Novel and Highly Recurrent Chromosomal Alterations in Sézary Syndrome


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Abstract

This study was designed to identify highly recurrent genetic alterations typical of Sézary syndrome (Sz), an aggressive cutaneous T-cell lymphoma/leukemia, possibly revealing pathogenetic mechanisms and novel therapeutic targets. High-resolution array-based comparative genomic hybridization was done on malignant T cells from 20 patients. Expression levels of selected biologically relevant genes residing within loci with frequent copy number alteration were measured using quantitative PCR. Combined binary ratio labeling-fluorescence in situ hybridization karyotyping was done on malignant cells from five patients. Minimal common regions with copy number alteration occurring in at least 35% of patients harbored 15 bona fide oncogenes and 3 tumor suppressor genes. Based on the function of the identified oncogenes and tumor suppressor genes, at least three molecular mechanisms are relevant in the pathogenesis of Sz. First, gain of cMYC and loss of cMYC antagonists (MXII and MNT) were observed in 75% and 40% to 55% of patients, respectively, which were frequently associated with deregulated gene expression. The presence of cMYC/MAX protein heterodimers in Sézary cells was confirmed using a proximity ligation assay. Second, a region containing TP53 and genome maintenance genes (RP1A/HIC1) was lost in the majority of patients. Third, the interleukin 2 (IL-2) pathway was affected by gain of STAT3/STAT5 and IL-2 (receptor) genes in 75% and 30%, respectively, and loss of TCF8 and DUSP5 in at least 45% of patients. In sum, the Sz genome is characterized by gross chromosomal instability with highly recurrent gains and losses. Prominent among deregulated genes are those encoding cMYC, cMYC-regulating proteins, mediators of MYC-induced apoptosis, and IL-2 signaling pathway components. [Cancer Res 2008; 68(8):2689–98]

Introduction

Sézary syndrome (Sz) is an aggressive type of cutaneous T-cell lymphoma/leukemia of skin-homing, CD4+ memory T cells and is characterized by erythroderma, generalized lymphadenopathy, and the presence of neoplastic T cells (Sézary cells) in the skin, lymph nodes, and peripheral blood (1). Sz has a poor prognosis, with a disease-specific 5-year survival of ~24% (1).

Significant effort has been directed toward elucidating the molecular genetic events underlying this malignancy with the goal of facilitating early diagnosis and providing targets for directed therapeutic intervention. Cytogenetic and conventional comparative genomic hybridization (CGH) studies have uncovered a number of structural and numerical chromosomal aberrations in Sz, but highly recurrent genetic lesions have not been identified (2–7). Gene expression profiling of Sézary cells has revealed deregulated expression of several oncogenes and tumor suppressor genes, including TGF-β receptor II, JUNB, STAT4, MMP9, MXII, and TWIST (8–11).

Although many genetic alterations have been identified in previous studies, the small number of included patient samples and variation of the criteria used for the diagnosis of Sz have resulted in differing results and has impeded the delineation of genetic alterations typical of this malignancy. In addition, the low resolution of the cytogenetic analyses applied has limited the identification of presumed Sz-relevant genes located in regions with chromosomal alteration. In this multicenter study, we aimed to obtain a detailed catalogue of numerical and structural chromosomal abnormalities in a large group of well-defined Sz patients using high-resolution array-based CGH in combination with combined binary ratio labeling (COBRA), fluorescence in situ hybridization (FISH), and in situ proximity ligation assays (PLA).

Materials and Methods

Selection of patients. Cryopreserved or fresh blood samples from 20 patients with Sz (10 males, 10 females; median age, 69 y) were available for inclusion in this study (clinical characteristics are summarized in Supplementary Table S1). Diagnosis of Sz was based on criteria defined in the WHO/European Organization for Research and Treatment of Cancer classification (1). All patients presented with erythroderma and showed highly elevated CD4/CD8 ratios and clonality of T cells in peripheral blood. Follow-up data revealed that nine patients had died of Sz; the median survival time was 31 mo. In all patients, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density centrifugation. From patients 3, 4, 5,
and 7, CD4+ T cells were subsequently isolated from PBMCs by negative selection with magnetic beads (CD4+ T-cell isolation kit, Miltenyi Biotech). CD4+ T cells isolated with MACS beads from peripheral blood of three patients with an erythroderma secondary to atopic dermatitis and seven healthy donors (of which six were in vitro activated as previously described; ref. 12) were used as controls for quantitative PCR experiments.

Approval for these studies was obtained from the respective institutional review boards. Informed consent was provided according to the Declaration of Helsinki.

**Extraction of DNA and RNA.** DNA was isolated from 1 × 10^6 Ficoll purified lymphocytes with the Genomic-tips 20/G kit (Qiagen). RNA was extracted from isolated CD4+ T cells (Sz patients or controls) with the RNeasy kit (Qiagen).

**Array-based CGH and data analysis.** Genome-wide analysis of DNA copy number changes of patient samples was done with high-resolution array-based CGH. Fabrication and validation of the array, hybridization methods, and analytic procedures have been described elsewhere in detail (13, 14). The particular bacterial artificial chromosome (BAC) set used to produce these arrays is distributed to academic institutions by the Wellcome Trust Sanger Institute (Cambridge, United Kingdom) and contains 3,500 individual BACs each harboring ≈100 kb targets spaced at ≈1 Mb density, thus covering 10% of the full genome. Genomic positions were established based on National Center for Biotechnology Information Build 35, Ensembl database version 36.35. Data were analyzed using CAPWeb and visualized using VAMP (15). Candidate genes were selected from the "Atlas of Genetics and Cytogenetics in Oncology and Haematology," whereas bona fide oncogenes and tumor suppressor genes within this list were identified using the Cancer Gene Census list.

Several seemingly isolated gains and losses involving single reporter BAC clones only (e.g., 5p15.33, 1p21.1) were excluded from the analysis because they reside in regions with described copy number variations.

**Quantitative real-time PCR.** cDNA synthesis was done on 1 μg of total RNA after treatment with RQ1 DNase I (Promega), using iScript reverse transcriptase (Bio-Rad), oligo(dT)12–18, and random hexamer priming (Bio-Rad) in a final volume of 20 μL. Real-time PCR was done with the MyiQ instrument and the SYBR Green Supermix (Bio-Rad). Primer sequences for amplification of selected transcripts are given in Supplementary Table S1. In all samples, chromosome condensation was evaluated using the MyIQ software (Bio-Rad) and the second derivative maximum algorithm, whereas confirmation of the specificity of the PCR product and standard curves were done as previously described (16).

**COBRA-FISH.** COBRA-FISH analysis was done on short-term cultures of peripheral blood samples from five patients (numbers 2, 3, 5, 6, and 7 in Supplementary Table S1). In all samples, chromosome condensation was chemically induced and resulting chromosome preparations were hybridized according to previously described protocols (17, 18).

In situ PLA. MACS-isolated CD4+ Sézary cells (patient 1, 3, and 7) or control CD4+ T cells from a healthy control were either stimulated as previously described; ref. 17, 18) or deposited directly on glass slides using a 63× objective (Plan-Neofluar, Zeiss) for in situ analysis. Proximity probes (oligonucleotides attached to antibodies against the two target proteins) guide the formation of circular DNA strands when bound in close proximity. The DNA circles in turn served as templates for localized rolling-circle amplification, allowing individual interacting pairs of protein molecules to be visualized and counted.

**Results**

**Chromosomal gains are more frequent than losses in Sézary syndrome.** In all 20 Sz patients, multiple chromosomal imbalances were identified. Overall, gains were more frequent than losses. A ratio plot of a single Sz patient (number 2 in Table S1), an cumulative dot plot/heatmap of all data, and a corresponding frequency plot of gains and losses for all Sz samples are shown in Fig. 1A to C. These data were used by VAMP to demarcate recurrent minimal genomic alterations (15, 21) in tumor cells using a threshold of 35% and excluding the X and Y chromosomes (Fig. 1D; Table 1).

Two broad regions and several focal areas of gains were frequently observed in Sz samples. Most involved was the long arm of chromosome 17 with a complex pattern. In at least 80% of the patients, gains were observed in the region 17q21.31–17q23. Gains at 8q24.1–q24.3 were detected in 15 of 20 (75%) samples; 5 minimal common regions of gain at chromosome 8 could be distinguished at megabase positions 42.15–42.67, 50.88–58.10, 62.41–79.80, 101.25–113.66, 127.56–136.65, and 139.31–146.17. Using the frequency of amplifications, gains and losses (FrAGL) option of VAMP, highly recurrent isolated gains were found at 2p11.2, 4p16.1, and 7p21.1.

The most frequent regions of loss were found at chromosomes 17 and 10 and (albeit to a lesser extent) at chromosomes 2, 5, 12, and 13. The most frequently lost region consisted of 17p13 to 17p12 detected in 15 of 20 (75%) samples, with a peak at 17p13.1–17p12.1 (Mb 6.07–7.68). Multiple discrete minimal overlapping regions of loss could be identified at chromosome 10: one at the short arm [10p11.22 (Mb 29.84–33.17)] and seven at the long arm of the chromosome in the region from 10q22–10q26 (see Table 1). In addition, highly recurrent isolated losses were observed at 3q26.33, 5q14.3, 7p14.1, 9p13.1–9p12, and 16p11.2.

**COBRA-FISH.** In five patients, COBRA-FISH was done to analyze structural chromosomal alterations. This detailed karyotyping complements the array-CGH data by detecting polyplody (e.g., 3n or 4n) and balanced rearrangements (translocation, inversion). As in previous studies (2, 3), several structural rearrangements were observed in all five cases, but no recurrent translocation breakpoint were identified (example given in Supplementary Fig. S1; results summarized in Supplementary Table S3). The most frequently observed alterations involved chromosomes 10 and 17, detected in all five cases; chromosome 8, detected in four of five cases; and chromosome 12, detected in three of five cases. Recurrent interstitial deletion of chromosome 10q24 region was observed in three of five patients, confirming array-CGH results.

**Candidate genes residing in loci with copy number alteration.** Copy number alterations may have a causative role in oncogenesis.
through deregulation of the expression of genes residing in the affected chromosomal region. Using the high-definition picture of the Sz genome as summarized in Table 1, we examined minimal common regions in more detail. We screened the data for consistency of recurrences of particular genes and identified 15 bona fide oncogenes and 3 tumor suppressor genes, as well as genes involved in cytokine signaling and in the control of integrity of the genome in the affected loci (Supplementary Table S4).

**Oncogenes and tumor suppressor genes.** The bona fide oncogene most frequently affected by gain in the Sz genome was the \( c\text{MYC} \) oncogene (15 of 20 cases, 75%) on chromosome 8. Moreover, we found losses of the cMYC regulating genes \( MXI1 \) (on chromosome 10q25) and \( MNT \) (on chromosome 17p13) in at least 8 and 11 cases, respectively. Gain of \( c\text{MYC} \) was accompanied by loss of \( MXI1 \) in at least 7 of 20 patients, loss of \( MNT \) in 9 of 20 patients, and a combination of \( MXI1 \) and \( MNT \) loss in 6 of 20 patients. Isolated loss of \( MXI1 \) or \( MNT \) was found in one patient. In aggregate, 18 of 20 patients had defects in \( c\text{MYC} \) and/or \( c\text{MYC} \) antagonists.

To protect cells from the oncogenic potential of continuous cMYC activity, cMYC protein promotes apoptosis by induction of BIM leading to the release of cytochrome c from mitochondria and through the p14ARF-Mdm2-TP53 pathway (22, 23), which is controlled by TWIST (24, 25). We detected loss of 17p13.2 harboring \( TP53 \) and loss of 2q12, leading to loss of \( BIM \) in 15 of 20 and 1 of 20 patients, respectively. There was gain of a chromosomal region on 7p21 containing the \( TWIST \) gene in at least 1 but potentially 8 of 20 (40%) patients. Loss of 9p21, affecting \( CDKN2A \) (encoding p14ARF and p16), was detected in 3 patients. Combined, recurrent genetic lesions in \( TP53, BIM, TWIST, \) or \( CDKN2A \) that may contribute to abrogation of the cMYC-induced apoptotic response was observed in 16 of 20 (80%) of patients.

Finally, we noticed loss of the tumor suppressor gene FAS (on chromosome 10q24), a key regulator of apoptosis in mature T cells (26), in nearly half of the Sz patients.

**Aberrations in genes affecting cytokine signaling.** T-cell activation and proliferation is critically dependent on autocrine/paracrine stimulation through the IL-2 receptor (IL-2R). We identified gains of regions harboring the \( IL-2, IL-2R\alpha, \) or \( IL-2R\beta \) genes in 10% to 30% of Sz patients. Gain of both \( IL-2R\alpha \) and \( IL-2R\beta \) or gain of one of the receptor chains and the \( IL-2 \) gene was encountered in three patients. In addition, deletion of \( TCF8 \) (also known as \( ZEB1 \)), a gene encoding a zinc finger transcription factor that represses IL-2 expression in T cells, was detected in at least 9 of 20 (45%) of the patients. In seven patients, gain of IL-2R components was accompanied by deletion...
Table 1. Minimal recurrent chromosomal aberrations in Sézary syndrome

<table>
<thead>
<tr>
<th>Cytogenetic band</th>
<th>Adjacent clones</th>
<th>Mb Size</th>
<th>Candidate genes</th>
<th>Sézary syndrome (n = 20)</th>
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<td>2p11.2*</td>
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<td>8p11.2–8p11.1</td>
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**Loss and deletion**

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<tr>
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<th>Candidate genes</th>
<th>Sézary syndrome (n = 20)</th>
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<td>2q37</td>
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<td>3q26.33*</td>
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<td>5q13</td>
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<td>5q14.3*</td>
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<td>RP11-326F3 RP11-345K20</td>
<td>72.65 75.73 3.08</td>
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(Continued on the following page)
of TCF8. Taken together, in 13 of 20 (65%) of Sz patients, genetic alterations potentially leading to increased IL-2 signaling were found.

Gain of the STAT3 and STAT5A/STAT5B genes, the major transducers of the IL-2 cytokine signaling pathway that are localized as a tandem on the genome, was detected in 15 of 20 (75%) of Sz patients. Finally, loss of DUSP5, an inhibitor of IL-2 signaling (27), was found in 11 of 20 (55%) of patients. Combined, gains of STAT3/STAT5 and/or loss of DUSP5 was found in 17 of 20 (85%) of Sz patients. Finally, loss of DUSP5, an inhibitor of IL-2 signaling, localized as a tandem on the genome, was detected in 15 of 20 (75%) of Sz patients. This study as well as previous cytogenetic studies shows that the Sz genome harbors numerous chromosomal imbalances, indicating that chromosomal instability is an important characteristic of this malignancy. We therefore focused on genes present in minimal common regions, loss of which may contribute to genetic instability. In 11 of 20 (55%) Sz patients, a loss of 17p13.3–13.1 on the short arm of chromosome 17 was detected. This region harbors at least two candidate tumor suppressor genes involved in genome maintenance: hypermethylated in cancer 1 (HIC1) and replication protein 1 (RPAI; refs. 28, 29).

Table 1. Minimal recurrent chromosomal aberrations in Sézary syndrome (Cont’d)

<table>
<thead>
<tr>
<th>Cyto genetic band</th>
<th>Adjacent clones</th>
<th>Mb</th>
<th>Size</th>
<th>Candidate genes</th>
<th>Sédary syndrome (n = 20)</th>
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<td><strong>Start</strong></td>
<td><strong>Stop</strong></td>
<td><strong>Total (%)</strong></td>
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<td><strong>Start</strong></td>
<td><strong>Stop</strong></td>
<td><strong>Total (%)</strong></td>
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| **+Denotes aberrations identified by FrAGL (threshold, >35%); other aberrations are identified using the minimal common region option of VAMP (threshold, 35%). Genes discussed in the text are in bold; oncogenes and tumor suppressor genes are underlined.**

Confirmation of alterations in gene expression by quantitative real-time PCR. For eight Sz patients, the expression of cMYC, MNT, MXI1, and TWIST (all belonging to the cMYC network) was compared with CD4+ T cells isolated from peripheral blood of patients with erythroderma secondary to atopic dermatitis (n = 3) and normal naive (n = 1) and in vitro activated CD4+ T cells from healthy controls (n = 3; Fig. 2).

In seven of eight Sz patients, cMYC levels were higher when compared with naive or in vitro activated CD4+ T cells, whereas in CD4+ T cells from erythrodermic atopic dermatitis patients, comparable to slightly higher cMYC levels were found (Fig. 2). Expression of TWIST was only detected in Sz patients and was observed in seven of eight Sz patients tested. MNT and MXI1 were expressed at low levels in six and eight Sz patients, respectively. In benign controls, higher expression for MXI1 was observed in two patients with atopic dermatitis, and higher expression for MNT was found in three atopic dermatitis patients as well as two samples of activated T cells.

Detection of cMYC expression and heterodimerization with MAX using in situ PLA. Expression of cMYC protein and heterodimerization with its obligatory partner MAX were visualized by in situ PLA (Fig. 3). Quantification of the number of in situ PLA signals per cell revealed significantly higher expression of cMYC/MAX heterodimers in three Sz patients (Sz patients 1, 3,
and 7) compared with controls (CD4+ T cells). In addition, in a time course experiment done with Sz cells from patient 7, we observed increased expression of cMYC/MAX heterodimers in Sz cells 2, 8, and 24 hours after stimulation with PHA and IL-2 compared with CD4+ T cells.

Discussion

To identify genetic events underlying the pathogenesis of Sz, we conducted a high-resolution analysis of recurrent copy number alterations and structural chromosomal abnormalities in tumor cells from 20 patients diagnosed with Sz according to the criteria of the WHO-European Organization for Research and Treatment of Cancer classification (1).

Our array-CGH method provides improved mapping resolution over previously reported cytogenetic studies and permitted the identification of candidate genes contributing to the pathogenesis of Sz. In addition to fine-mapping of previously described chromosomal alterations, we identified several novel recurrent genetic lesions. Using COBRA-FISH, we detected unbalanced translocations affecting 17p10~p11 and 6q22~23 in four of five and two of five patients with deletion of the proximal or distal regions, respectively.

The chromosomal regions showing gain most frequently include 17q23–17q25 and 8q24.1–8q24.3. Chromosomal loss was detected most frequently at 17p13.1 and 10q25. Previous cytogenetic and allelotyping studies in Sz identified 10q22–26 as the chromosomal region most frequently affected by both deletions and unbalanced translocations (2, 4). The present study enabled identification of eight discrete regions of highly recurrent loss in chromosome 10: a novel one at the short arm (10p11.22) and seven at the long arm between 10q22 and 10q26. The deletions at 10q23.32 and 10q24–10q25.1 were described before in a fine-mapping allelotyping study (30). The other losses represent novel genetic lesions in Sz. Of interest, the loss at 10q23.31 contains the FAS gene, and the loss on 10q25.1–10q25.2 includes the MXI1 and DUSP5 genes. The region on 10p11.22 harbors the TCF8 gene.

A recent publication indicated that deletions or translocations affecting the NAV3 gene encoded at 12q21 are found in most Sz patients (9). In the present study, we identified deletions in this region in a minority of patients (at most 6 of 20, 30%; see Supplementary Table S4). We recognized a previously described deletion in the 6q22–24 region (3) in a minority of our patients (25%); according to our analysis, it encompasses a 14.35-Mb fragment between Mb 137.13 and 151.49.

Taking advantage of the high resolution of our analysis, we sought to identify genes, deregulation of which may contribute to the development or progression of Sz. First, we focused on oncogenes and tumor suppressor genes, including cMYC family genes and TP53, residing in minimal common regions within highly recurrent copy number alteration. Given the central role of the IL-2 pathway in the proliferation of mature T cells from which Sz cells are derived, we additionally examined copy number alteration and expression of genes encoding components of this pathway.

Figure 2. mRNA expression in Sz samples and controls as measured by quantitative real-time PCR. Columns, mean of three independent quantitative real-time PCR experiments, depicted relative to the reference gene RPS11; bars, SE.
cMYC family proteins, cMYC antagonists, and cMYC-induced apoptosis. In the present study, we identified increased expression and gain of chromosomal regions harboring cMYC and loss and decreased gene expression levels of the cMYC antagonists MXII and/or MNT in the large majority of Sz patients. This is in accordance with previous gene expression studies by our group reporting low expression of MXII and MNT in Sz cells (11). Deregulated expression of cMYC has been implicated in the etiology of a wide range of hematologic malignancies (31, 32). Increased amounts of the oncogenic transcription factor cMYC contribute to tumorigenesis by promoting proliferation, inhibiting differentiation, and increasing genomic instability (33, 34). The cMYC protein requires heterodimerization with MAX to function as a transcription factor. MNT and MXII also interact with MAX thereby acting as transcriptional repressors of cMYC. Consistent with the proposed cMYC antagonist function, recent studies showed that in mice loss of Mnt and Mxi1 can promote proliferation and facilitate tumorigenesis, leading to disrupted T-cell development and ultimately T-cell lymphomas (35, 36). In Sz cells, gene expression levels of cMYC were comparable to CD4+ T cells from erythrodermic atopic dermatitis patients, which are known to be highly activated, proliferating T cells. However, in contrast to samples from Sz patients, the atopic dermatitis samples also

**Figure 3.** Detection of endogenous cMYC/MAX heterodimers in Sézary cells by in situ PLA.

A, cytospin of CD4+ T cells from Sz patient 3. cMYC/MAX heterodimers were visualized by staining cells with proximity probes directed against cMYC and MAX, followed by ligation and amplification as outlined in ref. 22. The hybridization probes were labeled with Alexa 555 (red), the cytoplasm was counterstained with FITC-labeled antibody to actin (green), and the nuclei were stained with Hoechst 33342 (blue). B, quantification of signals and comparison with a healthy donor. MACS-isolated CD4+ T cells from a Sézary patient (Sz 7) or from a healthy control were stimulated with PHA 1 μg/mL and IL-2 100 units/mL and samples were drawn at 0, 2, 8, and 24 h after stimulation. Signals were quantified as described (23).
expressed MNT and, to a lesser extent, MX11, in line with intact physiologic regulatory control of cMYC expression (35–37). In line with the hypothesized deregulation of cMYC in Sz, we detected increased expression of cMYC/MAX heterodimers in Sz cells compared with controls, suggesting that the increased levels of cMYC mRNA are associated with increased transcriptional activity.

cMYC-induced apoptosis acts as a threshold to malignant transformation, and additional genetic alterations that hamper cMYC-induced apoptosis through disruption of downstream effector molecules or by specific mutations in cMYC can profoundly accelerate cMYC-dependent tumor progression. Mutations of TP53 or p14ARF collaborate with cMYC in the induction of lymphomas (23), whereas high expression of TWIST can inhibit the proapoptotic effect of cMYC through inhibition of the p14 ARF/TP53 pathway in neuroblastoma (24). The present study suggests that in Sz, this cMYC feedback system is abrogated by deletions of 17p13.2, leading to loss of TP53, deletions of 2p21 containing CDKN2A, which encodes p14ARF, as well as gain of a region in 7p21 containing the TWIST gene. These observations are in line with previous studies showing that TWIST is highly expressed in Sz cells compared with non-malignant CD4+ T cells (11).

Taken together, 18 of 20 Sz patients harbored genetic lesions potentially leading to increased levels of cMYC that were accompanied by defects in the cMYC-induced apoptosis in 16 of 20 patients, thus strongly suggesting that deregulation of the cMYC signaling network is a pivotal event in the pathogenesis of Sz.

**Cytokine and STAT signaling.** Recent studies have led to the recognition that autocrine/paracrine signaling of IL-2, IL-7, and IL-15 cytokines, resulting in phosphorylation of STAT3 and STAT5 proteins, plays a crucial role in the pathogenesis of Sz (38–41). Our array-CGH results provide a rationale for the activation of this cytokine signaling pathway because gain of IL-2, IL-2Ra, and IL-2Rβ, combined with gain of the STAT3/STAT5 cluster and deletions of DUSP5 and TCF8, which inhibit IL-2 production and IL-2 signaling, respectively (27, 42), was shown in the majority of patients. Deregulated stimulation through the IL-2R combined with disrupted apoptosis (e.g., by loss of the FAS gene, which was detected in at least 45% of the patients) is likely to be responsible for uncontrolled proliferation. Gain of chromosomal DNA encoding the IL-7 gene in 65% of the patients suggests that the observed elevated IL-7 protein levels in cutaneous T-cell lymphoma patients might not only result from production by skin cells (43) but could, in part, also be the result of secretion by tumor cells themselves as was previously postulated (44).

**Chromosomal instability and epigenetic modifications.** Focal deletions at 17p13.3–17p13.1 leading to loss of TP53, RPA1, and HIC1 was detected in the majority of Sz patients and may play an important role in the widespread chromosomal and genetic instability that is a characteristic feature of this disease. Loss of HIC1 function promotes tumorigenesis by loss of direct transcriptional repression of SIRT1, leading to deacetylation of TP53, whereas loss of RPA1 directly impairs DNA double-strand break repair and maintenance of chromosomal stability (28, 29). The synergy between Hic1 and Tp53 in tumor suppression is illustrated by studies in knockout mice showing that germ-line disruption of one copy each of HIC1 and TP53 on opposite chromosomes (trans) or on the same chromosome (cis) resulted in an altered spectrum, earlier appearance, increased prevalence, and aggressiveness of tumors (45).

Recent experiments in mice showed that Rpa1 function is essential for DNA double-strand break repair maintenance of chromosomal stability and tumor suppression. Mice carrying a heterozygous missense change in one of the DNA-binding domains of Rpa1 developed lymphoid tumors characterized by large-scale chromosomal changes as well as segmental gains and losses (46). Combined, the loss of TP53, HIC1, and RPA1 in Sézary cells will probably contribute to genetic instability.

**Hypothetical model of the pathogenesis of Sézary syndrome.** Collectively, the results of this study show that the Sz genome is characterized by a combination of highly recurrent chromosomal alterations. Prominent among genes residing in minimal common regions are genes encoding cMYC, cMYC-regulating proteins, and mediators of cMYC-induced apoptosis. In
addition, these loci harbor IL-2 signaling pathway component genes known to encode critical regulators of T-cell proliferation and genes involved in the maintenance of genetic stability. Whether these specific chromosomal alterations occur at the first steps of tumor development or are selected during progression of the disease is at present unknown. Hypothetically, the combined signaling alterations that may occur as a consequence of the observed genetic alterations could lead to increased proliferation and diminished apoptosis of malignant T cells (see Fig. 4 for hypothetical model). Indeed, we show that several of these potentially biologically relevant genes show altered gene expression at RNA level (TWIST, MXI1, MNT, and cMYC) and protein level (cMYC). Nevertheless, to further substantiate the functional significance of the identified gene protein products in the pathogenesis of Sz, additional (functional) studies are clearly needed.

Most importantly, our data suggest that cMYC, TWIST, and STAT proteins provide promising novel therapeutic targets in the treatment of Sz. Several approaches to target cMYC are presently under investigation (47–49), and similarly, novel series of inhibitors of STAT proteins (e.g., cucurbitacin) are currently tested in clinical trials and might, if proven safe and effective, lead to new therapeutic approaches in Sz (50, 51). Because present treatment of Sz is largely unsatisfactory, additional studies to validate these novel therapeutic targets in larger numbers of patients are clearly warranted.

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