Cutaneous T-Cell Lymphoma: Molecular and Cytogenetic Findings

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Chromosomal changes have been identified early in the disease process in cutaneous T-cell lymphoma (CTCL): both losses and gains of chromatin have been found on several chromosomes. The extent of chromosomal aberrations increases with disease stage and in more aggressive subtypes of the disorder. Changes in specific genes, such as NAV3, have been characterized in patients with CTCL, and may produce a proliferation advantage for affected cells. In addition, the expression of some genes can discriminate between controls and patients with CTCL. Therefore, the over- or under-expression of certain genes may be of diagnostic and pathogenic relevance in this disease, and may allow the development of selective treatments.

Chromosomal changes have long been recognized as a feature of cutaneous T-cell lymphoma (CTCL). These changes include losses of chromatin in chromosomes 1, 2, 8, 10, and 17 and, in contrast to B-cell lymphomas, for example, the T-cell-receptor genes are not frequently involved. Importantly, these cytogenetic changes can be seen very early in the disease process. Data from Karenko et al have demonstrated that chromosomal changes characteristic of CTCL can be found even in patients for whom the differential diagnosis of large-plaque parapsoriasis or early mycosis fungoides (MF) is unclear.[1] Thus, some cytogenetic changes appear prior to the clinical and histologic onset of the disease.

Karyotyping in Patients With CTCL
Conventional karyotyping of cells from patients with Sézary syndrome (SS) showed many changes, including the presence of several chromosomes that are impossible to assign.[2] We therefore used comparative genomic hybridization (CGH) to conduct a detailed analysis of the chromosomal imbalances in patients with CTCL.[3] Chromosomal aberrations were found in 21 out of 32 (66%) patients, and chromatin losses were observed somewhat more frequently than gains (54% vs 46%, respectively). Euchromatic loss (diminished chromatin) was observed most frequently (> 16%) in the short arm of chromosome 17 (17p), which was found in 28% of patients showing aberrations, in the long arms of chromosomes 13 (13q; 25% of patients), 10 (10q; 16%), and 6 (6q; 19%). In addition, we found gains of chromatin in chromosome 7 (25%), in the long arm of chromosome 8 (8q; 25%), and in chromosome 17 (17q11-q22; 16%). These data are similar to those from other groups who have investigated patients with CTCL using CGH.[4,5]

In 5 out of 21 (24%) patients, a gain in chromosome 7 was associated with a loss in chromosome 13q, reflecting possible recurrent associations of certain chromosomal changes. Four of these five patients had additional aberrations, such as deletions in chromosome 6q and partial trisomy in chromosome 8q. In the patients showing this phenotype of cytogenetic changes, we were able to observe a sequence for the changes, which may provide some information about the pathogenic process in CTCL, with certain chromosomal aberrations appearing to be associated with different disease stages. In addition, at the different stages of CTCL, the number of affected chromosomes increased from none at stage IA to a mean of 9.33 (standard error of the mean [SEM] 3.76) at stage IVA and 8.75 (SEM 1.75) at stage IVB (Figure 1).
Furthermore, the overall number of chromosomal aberrations was correlated with patients' prognoses. In patients with tumor samples showing more than five chromosomal imbalances, 5-year survival was lower than in patients with less than five chromosomal imbalances (≤ 5% vs 72%, respectively). As there are larger numbers of chromosomal changes at late compared with early stages of the disease, these alterations may simply reflect a correlation between stage of the disease and prognosis. Nevertheless, chromosomal imbalances were found to occur more frequently in aggressive subtypes of CTCL (9.33, SEM 2.16) than in the indolent subtypes (2.88, SEM 0.8).

Aberrations in a number of individual chromosomes were also associated with poor survival. Loss of chromatin in chromosomes 6q, 10q, and 13q and gains in chromosomes 7 and 8q were all associated with lower 5-year survival rates than in patients who did not show these changes. However, the most common chromosomal aberration—losses in chromosome 17p—was not associated with a reduction in 5-year survival.

A Gene Site for Chromosomal Aberrations
In peripheral blood clones from seven patients with SS and six with MF, using multifluorescent in situ hybridization and spectral karyotyping, Karenko et al[6] demonstrated that the most frequently affected chromosome was 12. All of the structural clonal aberrations of chromosome 12 involved bands q21 or q22. Using three samples that showed large deletions of chromosome 12 (two cases) and a balanced translocation between chromosomes 12 and 18 (one case), they were able to analyze the breakpoint in chromosome 12 and fine-map the affected gene. By sequencing the bacterial artificial chromosomes used as probes for the breakpoint, they found that the affected gene was NAV3 and the breakpoint was in exons 10-11 (Figure 2).
These deletions in NAV3 were found not only in blood lymphocytes, but also in interphase cells of skin lesions from patients with CTCL (both SS and MF) and in skin lesions in 50% of patients with early stages of MF. Thus, this deletion appears to be a relatively early event in the pathogenesis of CTCL. Moreover, in patients without gross chromosomal changes, nonsense mutations of the NAV3 gene could be found. Therefore, there appears to be an even higher percentage of patients with CTCL who have aberrations of the NAV3 gene than might be estimated from these cytogenetic analyses. The changes in the NAV3 gene did not appear to have been produced by therapy, as these mutations and cytogenetic changes could also be found in untreated patients.

Other genetic aberrations of tumor suppressor genes that are found in CTCL include those affecting PTEN, which is a mutation found in Cowdens syndrome; P15 and P16, which are found in malignant melanoma[7,8]; and P53, which is the most frequently mutated tumor suppressor gene.[9,10] However, all of these mutations have a lower frequency in CTCL compared with that of the NAV3 gene.

Pathogenic Function of NAV3 Deletion
In Jurkat cells, lentiviral infection lowered the relative expression of NAV3 by 77%. This lentiviral silencing of NAV3 increased the proportion of IL-2+/GFP+ Jurkat cells, and so increased interleukin-2 production by these cells.[6] This may represent a proliferation advantage of cells with abolished NAV3 expression, so it may have some pathogenic relevance. The relevance of deletion of only one allele of the NAV3 gene is unclear, as one functioning allele of a tumor-suppressor gene is usually sufficient to keep the cell functioning normally. Nevertheless, there are now a number of published reports of gene-dose effects, which may lead to functional changes if one allele is lost.[11] Thus, it is possible that NAV3 is an example of such a haploinsufficient gene.

DNA Instability in CTCL
DNA instability has been detected in many solid cancers—for example, in inherited colonic cancer. These patients exhibit the so-called mutator phenotype and DNA instability because they are deficient in DNA mismatch repair enzymes. This deficiency leads to instability of microsatellite regions of DNA, which are repetitive sequences that are particularly difficult to amplify, so mutations will accumulate there.

In a recent study of 603 patients with non-Hodgkin's lymphoma, the mutator phenotype was found in only 12 (2%) patients.[12] This phenotype of high microsatellite instability was only found in patients who were either HIV-infected or iatrogenically immunosuppressed following transplantation. Thus, there is an immunodeficiency-related T-cell lymphoma in which the mutator pathway is a feature of this disease. Nevertheless, we have not found any evidence of microsatellite instability in CTCL.

Diagnostic Use of Gene Expression in CTCL
Kari et al were able to identify several groups of genes for which expression profiles discriminated SS from normal peripheral blood mononuclear cells, with 100% accuracy in patients with high tumor burden.[13] Five of these genes that showed differential expression between patients with SS and
high tumor burden and Th2-skewed healthy controls were further tested in 125 new samples (56 from patients with CTCL and varying levels of tumor burden, and 69 from controls). The five genes analyzed were STAT4, CD1d, GATA-3, TRAIL, and PLS3.[14] These genes were selected not for their functional significance in CTCL, but because they are differentially expressed in SS and controls and expressed at high enough levels that changes in expression could be reliably detected. Using these five genes, the authors were able to differentiate SS from controls with a sensitivity of 86% and a specificity of 95% in this data set (Figure 3). It was also possible to discriminate between controls and patients who had very little blood involvement of CTCL, and therefore mainly cutaneous disease. In addition, the pattern of gene expression was not found in control samples from patients with MF, those with remission SS, or those with atopic dermatitis.

STAT4 was found to be downregulated in patients with SS, not only in tumor cells but also in normal CD4+ cells, even in patients with a low tumor burden. Thus, STAT4 transcription seems to be actively suppressed in patients with SS, in tumor cells and in normal cells, possibly as an effect of the tumor cells. STAT4 expression is required for Th1 differentiation. GATA-3 is overexpressed in patients with SS, again not only in tumor cells but also in other cells. This gene product induces Th2 T-cell differentiation and suppresses the Th1 T-cell response that is important for tumor suppression and protection against infection. Functionally, these two gene changes mean that, in patients with SS, there is a shift toward Th2 expression and patients lack Th1. Thus, patients show weakened
anti-infective and antitumor responses.

Hypermethylation in CTCL

One of the ways that the functioning of the genome is regulated is by methylation of the promoters of the genes. Promotor hypermethylation in CTCL was investigated on a genome-wide scale by van Doorn et al.[15] This group found that malignant T cells in CTCL exhibit widespread promotor hypermethylation suggestive of epigenetic instability. The results also demonstrated promotor hypermethylation of several putative tumor-suppressor genes, associated with inactivation of these genes. This could lead to dysregulation of the cell cycle, defective DNA repair, disruption of apoptosis signaling, and chromosomal instability. This finding may provide a rationale for treatment of CTCL with demethylating agents.

Conclusions

Chromosomal aberrations have been noted in CTCL for more than 30 years. These changes, initially characterized by conventional karyotyping, have now been investigated in more detail using CGH, specific analysis of chromosomal breakpoints, and identification of deleted genes. In addition, genes have been identified that may be of diagnostic and pathogenic relevance to the disease process in CTCL. These findings should provide a better understanding of the pathophysiology of the disease, and eventually allow us to intervene specifically at certain points in the disease process.

Disclosures:

Dr. Sterry has performed clinical studies and is a consultant for Cephalon Europe.

References:


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