLs4 are a group of clinically heterogeneous malignancies of mature skin-homing T cells (1). The low mitosis index in the early stages of MF, the most common type of CTCL, and resistance to treatment with genotoxic agents suggest that defective apoptosis signaling plays a role in the pathogenesis of CTCL (2). One of the key regulators of apoptosis in mature T cells is Fas, a homotrimer cell surface receptor that mediates apoptosis upon cross-linking to Fas ligand. The Fas protein contains an intracellular region essential for transduction of the apoptotic signal termed the death domain; mutations in this domain dominantly interfere with Fas function (3, 4). Fas is a critical mediator of activation-induced cell death, a propriocidal mechanism involved in homeostasis of activated T cells (5). In addition, Fas signaling renders neoplastic cells susceptible to antitumor immune responses executed by cytotoxic T cells through cross-linking with Fas ligand. We previously reported loss of Fas protein expression in aggressive types of CTCL (6). In non-Hodgkin’s lymphoma, deleterious mutations of the Fas gene have been identified in 11% of patients (7). In the present study, we examined the occurrence of splice variants and mutations of the Fas gene as well as Fas protein expression in lesional skin biopsy specimens from patients with CTCL.

Materials and Methods

Lesional skin biopsy specimens were obtained from 15 patients with advanced CTCL, including 7 patients with tumor-stage MF (T3N0M0) and 8 patients with CD30− PCLTCL. For the detection of splice variants in early CTCL, skin biopsy specimens from 7 patients with plaque-stage MF (T2N0M0; Ref. 8) were obtained. Cultured keratinocytes, phytohemagglutinin-activated CD4+ and CD8+ T cells, and skin biopsy specimens from patients with benign cutaneous lymphocytic infiltrates (atopic dermatitis, graft-versus-host-disease, and chronic discoid lupus erythematosus) were used as controls. Total cellular RNA was extracted from homogenized samples before treatment with RQ1 DNase (Promega, Madison, WI). cDNA synthesis was performed using Expand Reverse Transcriptase (Roche, Mannheim, Germany) after priming with an oligo(dT)12–18 primer (Invitrogen, Breda, the Netherlands). PCR amplification of cDNA was performed with five overlapping primer pairs (FAS I-V) covering the entire coding region of the Fas gene used previously (9) and an additional primer pair encompassing intron 5 (FAS intron: sense, 5′ TCAAGGAATGCGA-CACCTCAC 3′; antisense, 5′ CCACAATATTAGGAAT 3′). PCR was performed under the following conditions: denaturing for 30 s at 94°C; annealing for 60 s at 58°C for primer pairs I and II and at 50°C for primer pairs III, IV, and V, and intron 5; and extension for 60 s at 72°C; for 35 cycles. PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and on a 12.5% acrylamide gel stained using PlusOne DNA Silver Staining Kit (Amersham Pharmacia, Uppsala, Sweden). PCR products were extracted from agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) for reamplification under identical conditions and direct sequence analysis using the dideoxy chain termination method. The presence of transmembrane helices was predicted using TMHMM version 2.0 software (CBS, Denmark). Snap-frozen skin biopsy specimens were stained with a monoclonal antibody to Fas (Fas6 antibody; kindly provided by Dr. L. A. Aarden) as described previously (6).

Results and Discussion

In three of seven (43%) patients with tumor-stage MF and four of eight (50%) patients with CD30− PCLTCL, an aberrantly spliced transcript of the Fas gene was identified that has not been described previously. Sequence analysis revealed that this splice variant displays selective retention of intron 5, a 152-bp sequence, leading to frameshift and formation of a truncated protein (Figs. 1 and 2). Aberrant splicing of Fas pre-mRNA was not due to splice site mutations because none were detected in intron 5 or in its boundaries. The presence of this particular splice variant was confirmed by using a different primer set designed to amplify the exonic sequences flanking intron 5 and cannot be due to contamination with genomic DNA because this would have generated a larger PCR product containing not only intron 5 but also intron 4. Subsequent examination of an additional group of seven patients with early patch/plaque-stage MF for this splice variant revealed that it was also present in six of seven (85%) patients. It could not be demonstrated in benign cutaneous lymphocytic infiltrates, keratinocytes (Fig. 1A), or phytohemagglutinin-activated CD4+ and CD8+ T cells (data not shown). This indicates that the aberrant
Fig. 1. Detection and analysis of Fas transcripts. A, reverse transcription-PCR analysis of human Fas mRNA using primer pair FAS III. In addition to the expected PCR product of 240 bp, a second PCR product of 392 bp (indicated by the arrow) was detected. Input cDNA template was synthesized from RNA isolated from the following sources. A1: Lane 1, H2O (no cDNA) control; Lane 2, cultured primary keratinocytes; Lanes 3, 5, 6, 7, 11, and 12, MF tumor-stage biopsies; Lanes 4, 8, 9, and 13–17, PCLTCL biopsies; Lane 10, benign lymphocytic skin infiltrate biopsy. A2: Lane 1, H2O (no cDNA) control; Lanes 2–6, benign lymphocytic skin infiltrate biopsies; Lanes 7–13, patch/plaque-stage biopsies. Lane M, molecular size marker (Generuler; MBI Fermentas, St. Leon-Rot, Germany). B, sequence analysis of reverse transcription-PCR products of 240 and 392 bp demonstrating the wild-type transcript and the alternative transcript that exhibits retention of a 152-bp sequence corresponding to intron 5.

Fig. 2. Top, schematic representation of the FAS-encoding exons, the FAS protein, and amplified regions of FAS cDNA in this study. Bottom, location of retained intron found in cDNA synthesized from RNA isolated from CTCL biopsies and schematic representation of altered protein encoded by mRNA with retained intron 5. CRD, cysteine-rich domains; TM, transmembrane domain. Nucleotides are numbered starting with the ATG encoding the initiating methionine as 1 (taken from GenBank accession number M67454).
splicing of Fas generating this particular variant is specific for CTCL and is an early event in lymphomagenesis. Detection of this transcript might therefore be useful in the early diagnosis of this group of lymphomas. From one patient with CD30− PCLTCL, we analyzed the presence of this splice variant in a skin biopsy specimen obtained at the time of diagnosis and in a separate skin biopsy specimen obtained 7 months later (Table 1, patient 15a and 15b). Analysis revealed the identical splice variant (Fig. 1A, Lanes 13 and 14), suggesting that alternative splicing of Fas is a persistent event.

Translation of the aberrantly spliced variant results in a protein that contains 32 novel amino acid residues and terminates prematurely at codon 201. The predicted translation product encodes a form of Fas that does not contain a functional transmembrane anchor domain and lacks the death domain (Fig. 2). Therefore, if the aberrantly spliced variant is translated exclusively in a cell, the resulting aberrant Fas receptor would not be expressed on the plasma membrane. If both the wild-type and alternative transcript are translated simultaneously, assembly and expression of dysfunctional mixed receptor complexes are predicted to occur (10, 11).

In conclusion, we found a splice variant of the Fas gene in CTCL, Fas protein could not be detected on neoplastic T cells in 9 cases (56%). In five of the seven skin biopsy specimens in which the splice variant was identified, Fas protein expression was not detectable (Table 1).

In addition, three missense point mutations and two silent point mutations were detected in the 15 patients with advanced CTCL. Two missense point mutations detected in one patient were located in the extracellular domain (G to C at position 406 causing substitution of Thr for Arg at codon 75) and the cytoplasmic domain and is predicted to interfere dominantly with the function of Fas. The observed low frequency of deleterious point mutations in the Fas gene in CTCL corresponds with the recent findings of Dereure et al. (12).

Although alternative splicing is used extensively by genes involved in apoptosis, a role for this mechanism in the pathogenesis of lymphoma has scarcely been described thus far (13, 14). The function of the Fas gene is regulated at the level of pre-mRNA splicing in human thymocytes, where five splice variants have been demonstrated (11). Another three different Fas splice variants have been detected in leukocytes from patients with silicosis (9). One of the regulators of alternative splicing of Fas is TIA-1, an apoptosis-promoting protein that has been shown to be expressed by neoplastic T cells in a subset of patients with MF (15, 16).

In conclusion, we found a splice variant of the Fas tumor suppressor gene, associated with formation of a dysfunctional protein that dominantly interferes with Fas-induced apoptosis, frequently and specifically occurring in patients with CTCL. This could be implicated in the pathogenesis of CTCL by allowing continued clonal expansion of activated T cells and in tumor progression by reducing the susceptibility to antitumor immune responses.

Acknowledgments

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References


Table 1. Clinical features, Fas mutations and alternative transcripts, and Fas protein expression of 15 patients with advanced CTCL.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Diagnosis</th>
<th>Age, sex</th>
<th>Disease course</th>
<th>Fas protein expression</th>
<th>Nucleotide change</th>
<th>Type of mutation</th>
<th>Protein alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MF tumor stage</td>
<td>46 F</td>
<td>D + 12 mo</td>
<td>–</td>
<td>836 C→T</td>
<td>Silent</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MF tumor stage</td>
<td>83 F</td>
<td>D + 18 mo</td>
<td>+</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MF tumor stage</td>
<td>88 M</td>
<td>D + 10 mo</td>
<td>+</td>
<td>Insertion of 152 bp at nucleotide 699</td>
<td>Frameshift</td>
<td>Termination at codon 201</td>
</tr>
<tr>
<td>4</td>
<td>MF tumor stage</td>
<td>68 M</td>
<td>D + 15 mo</td>
<td>–</td>
<td>836 C→T</td>
<td>Silent</td>
<td></td>
</tr>
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<td>5</td>
<td>MF tumor stage</td>
<td>57 M</td>
<td>A + 36 mo</td>
<td>+ (50%)</td>
<td>Insertion of 152 bp at nucleotide 699</td>
<td>Frameshift</td>
<td>Termination at codon 201</td>
</tr>
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<td>6</td>
<td>MF tumor stage</td>
<td>54 M</td>
<td>D + 9 mo</td>
<td>–</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>MF tumor stage</td>
<td>68 F</td>
<td>D + 89 mo</td>
<td>–</td>
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<td>Frameshift</td>
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<td>8</td>
<td>CD30− PCLTCL</td>
<td>87 F</td>
<td>D + 5 mo</td>
<td>–</td>
<td>406 G→C</td>
<td>Missense</td>
<td>R71T</td>
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<td>9</td>
<td>CD30− PCLTCL</td>
<td>74 M</td>
<td>D + 18 mo</td>
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<td>None</td>
<td>None</td>
<td></td>
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<tr>
<td>10</td>
<td>CD30− PCLTCL</td>
<td>63 F</td>
<td>D + 14 mo</td>
<td>–</td>
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<td>None</td>
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<td>11</td>
<td>CD30− PCLTCL</td>
<td>82 F</td>
<td>D + 27 mo</td>
<td>–</td>
<td>None</td>
<td>None</td>
<td></td>
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<td>12</td>
<td>CD30− PCLTCL</td>
<td>73 M</td>
<td>D + 31 mo</td>
<td>nd</td>
<td>Insertion of 152 bp at nucleotide 699</td>
<td>Frameshift</td>
<td>Termination at codon 201</td>
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<td>13</td>
<td>CD30− PCLTCL</td>
<td>81 M</td>
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<td>+</td>
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<td>None</td>
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<td>14</td>
<td>CD30− PCLTCL</td>
<td>67 M</td>
<td>D + 9 mo</td>
<td>–</td>
<td>Insertion of 152 bp at nucleotide 699</td>
<td>Frameshift</td>
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<td>15a</td>
<td>CD30− PCLTCL</td>
<td>87 M</td>
<td>D + 24 mo</td>
<td>+ (75%)</td>
<td>892 C→T</td>
<td>Missense</td>
<td>I233T</td>
</tr>
<tr>
<td>15b</td>
<td>CD30− PCLTCL</td>
<td>87 M</td>
<td>D + 17 mo</td>
<td>+ (75%)</td>
<td>Insertion of 152 bp at nucleotide 699</td>
<td>Frameshift</td>
<td>Termination at codon 201</td>
</tr>
</tbody>
</table>

a A+, alive with disease; D+, died of lymphoma; mo, months after taken biopsy; D−, died of other cause.

b nd, not done.


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