High-throughput mutation profiling of CTCL samples reveals KRAS and NRAS mutations sensitizing tumors toward inhibition of the RAS/RAF/MEK signaling cascade

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Cutaneous T-cell lymphomas (CTCLs) are malignancies of skin-homing lymphoid cells, which have so far not been investigated thoroughly for common oncogenic mutations. We screened 90 biopsy specimens from CTCL patients (41 mycosis fungoides, 36 Sézary syndrome, and 13 non–mycosis fungoides/Sézary syndrome CTCL) for somatic mutations using OncoMap technology. We detected oncogenic mutations for the RAS pathway in 4 of 90 samples. One mycosis fungoides and one pleomorphic CTCL harbored a KRASG12D mutation; one Sézary syndrome and one CD30+ CTCL harbored a NRASG61K amino acid change. All mutations were found in stage IV patients (4 of 42) who showed significantly decreased overall survival compared with stage IV patients without mutations (P = .04). In addition, we detected a NRASG61K mutation in the CTCL cell line Hut78. Knockdown of NRAS by siRNA induced apoptosis in mutant Hut78 cells but not in CTCL cell lines lacking RAS mutations. The NRASG61K mutation sensitized Hut78 cells toward growth inhibition by the MEK inhibitors U0126, AZD6244, and PD0325901. Furthermore, we found that MEK inhibitors exclusively induce apoptosis in Hut78 cells. Taken together, we conclude that RAS mutations are rare events at a late stage of CTCL, and our preclinical results suggest that such late-stage patients profit from MEK inhibitors. (Blood. 2011;117(8):2433-2440)

Introduction

Cutaneous T-cell lymphomas (CTCLs) are rare malignancies of skin-homing T lymphocytes. Curative modalities have thus far proven elusive. CTCL microarray studies have revealed novel clusters in association with prognosis.1 Array-based comparative genomic hybridization (CGH) combined with gene expression profiling identified highly recurrent chromosomal alterations both in mycosis fungoides (MF) and Sézary syndrome (SS) patient specimens.2,3 For example, FASTK and SKAP1 gene loci showed recurrent gains, and these genes also increased expression, whereas RB1 and DLEU tumor suppressor genes displayed diminished expression associated with loss. In another study, recurrent deletion of tumor suppressor genes BCL7A, SMAC/DIABLO, and RHOF in MF was observed.4 Genomic patterns of characteristic of MF differ markedly from SS.5 This might imply discriminative molecular pathogenesis and different therapeutic requirements.

The RAS-RAF-MEK-ERK signaling pathway regulates cell responses to environmental stimuli and plays a crucial role in many cancers.6 Thus, RAF and MEK are attractive therapeutic targets.7,8 RAS is a small guanine-nucleotide binding protein that is attached to the inner side of the plasma membrane. Activation of RAS causes RAF recruitment and activation by phosphorylation. Activated RAF kinase phosphorylates and activates MEK, which phosphorylates ERK. Three RAS (KRAS, NRAS, and HRAS), 3 RAF (ARAF, BRAF, and CRAF), 2 MEK (MEK1 and MEK2), and 2 ERK (ERK1 and ERK2) isoforms compose the “canonical” mitogen-activated protein kinase pathway. Somatic mutations that are found in many cancers, including colon carcinoma, melanoma, or pancreatic cancer, occur almost exclusively in BRAF, KRAS, or NRAS isoforms.9-11 Typical mutations affect glycine 12 (G12), glycine 13 (G13), or glutamine 61 (Q61) and keep RAS in an activated form. The RAS pathway regulates survival, proliferation, senescence, and differentiation. However, in tumor cells, mutated (oncogenic) RAS preferentially promotes survival and proliferation. Thus, RAF and MEK kinases serve as suitable drug targets. RAF is targeted by inhibitors in preclinical or clinical development, including, for example, RAF265 and PLX4720.12,13 However, targeting the RAF pathway is complex because of the modes of pathway activation and regulation. Recently, it was shown that RAF265 and PLX4720 block MEK-ERK signaling and tumor growth only in cancers harboring a BRAFV600E mutation but not in wild-type BRAF or tumors with a KRAS mutation.12,14,15 Further, treating wild-type BRAF tumors with BRAFV600E specific inhibitors induced tumor growth in vitro and in vivo.14 Thus, MEK...
inhibitors might be of interest in wild-type BRAF cells. Presently, these inhibitors are in dose-finding and early phase 2 studies.8,16,17 AZD6244, a non–adenosine triphosphate-competitive specific MEK inhibitor, was evaluated in a phase 1 clinical trial and reached an appropriate safety profile for further studies.16 It interferes with epidermal homeostasis.18 In a phase 2 clinical trial, AZD6244 showed similar efficacy with respect to progression-free survival as control treatment.19 In a phase 2 clinical trial of 200 patients with melanoma patients, AZD6244 monotherapy resulted in lasting remissions, mainly in patients with documented BRAF mutations.20 Another specific inhibitor targeting MEK is PD0325901. PD0325901 treatment was shown to affect retinal function in PD0325901, and U0126.

toward treatment with the specific MEK inhibitors AZD6244, PD0325901, and U0126. We show that this oncogenic mutation creates an “addiction” to the RAS-RAF-MEK signaling pathway and sensitizes the pathway. We show that this oncogenic mutation creates an “addiction” to the RAS-RAF-MEK signaling pathway and sensitizes the pathway. We show that this oncogenic mutation creates an “addiction” to the RAS-RAF-MEK signaling pathway and sensitizes the pathway. We show that this oncogenic mutation creates an “addiction” to the RAS-RAF-MEK signaling pathway and sensitizes the pathway.

Table 1. RAS mutations in CTCL patients: characteristics of patients with mutations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>CTCL, pleomorphic</td>
<td>Sézary syndrome</td>
<td>Mycosis fungoides</td>
<td>CTCL, CD30*</td>
</tr>
<tr>
<td>Mutation</td>
<td>KRAS (G13D)</td>
<td>NRAS (Q61K)</td>
<td>KRAS (G13D)</td>
<td>NRAS (Q61K)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age, y</td>
<td>70</td>
<td>75</td>
<td>45</td>
<td>61</td>
</tr>
<tr>
<td>TNMB stages</td>
<td>T3 N3 M0 B0</td>
<td>T4 N3 M0 B1</td>
<td>T3 N3 M1 B0</td>
<td>T3 N3 M0 B0</td>
</tr>
<tr>
<td>Staging</td>
<td>IVA2</td>
<td>IVA2</td>
<td>IVB</td>
<td>IVA2</td>
</tr>
<tr>
<td>Tissue</td>
<td>CATS</td>
<td>PBMCs</td>
<td>CATS</td>
<td>CATS</td>
</tr>
<tr>
<td>Skin</td>
<td>Disseminated papules and small tumors</td>
<td>Melano erythrodema without palmoplantar hyperkeratosis</td>
<td>Disseminated tumors with a size up to 5 cm diameter</td>
<td>Right shoulder singular 4 × 3-cm firm tumor</td>
</tr>
<tr>
<td>Lymphocytes, × 10^9 μL</td>
<td>1.1</td>
<td>1.48</td>
<td>3.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Sézary cells,%</td>
<td>None</td>
<td>20%-30%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>4.3</td>
<td>6</td>
<td>16.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

CATS indicates cryo-assayed tissue samples; and PBMCs, peripheral blood mononuclear cells.

OncoMap 3 Core mass-spectrometric genotyping

Briefly, samples were run through OncoMap 3, which addresses 396 somatic mutations across 33 genes (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Whole genome amplified DNA at 5 ng/μL was used as input for multiplex PCR as described previously.21,22 Products were resized and transferred to SpectroCHIPs for analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Allele peaks were analyzed manually (supplemental Figure 1); candidate mutations were validated using multibase hME extension chemistry as previously described.21,22 Primers and probes were designed using Sequenom MassARRAY Assay Design, Version 3.0 software, applying default multibase extension parameters but with the following modifications: maximum multiplex level input adjusted to 6; maximum pass iteration base adjusted to 200.

Illumina sequencing

PCR was performed on 90 formalin-fixed, paraffin-embedded samples using primers for KRAS codon 61, NRAS codons 12/13, and NRAS codon 61 amplification. Reverse primers were tagged at the 5’ end with a 6-bp barcode (primer sequences indicated in supplemental Table 2). To amplify KRAS codons 12/13, a nested PCR was performed; 1 μL of a 1:10 dilution was used as input for a second round of PCR. DNA gel electrophoresis was used to confirm the presence of 60-bp DNA products. Illumina sequencing was performed by the Harvard Biopolymers Facility, Harvard Medical School. The output consisted of approximately 30 million 40-bp fragments per lane; these were aligned to genomic chromosome (HG18) reference sequences using NovoAlign (Novocraft Technologies); alignment coordinates and mismatch positions for aligned fragments were provided by the Harvard Biopolymers Facility. Approximately 15 million reads yielded useful alignment information. Reads with insufficient quality at any base or with truncated barcode regions were excluded from analysis. Approximately 13 million fragments were deemed high quality.

Each high-quality fragment was assigned to its corresponding tumor sample using the identity of bases in the barcode region. The presence of mutation at the bases of interest in KRAS codons 12/13, KRAS codon 61, NRAS codons 12/13, and NRAS codon 61 was also noted, and average variant frequency and quality for each allele at the target base were calculated. A 5% background “noise” rate was assumed; variants with insufficient reads to rise above this baseline frequency were excluded from analysis.

Methods

Patient samples

Ninety CTCL samples were collected at the Department of Dermatology, University Hospital of Zurich (n = 78) and at the Department of Dermatology, Yale School of Medicine (n = 12). The study group included 40 females and 50 males (mean age, 66.4 years; minimum, 19.4 years; maximum, 95.6 years) and consisted of 41 MF, 36 SS, and 13 non-MF/SS CTCL patients (7 anaplastic large cell CD30+ CTCLs, 5 CD4+ small/medium pleomorphic CTCLs, and 1 peripheral aggressive CD8+ CTCL). Staging at diagnosis was as follows: 40 stage I, 4 stage II, 4 stage III, and 42 stage IV.23 Complete lifetime data (first diagnosis, last visit, or date of death) for survival function (ie, Kaplan-Meier curve) was available from 68 patients. Samples consisted of 44 skin (cryo-assayed tissue samples) and 46 blood (peripheral blood mononuclear cells) samples. The clinical presentation of each individual CTCL patient defined the source (skin or blood) of each sample. In general, cryo-assayed tissue samples were collected at early stages when the skin affection was predominant, and peripheral blood mononuclear cells were gathered at later stage CTCL when blood involvement was anticipated. DNA was extracted from all samples for diagnostic purposes and analyzed with a polymerase chain reaction (PCR)–based clonality assay (PCR-denaturing gradient gel electrophoresis). Clonality was present within the T-cell receptor-γ locus in all samples.24 All patients gave informed consent in accordance with the Declaration of Helsinki. The local ethical boards approved the study (Massachusetts Institute of Technology COUHES protocol #0806002814). Details of the patient samples with mutations are shown in Table 1.
Chemicals

U0126 was purchased from Sigma-Aldrich. AZD6244 and PD0325901 were provided by Dr C. Falk (Heidelberg). All inhibitors were solubilized in dimethyl sulfoxide at stock concentrations of 5mM.

Cell culture

CTCL cell lines SeAx, Hut78, MyLa, and HH cells were cultured in RPMI medium supplemented with 10% fetal calf serum and 1mM L-glutamine.

Western blot analysis

A total of 1 × 10⁶ CTCL cells were lysed for 10 minutes in ice-cold radioimmunoprecipitation assay lysis buffer (50mM Tris-HCl, pH 8.0, 120mM NaCl, 1% NP-40, 0.5% sodium dodecyl sulfate, 2mM ethylenediaminetetraacetic acid, 25mM NaF, 0.2mM NaVO₄, 120mM NaCl, 1% NP-40, 0.5% Na-desoxycholate, 0.1% sodium dodecyl radioimmunoprecipitation assay lysis buffer (50mM Tris-HCl, pH 8.0, 120mM NaCl, 1% NP-40, 0.5% sodium dodecyl sulfate, 2mM ethylenediaminetetraacetic acid, 25mM NaF, 0.2mM NaVO₄, 120mM NaCl, 1% NP-40, 0.5% Na-desoxycholate, 0.1% sodium dodecyl sulfate) and complete protease inhibitor cocktail from Roche Diagnostics). Cell debris was removed by centrifugation at 17 000 g for 30 minutes, and equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Then, separated proteins were blotted onto a nitrocellulose membrane (GE Healthcare) followed by blocking with 5% bovine serum albumin in phosphate-buffered saline/Tween (0.05% Tween-20 in phosphate-buffered saline). The following antibodies were used: anti-phospho-ERK (P-p44/p42 (Tyr202/204; Cell Signaling Technology), anti-NRAS (F155; Santa Cruz Biotechnology), anti-ERK2 (C-14; Santa Cruz Biotechnology), and anti-tubulin (Sigma-Aldrich).

PCR and sequencing of cell lines

Total cellular RNA was isolated using the Absolutely mRNA Purification Kit (Stratagene). Total RNA (5 μg) was reverse transcribed with a reverse transcription-PCR kit (Applied Biosystems); 5 μL of cDNA was used for a PCR of 50 μL volume. The following primers were used: KRAS forward, 5′-agggctctgaaataagct-3′; KRAS reverse, 5′-ccaaaaacccaagacagaa-3′; NRAS forward, 5′-ggggtctcaatttcctc-3′; NRAS reverse, 5′-ccagggca-gaaataca-3′; HRAS forward, 5′-ggggcagagacctgtag-3′; HRAS reverse, 5′-ttttaacagacactcata-3′; BRAF_exon15 forward, 5′-ctcaatgcttcgcatgga-3′; BRAF_exon15 reverse, 5′-ggagcttgaatcagtt-3′. PCR was performed, and 30 μL of PCR product was sent for sequencing to Genetic Analysis Technology Consortium. For sequencing, the same primers were used as for PCR.

siRNA transfection and knockdown. SeAx, Hut78, MyLa, and HH cells were transfected with Amaxa transfection kits (Lonza Walkersville) with AllStars Negative Control siRNA (Qiagen) or siRNA oligonucleotides specific for NRAS: siNRAS1 5′-GGCCACUGACAUAUCGCAGCU-3′; siNRAS2 5′-GGCAUAUCGGAUACAGCUCAGCA-3′. SeAx and MyLa cells were transfected with Amaxa transfection kit V according to the protocol established for Jurkat cell lines. Hut78 and HH cells were transfected with Amaxa transfection kit R (Lonza) according to the protocol established for the Hut78 cell line.

Cell death assays. For cell death induction, CTCL cells were stimulated with the indicated concentrations of inhibitors. Cell death was assessed by forward-to-side-scatter profile. Specific cell death was calculated using the following equation: specific cell death % = [(% experimental cell death − % spontaneous cell death)/(100% − % spontaneous cell death)] × 100.

Results

High-throughput mutation profiling and next-generation sequencing of CTCL samples reveals KRAS and NRAS mutations in advanced disease

We identified mutations in KRAS (G13D) and NRAS (Q61K) in 4 of 90 patients: one MF, one SS, one CD30⁺ CTCL, and one pleomorphic CTCL with lymph node involvement (Table 1). Interestingly, all patients harboring KRAS/NRAS mutations were stage IV (Table 1), whereas none of the earlier stage patient samples demonstrated point mutations (P = .04, Fisher exact test). Illumina sequencing of the same 90 samples showed the identical samples being mutated without revealing any additional mutated samples (supplemental Table 2). Illumina mutant allele frequency ranged from 10.69% to 56.24%. These results show that CTCL patients harbor mutations within the RAS pathway and that these mutations are found at a later stage of the disease.

Patients harboring samples with KRAS/NRAS mutations display a significantly reduced survival

Stage IV patients with RAS mutations showed a significantly reduced survival compared with stage IV patients without mutations (P = .04; Figure 1). Comparing stage IV patients to mutated samples to all patients without mutation (ie, stage IV patients without mutated samples plus stage I-III patients; ie, non–stage-corrected) revealed an even increased significance (P = .02; Figure 1). Taken together, we conclude that mutations within the RAS pathway in CTCL patients are associated with an unfavorable clinical outcome.

Direct DNA sequencing reveals NRAS mutation in the CTCL cell line Hut78

We further investigated the implications of RAS mutations on survival and signaling in CTCL. Therefore, we specifically screened for mutations in KRAS, NRAS, HRAS, and BRAF in the 4 CTCL cell lines SeAx, Hut78, MyLa, and HH. By direct DNA and cDNA sequencing, we found a homozygous thymidine-to-cytosine conversion at position 270 of HRAS in the SeAx cell line that resulted in a silent mutation at codon 25. More importantly, we discovered a heterozygous cytosine-to-adenosine conversion at position 436 in the Hut78 cell line. This conversion generates a glutamate-to-lysine exchange at codon 61 (Q61K) (Figure 2A). We did not detect other mutations in KRAS, HRAS, NRAS, or BRAF at critical sites, including codon 12, 13, and 61 for RAS or codon 600 for BRAF, respectively (Figure 2B). RAS kinases are best known as key regulators of the MEK-ERK cascade. Oncogenic NRASQ61K mutations result in hyperactive RAS, which activates the downstream MEK-ERK signaling cascade, thus mediating a prosurvival signal. To analyze for differential activation of the MEK-ERK cascade, we assessed the phosphorylation status of MEK and ERK in all 4 cell lines. Indeed, we observed that ERK phosphorylation
was most prominent in the HUT78 cell line, whereas ERK phosphorylation was less pronounced in SeAx, Myla, or HH cells (Figure 2C). Of note, nonphosphorylated basal ERK expression was comparable in all 4 cell lines (Figure 2C). Although nonphosphorylated basal MEK expression differed in all cell lines, the highest MEK phosphorylation was detected in Hut78 cells carrying the oncogenic NRASQ61K mutation (Figure 2D). Thus, our data show an activating NRASQ61K mutation in the CTCL cell line Hut78 inducing the MEK-ERK signaling cascade.

Oncogenic NRASQ61K is critical for survival of Hut78 cells

To investigate the role of oncogenic NRASQ61K for survival of Hut78 cells, we performed a specific NRAS knockdown by 2 different siRNAs. Delivery of specific siRNAs against NRAS resulted in a substantial decrease of NRAS expression compared with a non-sense control siRNA (Figure 3A). Of note, nonphosphorylated basal ERK expression was comparable in all 4 cell lines (Figure 2C). Although nonphosphorylated basal MEK expression differed in all cell lines, the highest MEK phosphorylation was detected in Hut78 cells carrying the oncogenic NRASQ61K mutation (Figure 2D). Thus, our data show an activating NRASQ61K mutation in the CTCL cell line Hut78 inducing the MEK-ERK signaling cascade.

MEK inhibitors cause growth inhibition in Hut78 cells

RAS mutations occur in approximately 4 of 36 CTCL patients at advanced disease stage IV. RAS mutations were associated with poor prognosis compared with nonmutant RAS patients at stage IV. This prompted us to ask whether inhibitors of the RAS/RAF/MEK/ERK pathway could be of relevance for treatment of RAS mutated patients. Because RAS and RAF mutations are found in nearly 30% of human cancers, several MEK inhibitors are under clinical development.26 AZD6244 and PD0325901 were both shown to be potent MEK inhibitors that inhibit cell growth in vitro and in vivo.27,28 Both inhibitors recently entered phase 2 clinical trials.16,17 AZD6244, PD0325901, and the MEK inhibitor U0126 blocked basal ERK phosphorylation in Hut78 cells (Figure 4A). This suggests that MEK inhibitors are taken up in cell lines and inhibit RAS-RAF-MEK-ERK signaling. Phosphorylation of MEK was slightly increased by AZD6244 and PD0325901 but not by U0126 in both cell lines (Figure 4A). In addition, we investigated the inhibitory effect of the MEK inhibitors on cell growth. We observed that Hut78 cells respond for significantly inhibitor concentrations compared with SeAx, Myla, and HH cells (Figure 4B-D). The 50% inhibitory concentration values for Hut78 were: 3.1nM for AZD6244, 68nM for PD0325901, and 39nM for U0126.
This shows that NRASQ61K sensitizes cells toward treatment with MEK inhibitors strongly affecting cell growth.

MEK inhibitors induce apoptosis uniquely in Hut78 cells

To check whether NRASQ61K also sensitizes toward induction of apoptosis in CTCL, we treated the CTCL cell lines with the 3 MEK inhibitors and measured cell death after 48 and 72 hours. As expected, AZD6244, PD0325901, and U0126 induced apoptosis up to 90% in the NRASQ61K harboring Hut78 cell line only, whereas cell lines without this mutation were refractory (Figure 5). Of note, apoptosis induction occurred already at low concentrations of the inhibitor (100nM to 500nM). These concentrations are clinically relevant plasma concentrations (eg, for AZD6244).29 Taken

Figure 4. NRASQ61K sensitizes for treatment with MEK inhibitors. (A) Hut78 cells were left untreated or treated with 1 μM AZD6244, 1 μM PD0325901, and 1 μM U0126 for 4 hours. Then, cells were lysed, and the basal phosphorylation level of ERK and MEK was assessed by Western blot with specific anti-phospho-ERK and with specific anti-phospho-MEK antibodies. Equal loading was verified by antitubulin antibodies. (B-D) CTCL cell lines were incubated with indicated concentrations of MEK inhibitors U0126 (B), AZD6244 (C), and PD0325901 (D) for 72 hours. Cell growth was measured by Cell Titer Glo according to the manufacturer’s instructions. The 50% inhibitory concentration values, at which 50% of the cell growth inhibition compared with dimethyl sulfoxide was observed, were calculated by GraphPad Prism Version 5 software. Data are representative for 3 independent experiments.

Figure 5. NRASQ61K sensitizes for apoptosis by MEK inhibitors. (A-C) All 4 CTCL cell lines were incubated with indicated concentrations of MEK inhibitors U0126 (A), AZD6244 (B), and PD0325901 (C) for 48 hours (left) and 72 hours (right). Then, apoptosis was determined, and specific apoptosis was calculated as described in “Cell death assays.” Data are representative at least 3 independent experiments.
together, these results show that NRAS mutations confer sensitivity toward different MEK inhibitors in CTCL.

Discussion

We analyzed 90 samples of CTCL patients by OncoMap and found 2 KRAS (G13D) and 2 NRAS (Q61K) mutations. Our results were validated by next-generation sequencing (Illumina).

Activating KRAS and NRAS mutations are among the most common oncogenic lesions detected in human cancer, including myeloproliferative disorders and leukemias (eg, acute lymphoblastic leukemia). In a mutation screen of key exons of a distinct set of genes (NRAS, KRAS, FLT3, PTPN11, and BRAF) in acute lymphoblastic leukemia at diagnosis and disease recurrence, it was shown that somatic mutations deregulating the mitogen-activated protein kinase pathway were present in 35% and 25%, respectively. Sabnis et al showed that KRAS initiates leukemia in hematopoietic stem cells. Earlier, immunohistochemistry displayed increased detection of RAS in advanced stages of MF compared with early stages. On the RNA or protein level, an overexpression of the raf2 gene (belonging to the RAS superfamily) was detectable in SS patients. These results were later confirmed for KRAS. In line with these reports, it is not surprising that CTCLs show mutations in KRAS and NRAS. We hypothesize that RAS mutations reveal a late event in the course of CTCL and are not mutations that induce this type of lymphoma.

We also identified a CTCL cell line that carries the same mutation for NRAS (Q61K) as detected in patient samples. This cell line (Hut78) was isolated from a patient with SS, a severe form of CTCL. The stage of this patient was not reported in the publication. The NRAS mutation correlated with stronger MEK-ERK signaling compared with other cell lines that do not show this mutation.

The overall survival of the patients with KRAS/NRAS mutations decreased significantly compared with patients without mutations within the same stage group. This observation is interesting compared with findings in other tumors. The RASCAL II study analyzed the impact of KRAS mutations on survival for 3439 colon carcinoma patients. KRAS mutations had no overall effect on survival. Only one specific mutation (glycine-to-valine conversion at codon 12) in KRAS, which occurs in approximately 9% of patients, predicted a poorer prognosis. The impact of RAS mutations on survival for acute myeloid leukemia is discussed controversially. Seven studies showed a similar clinical outcome, 3 studies showed a worse clinical outcome, and 2 studies a favorable prognosis for patients harboring RAS mutations. This suggests that RAS mutations have a very mild, or most probably no effect on survival of acute myeloid leukemia patients. Interestingly, acute myeloid leukemia patients carrying RAS mutations benefited from a higher chemotherapeutic dose, indicating that oncogenic mutations may confer higher sensitivity to certain treatments. It has been reported that NRASG12R mutations may sensitize to BRAF inhibition or that NRASG12A mutation confers resistance toward apoptosis. Thus, these data suggest that patients with RAS mutations benefit from treatment inhibiting the RAS-RAF-MEK-ERK pathway. We have observed that knockdown of NRAS by 2 different siRNAs induces apoptosis in Hut78 cells but not in cell lines harboring WT RAS, thus suggesting that Hut78 depends on hyperactive RAS-RAF signaling.

This dependency on RAS signaling could therefore have therapeutic implications for these patients. Indeed, we found that the NRAS mutation sensitizes toward treatment with MEK inhibitors AZD6244, PD0325901, and U0126. All 3 inhibitors caused apoptosis in Hut78 cells and not in the other cell lines devoid of the respective NRAS mutation. These results suggest that CTCL patients with RAS mutations may benefit from treatment with MEK inhibitors.

Whether KRAS mutations also can sensitize toward MEK inhibitors could not be studied because of the absence of this mutation in CTCL cell lines. However, other studies have shown that KRAS mutant cell lines are dependent on KRAS hyperactivity for cell growth and survival. Survival of mutant RAS CTCL patients was significantly different from wild-type RAS CTCL patients within the same stage. However, RAS mutations display a rare event, and other genetic or epigenetic alterations should be considered. Evidence of epigenetic silencing of several individual genes by methylation (besides p16) has previously been reported: p15 (CDKN2B), MLH1, thrombospondin 4 (THBS4), BCL7A, and PTPRG. Mao et al found heterogeneous abnormalities of CCND1 and RB1 in CTCL, suggesting impaired cell cycle control in disease pathogenesis. In a further study, some patients showed a deletion or translocation affecting NAV3. Using CGH, loss of heterozygosity has been described for phosphatase and tensin homolog, and amplified chromosomal regions for HER-2/neu (ERBB2) have been reported. Recently, microarray studies, performed to identify gene expression patterns in CTCL, highlighted the tumor necrosis factor signaling pathway, Th2-specific transcription factors (eg, JUN-B, GATA-3), MYC, and TP53, among others. CTCL might represent a malignancy in which epigenetic change rather than mutations are pivotal. The latter is consistent with the observation that CTCL therapies interfering with transcriptional regulation (eg, interferon, retinoids, and histone deacetylase inhibitors) show hitherto a promising outcome. Nevertheless, certain patient subgroups may benefit from target tumor therapy, as this study shows.

Recently, the combination of molecular cytogenetics (CGH) and microarray gene expression analysis has shown chromosomal arms where DNA and the corresponding RNA copy numbers are altered, including 1q, 3p, 3q, 4q, 12q, 16p, and 16q. These regions probably harbor further gene-level aberrations in CTCL. In the near future, the use of next-generation sequencing focusing on these chromosome arms could reveal novel pivotal genes in the pathogenesis, diagnostic classification, and therapy of CTCL.

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Authorship


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