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The Role of Molecular Analysis in Cutaneous Lymphomas

Janyana M.D. Deonizio, MD,* and Joan Guitart, MD†

The purpose of this review is to summarize the most important molecular techniques for the diagnosis of cutaneous lymphomas. When making a diagnosis, we are looking for the solid clinicopathological correlation. Molecular analysis includes immunophenotyping and clonality analysis, and is important for 2 principal reasons: (1) to confirm the diagnosis in cases where the clinical and/or pathological presentations are nondiagnostic, and (2) to further characterize the nature of the lymphoma. More specifically, we are trying to discern whether the lymphoma is primarily cutaneous or systemic with secondary skin involvement, and we are also attempting to subclassify the tumor. Recently, many techniques have provided a more accurate diagnosis of cutaneous lymphomas and some prognostic implications, including polymerase chain reaction, fluorescence in situ hybridization, and flow cytometry. Fluorescence in situ hybridization is not routinely used in the diagnosis of cutaneous lymphoma, but many studies have shown potential future applications in various areas. Other techniques, such as comparative genomic hybridization, are still confined to the research arena, but have added some insight into the molecular pathogenesis of cutaneous T-cell lymphoma.
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The gold standard in the diagnosis of cutaneous lymphomas is a solid clinicopathological correlation. Molecular analysis includes immunophenotyping and clonality analysis, and is important for 2 principal reasons: (1) to confirm the diagnosis in cases where the clinical and/or pathological presentations are nondiagnostic, and (2) to further characterize the nature of the lymphoma. The additional data are important because a more concrete diagnosis will help patients and their relatives face the consequences of lymphoma diagnosis. Knowing whether the lymphoma is primarily cutaneous or systemic and the subclassification of the tumor will allow the clinician to proceed with a more appropriate treatment plan.

*Department of Dermatology, Northwestern University, Chicago, IL.
†Dermatopathology Unit and Cutaneous Lymphoma Clinic, Northwestern University, Chicago, IL.
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Correspondence Author: Joan Guitart, MD, Department of Dermatology, Northwestern University, 676 N. Saint Clair Suite 1765, Chicago, IL 60611. E-mail: j-guitart@northwestern.edu

The Importance of Clonality

Although cancer can be preceded by oligoclonal expansion of premalignant cells, ultimately it is defined by the unrestricted growth of cells derived from a single cell or a clonal expansion. The detection of a monoclonal population may help distinguish a lymphoma from a reactive process. However, clonality by itself does not imply malignancy, and likewise, a negative clonality result does not imply a benign process. On exposure to antigen-presenting cells, lymphocytes are primed to react against specific epitopes. During this process, genes encoding the antigen receptor immunoglobulin (Ig) for B cells and T-cell receptor (TCR) heterodimer genes for T cells are rearranged. This process referred to as V(D)J recombination involves stepwise rearrangement of variable (V), diversity (D), and joining (J) gene segments. During this process, fragments of nucleotides are deleted and inserted, resulting in an enormous diversity of unique antigen receptors providing high specificity to a vast array of antigens.[1]

Because the evolution of mycosis fungoides (MF) is often slow and early lesions are often characterized by a reactive infiltrate with more benign reactive cells than malignant lymphocytes, the diagnosis of MF is often challenging for the

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dermatopathologist. These unresolved cases are frustrating for patients and physicians alike. The concept of cutaneous lymphoid dyscrasias (CLD) was introduced to address these circumstances. CLD encompasses a series of dermatoses, which are characterized by a chronic course and lack of a triggering event and often are recalcitrant to topical steroids. The biopsy results display a chronic lymphoid infiltrate without significant or only minimal atypia, but frequently a T-cell clone is detected. Parapsoriasis, pigmented purpuric dermatosis, idiopathic follicular mucinosis, pityriasis lichenoides, syringolymphoid hyperplasia with alopecia, and idiopathic generalized erythroderma (preSézary) are some of these conditions included in the CLD model. The importance of identifying CLD is that although most cases will never progress, they have the potential to transform into bona fide cutaneous T-cell lymphoma (CTCL).[2] The frequent clonal nature of these conditions elicited some debate about the relevance of T-cell clonality testing in cutaneous infiltrates and the lack of specificity of a T-cell clone in the diagnosis of CTCL. The recognition of these "premycotic" conditions should be taken as an opportunity to characterize a group of dermatoses, which have been difficult to categorize in the past. This hypothesis of a multistep progression of CTCL frequently preceded by a clinical and pathological benign stage is consistent with our modern understanding of the pathogenesis of cancer as a stepwise process, which evolves after an initial clonal event, with selection of more proliferative subclones as the cancer advances. The progressive nature of the T-cell clone with emergence and selection of more aggressive subclones has been demonstrated in MF by studying the genealogic evolution of the T-cell clone with microsatellite instability markers.[3] B-cell-rich variants of cutaneous lymphoid hyperplasia including nodular infiltrates after arthropod bite, tattoo, and vaccination have also been reported in exceptional cases of primary cutaneous B-cell lymphomas.[4,5]

T- and B-Cell Clonality Methods

T-cell clonality studies are based on the detection of specific T-cell receptor gene rearrangements (TCR-GR) by Southern blot analysis (SBA) or polymerase chain reaction (PCR).[6] Because lymphomas and leukemias are believed to be derived from a single transformed cell, we should expect that the tumor cells contain identical TCR-GRs, reflecting a monoclonal T-cell population. Conversely, heterogeneity of TCR-GRs in polyclonal reactive cases presents with a smear of DNA fragments without a dominant band.[1,7]

SBA used to be the gold standard for detection of T-cell clonality, but this procedure is laborious and lengthy, with the additional inconveniences of the need of fresh or frozen tissue and the use of radioactive probes. Besides, this method requires that the clonal population represents at least 5% of the total DNA extracted, which includes cells other than T cells, thus greatly decreasing the sensitivity of the test.[8-11] Presently, few laboratories rely on SBA for clonal detection.

The SBA has been gradually replaced by the PCR techniques. The overall sensitivity of PCR-based methods for the detection of T-cell clonality ranges approximately between 70% and 90%, with variable specificity depending on the sample population.[6] Clonal population representing as little as 0.001%-1.0% of the infiltrate can be detected using such methods. Other characteristics give greater applicability to the method, including speed, lower amount of DNA required, and new techniques allowing sufficient DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue.[1,7] The test amplifies extracted DNA using primers directed against the TCR beta, gamma, and delta chains.[7] The gamma chain gene is most commonly used because of the lower complexity of the gene with limited number of V and J region segments and lack of D segments, thereby requiring fewer specific primers and an optimal PCR product of < 400 bp.

In 2003, the EuroClonality (BIOMED-2 Concerted Action consortium) gathered many institutes from 7 European countries to develop and standardize PCR protocols and PCR primer sets for detection of Ig and TCR gene rearrangements, as well as guidelines for interpretation of the results. Following the new protocols, there was a marked increase in the detection rate of clonal rearrangements. Using 107 different primers in only 18 multiplex PCR tubes, it is possible to detect virtually all clonal B- and T-cell proliferations.[7] The gamma TCR primers are run in 2 separate tubes (A and B) and detect most combinations of TCR genes. Most laboratories only check the gamma TCR gene, but adding probes to the beta TCR gene allows for a higher sensitivity and specificity of clonality analysis. Approximately 10% of the false-positive results have been reported with both gene panels, but again, the specificity will vary depending on the characteristics and quality of the sample.

The genes encoding the antigen receptors for B cell or immunoglobulins include a heavy-chain gene (14q32), a kappa light-chain gene (2p12), and a lambda light-chain gene (22q11). There are 4 conserved framework regions that are reliable targets for immunoglobulin heavy-chain primers for PCR: 3 within the variable region (VH) and 1 within the join region (JH) (FR 1, 2, and 3) and 1 within the JH region. BIOMED-2 probes target all these regions, with oligonucleotides covering the entire potential repertoire of rearrangements.[7]

In our experience, the sensitivity of PCR for immunoglobulin heavy chain is lower than for TCR. Other researchers have noted the lower sensitivity, which they attribute to FFPE tissue usage and alterations in the DNA sequence, leading to a failure of primer annealing.[12] As an alternative, kappa and lambda Ig light-chain expression can be assessed in the cytoplasm of plasma cells on FFPE tissue sections using immunohistochemistry (IHC) and in situ hybridization techniques. The detection of light-chain restriction often referred as monotypic immunoglobulin expression is consistent with monoclonality.[12] However, there is one exception: in rare cases of monotypical expression not associated with clonal gene rearrangement, light-chain restriction is rarely found in certain benign B-cell infiltrates.[13] Monoclonality can also be demonstrated with flow cytometry targeting kappa and lambda light-chain expression at the cell membrane. In contrast, immunomarker methods are not practical for T-cell clonal detection and are mostly used as research tools.

PCR products are analyzed by different techniques. In most of the conventional PCR methods, monoclonality is defined by the presence of a discrete band after gel electro-

phoresis of the PCR product. Separation of the DNA products can be enhanced by using temperature- or a chemical-gradient gel. A smear pattern without specific bands indicates a polyclonal T-cell population.[14,15] Single-stranded confirmation polymorphism is another electrophoresis-based method that has been used for the analysis of clonality in skin biopsies, which separates fragments according to the unique shape of the single-stranded PCR products.[16]

Fluorescent fragment analysis using consensus primers for the TCR gene is becoming the standard method for T-cell clone detection in clinical pathology. The PCR product is denatured and separated by high-resolution capillary electrophoresis, and the fluorescence output is analyzed using GeneScan software (PE/Applied Biosystems, Foster City, CA). This method allows a base-pair measurement of the PCR product, providing a precise identification of the T-cell clone. Furthermore, clonal definition can be complemented using multiple PCR probes labeled with different fluorochromes. This allows for a more precise definition of the clone within a given variable region family.[15,17] This latest method is based on the same principle of molecular separation (charge, size, and hydrophobicity) as gel electrophoresis;[18] and it has the advantages of being faster, more accurate, sensitive, and easy to interpret.[8] Defining the clone is important for several reasons. The detection of a dominant T-cell clone, defined as the same PCR product at different sites, implies dissemination of a prevailing T-cell clone, and it has been associated with a higher incidence of tumor progression.[14] A dominant clone can be established by comparing the size, the variable region family usage, or the sequence of the PCR product obtained from more than one site (ie, 2 skin biopsies, skin and blood, skin and lymph node, etc.). Likewise, a nonreproducible clone or "pseudoclone" is infrequently associated with a truly malignant T-cell process. Clonal heterogeneity within 1 biopsy sample and at different sites supports the presence of various persistent and expanded T-cell clones, and has been reported in patients with early stages or indolent MF and in patients with CLD without a malignant process.[15]

The prognostic and diagnostic value of the detection of circulating clonal T cells in peripheral blood has been debated. Detection of circulating clonal T cells was uncommon among MF patients (12.5%), but much more common in those with erythodermic MF (42%).[19] Defining a circulating T-cell clone may also help in distinguishing a dominant CTCL clone from the innocent cytotoxic T-cell clones, which are occasionally detected in the peripheral blood of elderly patients.[20]

Detection of lymphoma by routine morphological examination (histologic score of LN3 or LN4) on palpable lymph nodes raises the tumor, node, metastasis, blood involvement stage to IVa, justifying a more aggressive therapeutic approach possibly including chemotherapy. The significance of a T-cell clonal detection in a palpable node with dermatopathic changes, but without obvious lymphoma (LN1 or LN2), is less clear. In 1 study using comprehensive gamma and beta TCR probes, a poor prognosis was detected in subsets of patients with nodal T-cell clonality despite negative histology results.[21] In the context of palpable lymphadenopathy, detection of the same T-cell clone in the lymph node and the skin CTCL lesions may indicate a poor prognosis, similar to the identification of lymphoma by histology (tumor, node, metastasis [staging system] stage IVa). The poor prognosis noted in patients with T-cell clonality by SBA is consistent with the lower sensitivity and higher specificity of the method. In other words, a substantial tumor burden is required for a positive test, which by itself tends to correlate with a poor outcome. T-cell clonal detection in skin biopsies using SBA was found to correlate with high tumor burden and poor survival in patients with CTCL.[9]

When Should We Check for T-Cell Clonality?

Ideally, TCR clonality should be checked at the time of diagnosis in the skin and blood. As previously mentioned, the detection of a dominant clone is important not only to confirm diagnosis but also for some prognostic guidance. T-cell clonality is particularly helpful when early stage MF is being considered in the differential diagnosis, which does not include sufficient clinical or microscopic evidence to reach a definitive diagnosis. The detection of a T-cell clone may lead us to a more definitive diagnostic call when the diagnosis is otherwise uncertain. The correlation of clonality and diagnosis is understandable, considering that in the more challenging early cases, there are relatively fewer malignant lymphocytes due to a less dilative effect of reactive T cells, and PCR detection may be more sensitive than histomorphology.[22] In one study, TCR gamma clonality was positive in 53% of the patch stage cases and in 100% of the plaque stage or tumor stage.[22] The TCR gamma-chain rearrangement using the GeneScan method was demonstrated in 100% of Sézary syndrome (SS) cases and in 84% of the MF cases. An increased rate of clonality was observed in connection with more advanced cutaneous disease burden (79% T1/T2; 100% T3/T4) and a higher histopathological diagnostic score (histopathological diagnostic score $< 5\%$: 76%; histopathological diagnostic score ≥ 5: 94%, with more than 5 points consistent with MF).[23]

It is also important to be aware of the limitations of T-cell clonality tests. False positive monoclonal or oligoclonal bands may be identified in inflammatory dermatoses, where the T-cell infiltrate is sparse. Amplification of few TCR-GRs derived from a limited number of T cells may result in a false positive nonreproducible clone or "pseudomonoclonality." A pseudoclone is infrequently associated with a malignant T-cell process.[15] Repeating the analysis using the same DNA template or fresh DNA extraction may solve the problem because in reactive conditions, the predominant PCR product typically vary in repeated PCR analyses of the same sample. In contrast, in neoplasic T-cell proliferations, dominant TCR clones are reproducible and should be routinely verified to confirm monoclonality.[24]

A correlation between TCR clonality by PCR methods and response to treatment has been suggested in some studies. The absence of a detectable clone in CTCL was associated with a higher rate of complete remission,[19] but was not necessarily associated to improved survival.[25]

To improve assessment of disease activity, immunophenotypic and immunogenotypic assays have been used to mon-

itor the response of CTCL to therapy, define remission, and detect early relapse.[11,20,23,26-28] Detection of the dominant clone appears to be associated with a high relapse rate in a similar way that a persistent pemphigus titer is likely to herald a flare of blisters.[29] The concept of minimal residual disease is defined as the persistence of the tumor T-cell clone in the tissue or blood despite clinical complete remission status.[28,30] One-third of MF patients in complete clinical remission have molecular residual disease.[28] Minimal residual disease may help identify patients at risk for relapses even when complete clinical and histological remission is achieved,[20] but the real prognosis implications of this finding are still uncertain.[26,28] It is well known that even on achievement of complete remission, patients with MF often relapse, which highlights the need to establish maintenance protocols to delay or prevent relapse. However, how fast the therapy should be tapered or when should it be discontinued has been an art more than a science. It is conceivable that in the future, the presence or absence of the dominant clone will guide our approach, allowing for more durable remissions while minimizing the adverse effects of therapy.

Flow Cytometry Analysis in Cutaneous Lymphomas

Flow cytometry analysis (FCA) is an efficient and sensitive method to detect and enumerate abnormal cells in the peripheral blood or any other cell suspension. Blood FCA is routinely performed in erythrodermic patients to rule out SS. This method is based on the abnormal expression of various surface markers of malignant T cells compared with normal T cells. Other helpful findings are the demonstration of overwhelming dominance of specific T-cell subsets (clusters of differentiation CD4 vs CD8) and the loss of one or more pan-T-cell antigens (ie, CD2, CD3, CD5, and CD7).[29] However, as previously stated, the presence of many reactive lymphocytes in early MF may limit the sensitivity and specificity of immunomarker-based assays. A high CD4:CD8 ratio of more than 10:1 and loss of CD7 and CD26 are the most reliable findings in SS. CD7 is the most commonly reported T-cell antigen to be lost in MF. However, low CD7 expression has low specificity because many inflammatory skin conditions also show low expression.[31,32] Deletion of CD2 and CD5 has been described as specific but with low sensitivity, and in our experience, these deletions tend to be associated with more advanced disease.[33] CD26 negativity was found in 59.3% of SS cases, in 33.3% of MF, in 14.2% of benign dermatoses, and in no control cases. The specificity of a CD26 result was inferior to that of T-cell antigen loss in differentiating SS from MF. The addition of CD26 to standard T-cell panels enhances the sensitivity of FCA in the diagnosis of SS.[34]

A simplified flow cytometric assessment using markers for CD3, CD4, CD26, and CD7 was sufficient to detect an abnormal T-cell population in 46% of patients with MF/SS and to correlate with disease extent.[35] The immunophenotypic correlation between a skin biopsy and a peripheral blood finding in patients with MF and blood involvement showed discrepancies in 55% of the cases. Compared with flow cytometry in blood, IHC analysis of the skin samples failed to detect partial deletion of CD2 (45%), CD3 (27%), and CD5 (27%) and overrepresented deletion of CD7 (18%). Additionally, CD8 + MF was not detected by IHC analysis, suggesting that CD 8+ MF might be more common than usually described.[33]

FCA can also be performed on leukocyte suspensions retrieved from skin biopsies. The main challenge of FCA in the analysis of MF is the difficulties in the extraction of viable tumor cells from the skin sample, which is a labor-intense process of mincing the skin biopsy and filtering the fragments with a pestle to obtain a cell suspension. Because immunophenotypic aberrations in MF can easily be detected by tissue IHC, this procedure is not routinely done. A dim expression of T-cell markers is a common finding in CTCL, which can be detected by FCA, but only rarely noted by IHC analysis. Flow cytometry not only allows for detection of surface antigens, but also provides important information about the size and complexity of the lymphocyte's nuclear membrane. In the early stages, MF and Sézary cells are characterized by a slight increase in nuclear size with increased complexity or convolution of the nuclear membrane or high scatter.[36] Flow cytometry can also be used to detect a clonal population by using a battery of antibodies against different subsets of T lymphocytes based on the expression of V beta family antibodies. This is used mainly as a research tool because the extensive panel of antibodies is expensive, incomplete, and does not include the entire spectrum of V beta families.[37] FCA assessment of T-cell clonality by V beta repertoire analysis has also been used to detect minimal residual disease in research protocols.[38] FCA can also provide prognostic information. Lower counts of circulating CD8 + lymphocytes and higher white cell counts in CTCL patients are associated with a less favorable prognosis.[39]

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) involves annealing of fluorescence-labeled nucleic acid probes with complementary DNA or RNA sequences and the subsequent detection of these probes within fixed cells. FISH is used to detect major chromosomal gains or losses, as well as specific translocations, and requires a target-specific probe. FISH can be performed with fresh-frozen or FFPE tissue, and the probes used will vary depending on the lymphomas under investigation. Nucleic acid probes are applied to the slide and incubated at a high temperature and high humidity to facilitate hybridization of the probe to its complementary sequence. After the excess probe is washed away, the slide is analyzed using fluorescence microscopy. FISH allows for the correlation between the location of the probe signal and the location of the probe within the cell/tissue of interest.[40] Although FISH is not routinely used in the diagnosis of cutaneous lymphomas, recent publications have shown the potential for future applications in various areas.

A high frequency of aberrant-gene expression of the cyclin-dependent kinase inhibitor gene 2A (*CDKN2A*) localized on chromosome 9p21 has been found in primary cutaneous large B-cell lymphoma, leg type (PCLBCL-LT). This gene locus encodes for p16 (INK4A), an inhibitor of the CDK4/retinoblastoma cell proliferation pathway, and for p14 (alternate reading frame), which controls p53-dependent pathways. The inactivation of p16 is associated with a worse prognosis in PCLBCL-LT. In most PCLBCL-LT, alterations of *CDKN2A*, deletion of p53, and deletion of Rb are found. Neither primary cutaneous follicle center lymphoma (PCFCL) nor primary cutaneous marginal zone lymphoma (PCMZL) shows alterations of *CDKN2A*.[41,42] The FOX-P1 (forkhead box P1 protein) is expressed in normal activated B cells and overexpressed in a subset of diffuse large B-cell lymphoma. *FOX-P1* gene (3p14.1) gains by FISH analysis were observed in 82% of the samples of PCLBCL-LT and in 37% of PCFCL.[43] This molecular analysis may become important in view of the poor prognosis of the PCLBCL-LT and the frequent clinical and pathological overlap with PCFCL.

The hallmark of nodal follicular lymphoma is the t(14;18)(q32;q21) chromosomal translocations, which lead to increased expression of the proapoptotic protein B-cell lymphoma 2 (BCL2) in the tumor cells. Not all cases of nodal follicle center lymphoma harbor this translocation, and among those cases that do, BCL2 protein is expressed in at least 50%.[44] The t(14;18) translocation may be detected by FISH or PCR, and it can be used to monitor the response to therapy and assess for residual disease. Approximately two-thirds of the cases with t(14;18) translocation detected by FISH express the BCL2 protein, as detected by IHC. Therefore, FISH analysis may become an important tool in the diagnosis, treatment, and follow-up of these malignancies.[45] Usually PCFCLs are BCL2 negative, and the FISH analysis of tumor cells fails to detect t(14;18) translocation.[46] The expression of BCL2 in PCFCL raises the possibility of secondary skin involvement from a nodal lymphoma and warrants a complete investigation to exclude a primary systemic lymphoma.

PCMZL is the skin counterpart of mucosa-associated lymphoid tissue (MALT) lymphomas with similar histopathological features, which may suggest a common pathogenic mechanism. The t(11;18)(q21;q21) translocation involving *API2* and *MALT1* genes occurs in 20%-30% of gastric MALT lymphomas and is associated with resistance to *Helicobacter pylori* eradication therapy and lack of progression.[47]

The implication of *MALT1* gene defects in the pathogenesis of PCMZL has been a matter of controversy. Using the FISH technique, the t(11;18) translocation was not found in PCMZL cases. The only MALT lymphoma translocation that could be identified in some PCMZL cases (3/12) was t(14;18)(q32;q21) translocation. These 3 cases were characterized by monocytoid morphology without blastic transformation and had an equally indolent course.[47] Other studies failed to demonstrate this translocation in PCMZL using FISH and reverse transcriptase PCR techniques.[48-50]

PCMZLs are entirely indolent, and although clonality by light-chain restriction and IgH analysis is detected in the majority of the cases, the low aggressive behavior raises the appropriateness of classifying such lesions as lymphomas.

Systemic anaplastic large-cell lymphoma (ALCL) is an aggressive CD30-positive lymphoma that can initially present with skin lesions or other extranodal sites.[51] Approximately one-half of the ALCL cases harbor the translocation t(2;5)(p23;q35), which leads to fusion of the nucleophosmin gene with the anaplastic lymphoma kinase (ALK), leading to its constitutive activation. Considering these facts, many efforts have been made to develop ALK inhibitors to treat this type of lymphoma.[52] The t(2;5)(p23;q35) translocation is detected in ALCL but not in lymphomatoid papulosis, another CD30-positive disorder. Although systemic ALCL can be ALK positive or negative, primary cutaneous ALCL and lymphomatoid papulosis are ALK negative.[53] However, some primary cutaneous ALK-positive ALCL cases have been recently described in children.[54]

Adult T-cell leukemia/lymphoma is associated with viral infection by human T-cell lymphotropic virus type 1 exhibiting clonal integration of the viral genome, which can be detected by SBA and long-inverse PCR.[18,55]

Genomic Analysis by Microarray Assays or Comparative Genomic Hybridization

In the context of research, new gene analysis techniques are providing more insight into the molecular events in CTCL. Although abnormal findings are identified in most cases, predictable and reproducible findings with clinical relevance are still scarce.

Conventional comparative genomic hybridization (CGH) allows for the identification of chromosomal imbalances but does not allow for the identification of specific genes involved owing to its low resolution.[56] The microarray-based CGH is more precise, and chromosomal imbalances can be quantified and defined appositionally.[40] Using array-based CGH for the identification of genomic differences between SS and MF, a high frequency of gains in chromosomes 1, 7, 8, and 17 and losses of chromosomes 5, 9, and 13 was demonstrated.[57]

Cytogenetic analysis in CTCL has shown a variety of gene defects without predictable findings.[58,59] This wide variation of findings has been attributed to geno-traumatic events, meaning genetic instability predisposes the tumor cells to further gene alterations and transformation. Even when multiple samples are obtained from a single MF plaque lesion, different cell subpopulations with complex chromosomal aberrations have been reported, reflecting genomic instability.[3,59] The cases of tumor-stage MF that have unstable genomes with a high number of chromosomal abnormalities have a statistically shorter survival.[56]

Chromosome abnormalities, mostly complex karyotypes, are seen in approximately 50% of patients with MF/SS. The most common abnormalities (7/9 cases) involved chromosome 10, followed by chromosome 6, although no recurrent translocation has been established so far.[60]

Cytogenetic features of large-cell transformation in MF were analyzed, and more gains were demonstrated than losses. Re-

Table 1 Comparison of Different Chromosome Changes Found in Cutaneous Lymphomas

Disease	Cytogenetic Band or Translocation	Genes	Comments
SS	8q24	*MYC*	Found in 75% of SS cases
	9p21	*CDKN2A*	Loss predicts unfavorable prognosis
ALCL	t(2;5)(p23;q35)	*NPM*	Absent in lymphomatoid papulosis
PCLBCL-LT	9p21	*CDKN2A*	Not found in PCMZL and PCFCL
	3p14.1	*FOXP1*	82% in PCLBCL-LT and 37% in PCFCL
Nodal follicular lymphoma	t(14;18)(q32;q21)	*BCL2*	Expression of BCL-2 in PCFCL warrants investigation of systemic lymphoma
PCMZL	t(14;18)(q32;q21)	*IGH and MALT1*	Other studies failed to demonstrate

ALCL, anaplastic large cell lymphoma; PCFCL, primary cutaneous follicle center lymphoma; PCLBCL-LT, primary cutaneous large B-cell lymphoma, leg type; PCMZL, primary cutaneous marginal zone lymphoma; SS, Sézary syndrome.

current chromosome alterations were noted for chromosomes 1, 2, 7, 9, 17, and 19. The most common imbalances were the gain of chromosome regions 1p36, 7, 9q34, 17q24-qter, 19 and the loss of 2p36-qter, 9p21, and 17p.[61]

Recent molecular studies are suggesting MF and SS are pathogenetically distinct entities and should not be considered a continuum for clinical trials or other studies. While using the CGH, it was demonstrated that numerical chromosomal alterations in MF include the gain of 7q21-36 and 1p36.2 and the loss of 5q13 and 9p21. On the other hand, in SS, the gain of 17q22-25 and 8q22-24 and the loss of 17p13 and 10q25 were observed. Besides amplification of the locus-containing *MYC* gene on 8q24, which is recognized as the important regulator of proliferation, growth, and differentiation, apoptosis was observed in 75% of the cases of SS and only in a minority of cases of MF.[57,62] It was demonstrated that a high-level amplification of the 8q24.21 region involving the *MYC* oncogene is correlated to a poor outcome.[56,57] The chromosomal region on 9p21 harbors the *CDKN2A* tumor-suppressor gene. Loss of this gene causes the reduced expression of this protein and predicts an unfavorable prognosis.[56,57] A comparison of different chromosome changes found in cutaneous lymphomas is shown in Table 1.

Conclusion

Molecular techniques can provide important information in the evaluation of cutaneous lymphoid infiltrate. The results from molecular analysis must be viewed in combination with morphological results, blood analysis and clinical presentation. By compiling all of these results, we can improve the accuracy of the diagnosis, as well as provide further data with prognostic and staging implication. While PCR based clonality techniques need to be interpreted with caution, modern capillaroscopy methods offer clone specific data that allow us to trace minimal residual disease at different compartments like skin blood or lymph node.

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