Somatic rearrangement of the TP63 gene preceding development of mycosis fungoides with aggressive clinical course

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Cutaneous T-cell lymphomas (CTCLs) comprise a heterogeneous spectrum of T-cell neoplasms with widely varying clinical presentation, biologic behavior and overall outcome.1,2 The most common CTCL is mycosis fungoides (MF), which can range from a localized, indolent process to an aggressive lymphoma with widespread cutaneous and extracutaneous involvement and large-cell transformation (MF-LCT). This biologic and clinical heterogeneity is a feature shared with other T-cell lymphomas, including systemic peripheral T-cell lymphomas (PTCLs). Also common to both CTCLs and PTCLs is a limited understanding of genetic mechanisms of pathogenesis and progression, the elucidation of which could facilitate classification, prognostication and individualized therapy. For example, accurate classification of CTCLs often requires careful clinical and pathological follow-up over time, and an enhanced understanding of CTCL genetics might allow earlier, more definitive classification. Furthermore, genetic biomarkers that identify patients at greatest risk of aggressive clinical behavior could allow initiation of earlier or more intensive treatment protocols that might lead to better outcomes. Finally, knowledge of CTCL genetics could improve biologic understanding of this group of diseases and facilitate the development of more specific, targeted therapies.

Although CTCLs and PTCLs are clinically distinct groups of diseases, genetic data have highlighted similarities that suggest biologic interconnectedness. For example, we have shown that chromosomal rearrangements of the DUSP22/IRF4 locus on 6p25.3 are seen in about 30% of both primary cutaneous anaplastic large-cell lymphomas (cALCLs) and systemic ALK-negative ALCLs.3,4 More recently, we identified recurrent rearrangements of TP63 on 3q28 in PTCLs that were associated with poor clinical outcomes.4,5 TP63 encodes p63, a member of the p53 family of transcription factors. Interestingly, although our study was primarily focused on patients with systemic PTCLs, we also identified two patients with unusually aggressive cALCLs that had TP63 rearrangements. Therefore, we undertook the current multi-institutional study to determine the frequency and clinical significance of TP63 rearrangements in an independent series of CTCLs.

We reviewed CTCL biopsy specimens from 136 previously unreported patients (mean age, 60 years; age range, 14–96 years; male/female, 1.4:1). Classification followed World Health Organization criteria and is summarized in Table 1. All cases were evaluated for p63 protein expression by immunohistochemistry using the 4A4 clone as previously described,5 defining positivity as nuclear staining in ≥30% of tumor cells. FISH using dual-fusion and/or breakapart TP63 probes was performed as previously described6 in all cases that were p63 positive by immunohistochemistry. The validity of using immunohistochemistry to select cases for FISH analysis is supported by our previous data, which showed that TP63 fusion transcripts encoded the 4A4 epitope and that these immunohistochemical criteria identified all T-cell lymphomas with TP63 rearrangements.4,5 In the current study, we also performed TP63 FISH in 21 additional p63-negative CTCLs and all lacked TP63 rearrangements.

Immunohistochemistry for p63 was positive in 8 of 136 CTCLs tested (6%), including 5 cALCLs, 1 case of Sézary syndrome, and 2 cases of MF-LCT (Table 1). No positivity was seen in any other subtype, including MF without LCT. One case of MF-LCT had a TP63 rearrangement, representing 1 of 14 (7%) of the cases of MF-LCT examined in this series. This case was a skin biopsy from the elbow of a 79-year-old female obtained in 2006 (Figure 1a). Morphologic examination revealed a dense dermal infiltrate of hyperchromatic tumor cells with >25% large cells, meeting criteria for MF-LCT (Figures 1b and c). The tumor cells showed strong nuclear positivity for p63 by immunohistochemistry (Figure 1d). FISH was positive in the majority of cells using both dual-fusion (Figure 1e) and breakapart probes (not shown), indicating the presence of TBL1XR1/TP63 fusion corresponding to inv(3)(q26q28). Extra copies of the non-rearranged TBL1XR1 and TP63 genes also were present.

We then examined the relationship of the TP63 rearrangement to the development of LCT. The patient first sought medical attention for her skin disease in September 2001. At that time she reported a 6-month history of pruritic scaly papules and plaques involving multiple anatomic sites. We obtained her original skin biopsy from 2001, which had been interpreted as suggestive of evolving MF. The biopsy showed clusters of atypical small lymphocytes, without prominent large cells (Figures 1f and g). Interestingly, many of the dermal lymphocytes were positive by p63 immunohistochemistry (Figures 1h), a finding absent in other cases of MF without LCT examined in the current series (Table 1). FISH demonstrated cells with both the TP63 rearrangement and extra copies of non-rearranged TBL1XR1 and TP63, identical to the subsequent MF-LCT specimen. These findings indicate that TP63 rearrangement and copy number abnormalities involving 3q occurred before the LCT.

The patient’s prior medical history was significant for stage Ib, grade 2 endometrial adenocarcinoma in 1999 for which she underwent total abdominal hysterectomy/bilateral salpingo-oophorectomy with pelvic and para-aortic lymphadenectomy. No metastatic carcinoma was identified in the lymph nodes. Because the origins of early cells leading to MF are poorly understood, we reexamined the lymphadenectomy specimen, which showed preservation of the nodal architecture and patent sinuses containing small lymphocytes (Figures 1j and k). Immunohistochemical staining performed retrospectively demonstrated CD3-positive T cells in and around the sinuses, some of which had nuclear irregularities and were positive for p63 (Figures 1l and m). FISH identified the presence of TBL1XR1/TP63 fusion and extra copies of both genes in these areas (Figure 1n) but showed a normal FISH signal pattern in background reactive cells and in the
Table 1. Frequency of p63 protein expression and TP63 rearrangement in cutaneous T-cell lymphomas

| Cutaneous T-cell lymphoma subtype | Number positive/number tested (%) | Present study | Blood 2012 | Total |
|---------------------------------|-----------------------------------|---------------|------------|-------|
|                                 | p63 protein expression            |               |            |       |
| Mycosis fungoides without       | 0/48 (0)                          | 0/48 (0)      | 0/5 (0)    | 0/53  |
| large-cell transformation        |                                   |               |            |       |
| Mycosis fungoides with          | 2/14 (14)                         | 1/14 (7)      | 1/2 (50)   | 2/16  |
| large-cell transformation        |                                   |               |            |       |
| Sézary syndrome                 | 1/6 (17)                          | 0/6 (0)       | 0/0 (0)    | 1/6   |
| Primary cutaneous anaplastic     | 5/22 (23)                         | 0/22 (0)      | 7/19 (37)  | 12/41 |
| large-cell lymphoma              |                                   |               |            |       |
| Lymphomatoid papulosis          | 0/32 (0)                          | 0/32 (0)      | 0/0 (0)    | 0/32  |
| Primary cutaneous peripheral     | 0/7 (0)                           | 0/7 (0)       | 0/0 (0)    | 0/7   |
| T-cell lymphoma, not otherwise   |                                   |               |            |       |
| specified                       |                                   |               |            |       |
| Subcutaneous panniculitis-like   | 0/4 (0)                           | 0/4 (0)       | Not tested | 0/4   |
| T-cell lymphoma                  |                                   |               |            |       |
| Extramodal NK/T-cell lymphoma,   | 0/2 (0)                           | 0/2 (0)       | 0/0 (0)    | 0/2   |
| nasal type                       |                                   |               |            |       |
| Primary cutaneous CD4-positive   | 0/1 (0)                           | 0/1 (0)       | 0/0 (0)    | 0/1   |
| small/medium T-cell lymphoma     |                                   |               |            |       |
| Total                            | 8/136 (6)                         | 1/136 (1)     | 8/26 (31)  | 16/162|

aNine cases of reactive dermatitis also were tested in the present study and all were negative. bProtein expression was defined by nuclear staining in ≥30% of tumor cells by immunohistochemistry. TP63 rearrangement was considered absent if protein expression was absent and/or negative by FISH, as supported by previous studies. cWithout including the single case (Figures 1F-i) identified retrospectively in a previous biopsy from a patient with mycosis fungoides with large-cell transformation that demonstrated both p63 protein expression and TP63 rearrangement.
Figure 1. MF with TP63 rearrangement. (a) Nodules and plaques on the upper extremity of a 79-year-old female with a history of MF. This photograph was taken in 2006, on the date of the biopsy included in the current series. (b) At low magnification (×100), a hematoxylin and eosin (H&E)-stained section of the biopsy showed an extensive, vaguely nodular lymphocytic infiltrate in the dermis. (c) At higher magnification (×1000), most of the cells were large, transformed lymphocytes, and mitotic figures were readily identified (arrow). These findings supported a diagnosis of MF with large-cell transformation. (d) Immunohistochemistry for p63 performed retrospectively as a part of the current study showed strong positivity in tumor cell nuclei. (e) Dual-fusion FISH demonstrated abnormal fusion signals (arrows), corresponding to TBL1XR1/TP63 fusion in tumor cell nuclei (T; ×600). Extra non-rearranged copies of both TBL1XR1 (green) and TP63 (red) also were observed. FISH also demonstrated nuclei with a normal signal pattern (N), showing two non-rearranged copies each of TBL1XR1 and TP63. (f) Review of a skin biopsy at the time of initial presentation in 2001 showed clusters of lymphocytes in the upper dermis with focal epidermal exocytosis (×200). (g) At higher magnification, the lymphocytes were mostly small and had irregular, hyperchromatic nuclei. (h) Scattered nuclei within the clusters of lymphocytes were positive for p63 by immunohistochemistry. (i) Dual-fusion FISH showed TBL1XR1/TP63 fusion and extra non-rearranged copies of TBL1XR1 and TP63, similar to the 2006 biopsy. (j) Retrospective review of pelvic lymph nodes obtained at the time of hysterectomy for endometrial carcinoma showed normal nodal architecture and expanded sinuses containing histiocytes and lymphocytes (×40). (k) At higher magnification, scattered medium-sized lymphocytes with irregular, hyperchromatic nuclei were observed. (l) Immunohistochemistry for CD3 performed retrospectively highlighted these atypical, sometimes cerebriform cells (arrow). (m) The atypical cells also were positive for p63. (n) Dual-fusion FISH showed TBL1XR1/TP63 fusion and extra non-rearranged copies of TBL1XR1 and TP63, similar to both subsequent biopsies.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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