Molecular Diagnosis of Cutaneous Diseases

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Objectives: To provide an update on the molecular procedures used increasingly in the study and diagnosis of a variety of dermatologic malignancies and inflammatory disorders and to explore the potential use of these techniques in clinical dermatology. Herein, we review assays such as G-banding, fluorescence in situ hybridization, comparative genomic hybridization, and spectral karyotyping in conjunction with the polymerase chain reaction and DNA microarrays.

Data Sources: PubMed was searched for published articles on molecular diagnosis and dermatologic diseases.

Study Selection: All English-language studies were selected if they provided useful methodologic information or highlighted the usefulness of molecular techniques.

Data Extraction: Only methodologic and qualitative information was extracted.

Data Synthesis: The information was synthesized into 2 sections: one describing the principles of different molecular diagnostic techniques, and the other highlighting the contributions of molecular diagnostic techniques to the understanding and diagnosis of several dermatologic diseases.

Conclusions: A basic understanding of the principles of molecular diagnostic techniques is crucial for the practicing dermatologist to benefit from the increasing number of molecular diagnostic articles appearing in the literature and potentially to apply these methods in clinical practice.

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A VARIETY OF MOLECULAR DIAGNOSTIC techniques are currently being used to detect an array of dermatologic malignancies and have potential use in other cutaneous diseases, including inflammatory disorders. Assays such as G-banding, fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and spectral karyotyping (SKY) are being used in conjunction with other assays (ie, polymerase chain reaction [PCR] and DNA microarrays) to diagnose primary cutaneous lymphomas, Kaposi sarcoma (KS), melanoma, basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and other dermatologic malignancies. These assays can be used to detect chromosomal aberrations such as polyploidy, aneuploidy, interstitial deletions and duplications, reciprocal and non-reciprocal translocations, and gene amplifications. Although the cost of required equipment has generally limited the use of these techniques to a research setting, molecular assays will likely have a future role in the identification and characterization of common malignancies in the clinic.

The diagnosis of certain dermatologic malignancies such as melanoma and cutaneous T-cell lymphoma (CTCL) can be difficult, even with clinical and histopathologic correlation. Molecular techniques can provide a level of specificity and objectivity unachievable with methods of diagnosis that rely on clinical and histopathologic observation alone. They can also detect molecular changes before a disease becomes clinically evident. The detection of disease-specific genes or chromosomal aberrations can aid in the diagnosis of the disease and may also play a role in determining prognosis and potential treatment. Thus, multiple studies have been completed or are in progress to use these genes or molecular markers for potential targets of diagnosis and therapy.

The present review is meant to serve as a primer of molecular techniques currently being used in dermatologic research that have the potential to be de-
veloped into clinical tests (Table). To this end, we describe in detail several molecular diagnostic techniques and their potential roles in elucidating the genetic pathophysiology of dermatologic malignancies. The contributions that molecular diagnostics have already made to the field are highlighted as well.

MOLECULAR METHODS IN DERMATOLOGY

G-Banding

G-banding is currently widely used to diagnose a variety of diseases. In G-banding, trypsin is used to partially digest histones, which are small multimeric proteins responsible for holding chromosomes together. This process allows the chromosomes to relax or uncoil to be dyed with Giemsa stain. After staining, a distinctive banding pattern is produced on each chromosome that reveals any numeric or structural chromosomal abnormalities (Figure 1). Detection of abnormalities in karyotypes allows not only for the diagnosis of disease but also for patient prognosis and management. Studies using G-banding in conjunction with other molecular techniques have identified multiple chromosomal aberrations in CTCL and correlated these changes with different prognoses.

G-banding has multiple advantages: it is easy and inexpensive to use; the entire genome can be screened for chromosome-level anomalies; and the results are reproducible. These characteristics have allowed the establishment of karyotype-phenotype correlations for many hematopoietic and soft tissue neoplasms. Clinically, G-banding has been used to detect chromosomal abnormalities in CTCL. Limitations of G-banding include the requirement for a high rate of cell division and good chromosomal structure to allow the analysis to be performed on metaphase chromosomes. The analysis for G-banding can be time-consuming and labor-intensive. G-banding is also unable to identify subtle translocations and small insertions or deletions. Despite these limitations, chromosome analysis through G-banding will remain a valuable diagnostic tool in the future.

Fluorescence In Situ Hybridization

In situ hybridization was a technique first introduced more than 15 years ago. Since then, advances in technology have led to the development of FISH. This technique can be used to detect chromosomal abnormalities and to map genes by painting chromosomes or portions of chromosomes with fluorescent molecules. The value of FISH in dermatology lies in its ability to locate chromosomal aberrations present in a variety of cancers and some inherited skin disorders.

The basis of this technique involves DNA sequences that are complementary chromosome-specific probes. Initially, short single-stranded DNA probes are created that are complementary to the DNA sequence of interest. These probes are then labeled with a fluorescent tag or a reporter molecule and allowed to hybridize with the complementary target chromosomal DNA. The fluorescence generated in the regions where hybridization has occurred is identified through a fluorescence microscope, which allows researchers to identify the location of the sequence of interest and/or copy number changes that are present in the target chromosomes. The FISH technique can be performed on metaphase or interphase chromosomes for the analysis of possible abnormalities and allows the use of nondividing cells (Figure 2 and Figure 3). Many different variations of FISH exist, including multicolor FISH (mFISH), multicolor-banding FISH (mBAND FISH), and dual-fusion FISH (D-FISH).

In mFISH, 24 differentially labeled whole chromosome-specific probes are created with different combinations of 1 to 5 fluorophores giving each probe a unique fluorescence signature. The chromosomes are hybridized with the different probes, and each fluorochrome to a different intensity that depends on the specific combination used. The chromosomes are then pseudocolored, separated, and identified by the computer from their gray-scale image using a labeling algorithm. The finished product is a multicolored karyotype in which each chromosome pair is assigned its own distinct color by the computer. This technique allows the detection of occult or complex chromosomal rearrangements and is similar to SKY analysis.

Similar to mFISH, mBAND FISH involves chromosome painting, but in mBAND FISH a specific region of a partial chromosome paint is resolved into multicolored bands. This allows analysis of intrachromosomal rearrangements that are not detectable using mFISH.

A modified version of FISH, D-FISH is used for the detection of reciprocal translocations. The standard FISH technique for detecting translocations uses 2 differently labeled probes, one complementary to the DNA sequence proximal to the break point of the involved chromosomes and the other complementary to the region distal to the translocation break point on the other chromosome involved in the translocation. In this approach, a dual-color fusion signal is generated in nuclei containing the translocation, which represents 1 of the derivative chromosomes created by the translocation. Each of the 2 normal alleles will display its respective single-color signal.

The weakness of the standard FISH technique is that it lacks specificity because the random spatial colocalization of normal signals with different colors generates false positives in 1% to 5% of screened nuclei. While this level of false positives is easily tolerated in patients with active disease in whom aberrant nuclei are abundant, it becomes problematic in patients being observed for recurrence. With D-FISH, this standard FISH limitation is overcome by using 2 sets of differentially labeled DNA probes complementary to sequences proximal and distal to the translocation break points on both chromosomes involved in the translocation. This results in fused signals in both derivative chromosomes and allows the translocation to be detected on both of the involved chromosomes as well as a single-color signal on each of the normal alleles (Figure 3). This pattern generated by D-FISH is unlikely to be duplicated randomly and imparts increased specificity over the standard technique.

Break-apart FISH can detect translocations involving multiple chromosomes, which are more common than reciprocal translocations in solid malignancies. Break-apart FISH uses differentially labeled DNA probes complementary to sequences proximal and distal to the break point within 1 critical gene. In normal nuclei, copies of the selected gene will be marked with a fusion signal. Nuclei containing a translocation that disrupts the selected gene will display 1 fusion signal for the normal allele and 2 single-color signals that have “broken apart” from each other, labeling each of the derivative chromosomes (Figure 3).

Different applications of FISH can be used to analyze a variety of chromosomal aberrations. Probes and techniques exist to identify abnormalities in chromosomal loci, translocations, and copy number changes. The availability of these different techniques and types of probes allows FISH to analyze the entire genome as well as localized regions of chromosomes. Additionally, FISH is faster and more sensitive and specific than conventional cytogenetic techniques. This multifunctional ability of FISH has opened a new dimension not only in cancer research, but also in gene mapping, prenatal research, and infectious disease research. In addition, FISH has been used to detect genetic changes involved with melanoma as well as to diagnose X-linked ichthyosis and its carrier state. It has also been...
### Table. Summary of Molecular Diagnostic Techniques

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<tr>
<th>Molecular Technique</th>
<th>Method</th>
<th>Molecular Applications</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Use in Dermatology</th>
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<tr>
<td>G-banding</td>
<td>Chromosomes are uncoiled via trypsin digestion of histones and then stained with Giemsa stain to produce a distinctive banding pattern.</td>
<td>Numeric and/or structural abnormalities can be identified.</td>
<td>Allows screening of entire genome. Easy and relatively inexpensive to use.</td>
<td>Requires dividing cells. Karyotype analysis can be time-consuming. Unable to identify subtle translocations, and small deletions or insertions.</td>
<td>Cutaneous T-cell lymphoma.</td>
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<tr>
<td>Fluorescence in situ hybridization (FISH)</td>
<td>Fluorescently labeled DNA probes hybridize with DNA sequence of interest.</td>
<td>Gene location, copy number changes, and translocations can be identified.</td>
<td>Can be used on interphase cells. Entire genome can be analyzed.</td>
<td>Can only identify abnormalities for which a specific probe exists.</td>
<td>Melanoma, X-linked ichthyosis, psoriasis.</td>
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<td>Multicolor FISH (mFISH)</td>
<td>Each human chromosome is painted a different color using 24 differentially labeled chromosome-specific probes.</td>
<td>Occult or complex chromosomal rearrangements may be identified.</td>
<td>Entire genome can be analyzed. Specific chromosomes involved in rearrangements are easily identified.</td>
<td>Requires dividing cells. Cannot identify intrachromosomal rearrangements.</td>
<td>Melanoma subtypes.</td>
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<tr>
<td>Multicolor banding FISH (mBAND FISH)</td>
<td>A specific region of a partial chromosome paint is resolved into multicolored bands.</td>
<td>Intrachromosomal rearrangements can be identified.</td>
<td>Can detect changes within and between chromosomes.</td>
<td>Requires specialized equipment. Can only analyze 1 chromosome at a time.</td>
<td>Melanoma.</td>
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<tr>
<td>Dual-fusion FISH</td>
<td>Probes proximal and distal to the region of interest.</td>
<td>Reciprocal translocations can be identified.</td>
<td>Increased specificity over traditional FISH.</td>
<td>Cannot identify translocations involving more than 2 chromosomes.</td>
<td>Leukemias.</td>
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<tr>
<td>Comparative genomic hybridization (CGH)</td>
<td>The ratio of differentially labeled sample and reference DNA hybridized to normal human metaphase chromosomes is graphically analyzed to detect copy number changes.</td>
<td>Copy number changes (gains or losses) in neoplastic or tumor cell DNA can be identified.</td>
<td>Entire genome can be analyzed. No cell culture needed. May be used on fresh or preserved specimens.</td>
<td>It can be technically difficult. The smallest detectable copy number change and mapping precision are relatively low.</td>
<td>Melanoma, Spitz nevi, genodermatoses.</td>
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<td>DNA microarrays</td>
<td>Segments of DNA serving as probes are arranged on a support to form the microarray. RNA from tissue samples are reverse transcribed, labeled, and hybridized to the microarray slide.</td>
<td>Copy number changes and single-nucleotide polymorphisms can be identified, and genetic mutations can be characterized.</td>
<td>Entire genome can be analyzed, and information about expression levels and functionalities of several thousand genes can be obtained simultaneously in a single experiment.</td>
<td>It depends on high-quality extraction of messenger RNA, which can be technically difficult. Array fabrication and analysis can also be difficult, and the required equipment is expensive.</td>
<td>Squamous cell carcinoma in patients with epidermolysis bullosa, atopic dermatitis, and psoriasis.</td>
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<tr>
<td>Spectral karyotyping (SKY)</td>
<td>Each human chromosome is painted a different color using 24 differentially labeled chromosome-specific probes prepared by fractionating flow-sorted human chromosomes.</td>
<td>Chromosomal translocations and rearrangements can be identified.</td>
<td>All chromosomes and their abnormalities can be visualized at one time.</td>
<td>Cannot detect insertions and deletions within the chromosome.</td>
<td>Malignant fibrous histiocytoma, head and neck cancers.</td>
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<tr>
<td>PCR</td>
<td>PCR is a rapid replication process in which a specific DNA segment from a tissue (or blood) is amplified, producing a large amount of the DNA fragment.</td>
<td>Detection of pathogens, genotyping, and screening for gene mutations.</td>
<td>Highly sensitive, requires small amount of DNA (ie, a small quantity of tissue).</td>
<td>High sensitivity necessitates special precautions to prevent contamination.</td>
<td>CTCL, CBCL, ALCL, LyP, Kaposis sarcoma, BCC, SCC, melanoma, variety of infectious agents.</td>
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<td>RT-PCR</td>
<td>A version of PCR in which cDNA is generated from RNA via reverse transcription. The cDNA is then amplified via PCR.</td>
<td>Detection and quantification of gene transcription.</td>
<td>Sensitive, amplifies very small amounts of any kind of RNA.</td>
<td>The accuracy of quantification of transcription is limited. Pure DNA-free RNA preparations are preferred. Special precautions needed to prevent contamination.</td>
<td>CTCL, melanoma, Kaposis sarcoma, BCC, SCC.</td>
</tr>
<tr>
<td>Real-time PCR (and RT-PCR)</td>
<td>Monitoring the real-time progress of PCR makes possible an accurate approach to PCR-based quantification of DNA and RNA.</td>
<td>Quantification of DNA or RNA: detection of gene amplification, mutation, DNA copy number of pathogens, and gene transcription.</td>
<td>Sensitive, accurate quantification.</td>
<td>Similar to PCR and RT-PCR, but requires more specialized instruments and facilities.</td>
<td>CTCL, ALCL, LyP, Kaposis sarcoma, melanoma, BCC, SCC, variety of infectious agents.</td>
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</table>

Abbreviations: ALCL, anaplastic large-cell lymphoma; BCC, basal cell carcinoma; CBCL, cutaneous B-cell lymphoma; cDNA, complementary DNA; CTCL, cutaneous T-cell lymphoma; LyP, lymphomatoid papulosis; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; SCC, squamous cell carcinoma.
used to evaluate the effect of acitretin therapy on human lymphocytes in the treatment of psoriasis.17

Limitations of FISH consist primarily of cost. The high cost of equipment and materials involved in FISH has hindered its introduction into the clinical setting. Also, many comprehensive probes are not commercially available, which further limits its use. Unlike conventional cytogenetic techniques that can identify many chromosomal abnormalities at one time, traditional FISH can only identify chromosomal abnormalities for which the researcher has a specific probe.9 However, these limitations are being overcome with the development of techniques like mFISH, SKY, and CGH.

Comparative Genomic Hybridization

Comparative genomic hybridization is a FISH-based technique that requires no cell culture and allows for identification of genomic copy number changes (gains or losses) in neoplastic or tumor cells. Because the entire genomic DNA of the neoplasm can be analyzed, CGH is used frequently in the study of a variety of cancers. This technique involves fluorescent labeling of the sample DNA and normal reference DNA by the process of nick translation. In this process, the DNA to be labeled is allowed to react with a deoxyribonuclease (creating a nick) and a polymerase (allowing transcription). Subsequently, the DNA is incubated with the 4 regular nucleotides as well as a high concentration of a modified nucleotide such as biotin-16-deoxyuridine triphosphate (dUTP) and digoxigenin-11-dUTP for the reference and sample DNA, respectively. These modified nucleotides will be preferentially incorporated into their respective DNA during transcription and will serve to label them with different colors.

The tumor and reference DNA are then coprecipitated in equal amounts and hybridized to normal human metaphase chromosomes, which serve as the reading devices.18 The cohybridization with normal reference DNA functions to provide an internal reference for the fluorescence generated.19 Sample and reference DNA hybridize proportionally to specific chromosomal regions relative to their differential representation in the DNA mixture. The fluorescence generated at each chromosomal segment is then analyzed as a ratio of tumor-to-normal signal against the baseline of the metaphase chromosomes. Chromosome segments in which there is no difference between sample and reference DNA will display both fluorescence markers in balanced proportions. Deletions of specific sequences in the tumor DNA will display an increased ratio of reference marker over tumor marker (Figure 4). Similarly, gains in copy number in the tumor DNA are represented with an excess of tumor marker over reference marker.20 These differences are displayed graphically through computer analysis.9

Comparative genomic hybridization has been used to help differentiate between melanoma and Spitz nevi21 and to identify chromosomal changes in genodermatoses.22 The main advantage of CGH is that it allows for the entire genomic material of the tumor to be analyzed. It can also be applied to fresh or preserved specimens and does not require cell culture. Limitations of this technique include the potential to be technically difficult, the inability to detect balanced rearrangements, and relatively low mapping precision. Also, the smallest detectable copy number change is relatively large.19,23 These limitations are being overcome through the use of microarray-based CGH, in which the metaphase chromosomes are replaced with defined nucleic acid target sequences or whole genome arrays. This incorporation of microarray technology with CGH

Figure 1. In G-banding, trypsin is used to partially digest histones and allow the chromosomes to relax and be dyed with Giemsa stain, which produces a distinctive banding pattern on each chromosome. A karyotype is generated that reveals any numeric or structural chromosomal abnormalities.
enables a greatly improved resolution for the detection of high or low copy number changes, direct mapping of aberrations to the genome sequence, and higher throughput.20

DNA Microarrays

DNA microarray analyses involve miniaturized arrangements of sequences from thousands of genes immobilized at specific positions on a solid support and analyzed via specific hybridization.24 Segments of DNA serving as probes for detection are arranged in a specific order on a nylon or glass support, which forms the microarray. A given microarray may include the entire genome on a slide or just a few specific genes. In general, there are 2 types of microarrays, depending on the material arrayed (eg, complementary DNA or oligonucleotides). Complementary DNA arrays range in size from hundreds of bases to several kilobases and are used for RNA expression analysis; oligonucleotide arrays can have probes that range from 20 to 25 bases (short array) to between 50 and 80 bases (long array) and can be used for both RNA expression and DNA sequence analysis.24,25 After preparation of the desired microarray, RNA from tissue samples are reverse transcribed, labeled appropriately, and hybridized to the microarray slide (Figure 5).24

Microarray technology has numerous applications, including functional genome analysis (which attempts to decipher the function of multiple genes) and comparative gene analysis. Comparative gene analysis can be used to determine differences in gene expression between normal and disease-state tissues. In this technique, sample and reference messenger RNA (mRNA) is reverse transcribed, differentially labeled, and allowed to hybridize with a DNA microarray. Analysis of the fluorescence pattern generated allows the researcher to determine which genes are being expressed in both sample and reference tissues as well as which genes are uniquely expressed or absent in the sample tissue.26 Comparative gene analysis has been used to detect differences in cytokine elaboration between atopic and psoriatic skin.27

DNA microarrays have several uses, including genomewide quantification of DNA copy number, characterization of genetic mutation, and the detection of single-nucleotide polymorphisms. However, the primary application of microarray technology has been to simultaneously obtain information about expression levels and functionalities of several thousand genes in a single experiment.25 Clinically, gene analysis by microarray offers new models for approaching disease diagnosis, prognosis, and treatment. Unique subsets of disease-specific genes can be detected using DNA microarrays to compare changes in gene expression patterns between normal and diseased cells and/or tissues.25 DNA microarrays have been used to study SCC in patients with epidermolysis bullosa29 as well as to evaluate gene expression in atopic dermatitis and psoriasis.30,31 Along with uses in dermatology, DNA microarrays have also been used to evaluate HLA types in patients undergoing transplantation.32 Molecular signatures have been elucidated in several instances from the outcome of specific therapies, and this type of analysis holds the potential to predict treatment response and prognosis in advance of the initiation of therapy. The ability to assess the effectiveness of anticancer drugs would avoid potentially unnecessary treatments and their inherent toxic effects.25

The main advantage of DNA microarrays is that they can be used to study the entire transcriptome simultaneously. They can be performed relatively quickly and are adaptable. Also, only small volumes are needed for hybridization and proportionately smaller amounts of sample for analysis.24 Limita-
tions include the need for high-quality extraction of mRNA, which can be technically difficult, the difficulties of fabricating the array, the cost of the equipment, and the difficulty of analysis. These limitations may be partially overcome by using smaller arrays, allowing higher densities of sample, and producing commercial arrays.

Figure 3. An illustration of the various types of fluorescence in situ hybridization (FISH). Blue circles represent interphase nuclei; stars, probes. A, In standard FISH, 2 differently labeled probes are generated and hybridized with interphase nuclei: one (yellow) complementary to the DNA sequence proximal to the break point of the involved chromosomes, and the other (red) complementary to the region distal to the translocation break point on the other chromosome involved in the translocation. In the normal cell, each normal allele displays its own single-color signal. In the abnormal cell where a reciprocal translocation has occurred, the derivative chromosome produces a dual-color fusion signal. B, In dual-fusion FISH, 2 sets of differentially labeled DNA probes complementary to sequences proximal and distal to the translocation break points on both chromosomes involved in the translocation (1 yellow, 1 red) are generated and hybridized with interphase nuclei. In the normal cell, strong, single-color signals (large stars) are produced by each normal allele. In the abnormal cell where reciprocal translocation has occurred, fused signals in both derivative chromosomes are displayed, with the remaining normal alleles showing a strong single-color signal. C, In break-apart FISH, 2 differentially labeled DNA probes complementary to sequences proximal and distal to the translocation break point on each chromosome involved in the translocation (1 red, 1 yellow) are generated and hybridized with interphase nuclei. In the normal cell, both copies of the selected gene are marked with a fusion signal (yellow and red). The abnormal cell, where the gene has been interrupted by translocation, displays 1 fusion signal for the normal allele and 2 single-color signals that have “broken apart” from each other, which serves to label each of the derivative chromosomes.

Figure 4. In comparative genomic hybridization, the ratio of differentially labeled sample (red) and reference (green) DNA hybridized to normal human metaphase chromosomes (blue) is graphically analyzed to detect copy number changes.
pared by fractionating flow-sorted human chromosomes. The probes are labeled with different-colored fluorophores, alone or in combination, and are hybridized to a metaphase chromosome preparation. On excitation, each homologous chromosome pair emits a unique wavelength; and after computer analysis, each pair is pseudocolored, and a color-coded karyotype is generated. Chromosomes can then be analyzed to detect abnormalities by using the fluorescent colors to highlight aberrations.

Spectral karyotyping can be used for a multitude of purposes, including identification of chromosomal translocations and rearrangements. It has been used to characterize large chromosomal markers in malignant fibrous histiocytoma as well as to map human papillomavirus integration sites in head and neck cancers. In addition, SKY can be used to monitor the progression of disease. However, SKY, like FISH, is limited by its inability to detect aberrations such as insertions and deletions within the chromosome. It is a relatively new technique compared with FISH and CGH, and its potential applications are only beginning to be discovered.

**PCR and Real-Time PCR**

Polymerase chain reaction was first introduced by Mullis et al in 1986. Since then, it has become one of the most pervasive techniques in biological research for detecting DNA and RNA (via reverse transcriptase PCR [RT-PCR]) from a large variety of biological sources. It has several advantages over other detection technologies: it is fast, very sensitive, needs little starting material, and is relatively inexpensive. However, given the geometric amplification that occurs, specificity depends on a primer pair that has high fidelity for the template. If the primers misprime, which usually occurs during the first few cycles, inappropriate PCR products may be made. The degree to which this occurs depends on how well the primers were designed, the reaction conditions, and the cycling parameters used.

One way to eliminate extra-assay PCR products is to use a nested PCR protocol. In this method, 2 pairs of primers are used sequentially. The first primer pair amplifies an initial sequence (amplicon) that flanks and is larger than the intended target sequence using a complex template (eg, genomic DNA or complementary DNA from mRNA). Following the first PCR, a small aliquot of the product is used as a template for a second PCR. The complexity of products following the first PCR is orders of magnitude lower than for the first PCR. The second primer pair is internal to the first pair and designed to amplify the region of interest. The second primer pair will only amplify the target sequence from the correct PCR product if it is present as a reaction product from the first PCR.

Historically, the detection method for PCR products has been an EtBr (ethidium bromide)–stained gel, agarose, or acrylamide, depending on the size of the amplicon. A gel is required because potentially more than 1 product can be made. Determining the size of the product is as important as the intensity of the band. For experiments where template/no-template questions are asked, a gel is sufficient. However, for
experiments that require quantitative data, gels have a limited dynamic range and sensitivity. More importantly, end-point PCR (eg, quantification of PCR products following 30 cycles) is not quantitative.

Competitive PCR was developed in an attempt to overcome the problems of quantification. In this technique, an artificial template is constructed that shares the same primer binding sequences on the ends but has a different size and sometimes different intervening sequences than the target sequence. It is beyond the scope of this review to explain why this technique is not totally satisfactory. It is certainly much closer to a quantitative technique than end-point PCR. However, data from the PCR products are still run on gels with the same limitations in sensitivity and accuracy.

In 1996, Applied Biosystems (Foster City, Calif) introduced the first real-time PCR instrument, the 7700 Sequence Detector System. This instrument combines a very strong light source (argon laser) with a novel fluorometric optical system that monitors the emitted light intensity individually in 96 wells multiple times during the PCR using a standard thermocycler. For the first time, it was possible to see what the progression of an amplification curve looked like over a 40-cycle experiment. It was immediately obvious that some assays were better than others.

Besides the novel hardware, a novel approach to template detection was also introduced by TaqMan chemistry (Applied Biosystems). Each TaqMan assay has 3 components, the 2 primers and a dual-labeled fluorescent probe oligonucleotide that anneals to the sequence between the primer pair. The initial probes were composed of a fluorescein derivative on the 5’ end (6-FAM [6-carboxy-fluorescein]) as a reporter dye and a rhodamine dye on the 3’ end (TAMRA [6-carboxy-tetramethylrhodamine]) as a quencher dye. When these 2 dyes come into close proximity in solution, they form a fluorescence resonance energy transfer (FRET) pair in which the emitted light from the excited reporter (6-FAM) is absorbed (at 1-10 nm) by the nearby quencher dye (TAMRA), exciting it and resulting in light being emitted at the TAMRA wavelength instead of the 6-FAM wavelength. Thus, the TAMRA dye has quenched the 6-FAM fluorescence signal. During the PCR, the probe will anneal to a melted single-stranded template DNA followed by 1 of the primers 5’ to the probe. Taq polymerase then binds and synthesizes a new sequence initiated by the primer toward the probe. When the enzyme reaches the probe, it is displaced and degraded by an exonuclease activity that is separate from the DNA polymerase. The probe is cleaved, and the 6-FAM is physically released from its proximity to the TAMRA. As the PCR progresses, this leads to a steady increase in the 6-FAM reporter signal that is directly proportional to the number of new DNA molecules made.

Real-time PCR is the first instance where PCR can be used as a truly quantitative technique without the caveats of the competitive PCR method. By running a dilution series of a known, independently quantified standard template at the same time as the unknown sample, the researcher can quantify the unknowns against the standard by simple interpolation from the dilute curve. The maximum dynamic range of most RT-PCR or PCR assays is 7 logs. The number of molecules, which is the standard of measure for real-time PCR, can range from tens of millions down to hundreds in the same assay and still be accurately measured and quantified. Very few other techniques have this kind of sensitivity and dynamic range.

Today, there are a whole host of real-time PCR instruments on the market. The chemical analysis techniques have also expanded such that there are many choices of dyes and mechanisms. However, the TaqMan system is still one of the best. Real-time PCR has become the gold standard for the detection and quantification of nucleic acids from multiple sources.

APPLICATIONS OF MOLECULAR TECHNIQUES IN DIFFERENT SKIN DISEASES

Although molecular techniques have potential use in all cutaneous diseases, they are primarily used in a limited number of skin diseases at this time. The most frequent uses in clinical dermatology are for primary cutaneous lymphomas, T-cell receptor gene and immunoglobulin gene rearrangements, KS, melanoma, SCC, and BCC.

Primary Cutaneous Lymphomas

Cutaneous T-cell lymphoma includes a heterogeneous group of non-Hodgkin lymphomas typically arising from malignant T-cells that are usually a CD4+ phenotype. The most common forms of CTCL include mycosis fungoides (MF), in which tumors form in the skin, and Sézary syndrome (SS), in which large numbers of the tumor cells are found in the blood. The use of molecular diagnostics in this area is enabling a better understanding of the disease pathogenesis, and as a result new treatments are being discovered. Reverse transcriptase PCR is being used to detect the presence of possible cancer-germline genes. It allows the researcher to amplify the mRNA produced in the tissue of interest and thus to analyze gene expression in different disease states. For example, the expression of LAGE, cTAGE, MAGE-A9, and GAGE-3-7 genes has been discovered in patients with CTCL. These genes are examples of cancer-testis antigens, which are a group of genes predominantly expressed in cancers and gametogenic tissues. These multigene families of coding sequences, many of which map to the X chromosome, are heterogeneously expressed in cancer and are immunogenic in patients with cancer, making them possible targets for cancer diagnosis and therapy.

The development of real-time PCR has further expanded the scientific armamentarium. Unlike traditional PCR, which detects the amplicon product at the end point of the reaction cycles through the use of a gel, real-time PCR monitors the amplicon product in real time during each PCR cycle through the amount of fluorescence emitted during the reaction. This allows more accurate quantification of the amount of each gene produced, automatic data analysis, standard curve generation, and copy-number calculation. Real-time PCR has been used to detect the expression of the oncogene JUNB, a transcription factor involved in cell cycle control, in patients with MF and SS. The identification of these genes may be a potential target for future immunotherapeutic agents.

A better understanding of the underlying pathogenesis of CTCL is also occurring with the aid of molecular diagnostics. For example, loss of heterozygosity on chromosome 10q and microsatellite instability have been noted in MF, indicating that perhaps tumor suppressor genes, such as PTEN, may be involved in disease progression. Microsatellite instability is an aberration in the small repeat sequences that occur in DNA replication secondary to defects in DNA-mismatch repair. Germ line mutations in the DNA-mismatch genes have already been discovered in other cancers; therefore, their discovery in MF may impact patient prognosis and lead to new dis-
coverage in therapeutic agents for this disease. In addition, PCR is being used for the amplification of chromosomal break points for the possible detection of t(2p23; 5q35) in MF, a translocation that is more commonly associated with nodal anaplastic large-cell lymphoma (ALCL). In addition to PCR, FISH, CGH, and G-banding have also detected aberrations in MF and SS on chromosomes 1, 6, 8, 9, 10, 11, 13, 15, and 17. Comparative genomic hybridization has identified losses most commonly on chromosomes 10q and 13q and losses or gains on chromosomes 8q and 17q.

With a combination of molecular diagnostic techniques, the underlying genetic components of CTCL are continually being investigated and determined.

Lymphomatoid papulosis (LyP) is a chronic lymphoproliferative disorder that is typically histologically malignant but clinically benign. It is characterized as a type of CD30+ T-cell lymphoma, and molecular diagnostic techniques are uncovering new details about this low-grade malignancy. Polymerase chain reaction has been used to investigate the role of t(2p23;5q35), which is commonly associated with ALCL in LyP. The role of this translocation in LyP is controversial in that studies have had contradicting results regarding its importance in this disorder.

The detection of such a translocation may play a role in prognosis: patients with this translocation in ALCL tend to have a better clinical course. Studies have also demonstrated that a t(8;9) translocation may be involved in the development of LyP, further enhancing the possibility of the involvement of genetic aberrations with this disorder.

Anaplastic large cell lymphoma is a rare type of CD30+ T-cell non-Hodgkin lymphoma that occurs in 2 distinct forms: systemic ALCL, which involves lymph nodes or extranodal sites; and primary cutaneous ALCL, which involves skin nodules. Combinations of molecular diagnostic techniques have been used to further characterize ALCL. Imbalances in chromosomes 1, 5, 6, 7, and 8 have been detected with CGH, and oncogenes such as FGR1 (8p11), CTBS, RAF1, REL (2p13p12), and JUNB (19p13.2) have been identified with real-time PCR and microarray-based CGH. Molecular diagnostics are also being used to determine if a t(2;5) translocation (which is commonly associated with nodal ALCL) is also present in primary cutaneous ALCL. This translocation results in the fusion of the nucleophosmin (NPM) gene and the anaplastic lymphoma kinase (ALK) gene, a novel receptor tyrosine kinase gene. Studies have been conflicting as to the presence of this translocation: some studies indicate that the presence of such a translocation may indicate that cutaneous ALCL is a subset of ALCL rather than a distinct disease.

Primary cutaneous B-cell lymphoma (CBCL) is a unique type of low-grade lymphoma occurring in the skin. The classification and treatment of primary CBCL remains controversial. As with primary cutaneous ALCL, CGH and PCR have been used to investigate genetic abnormalities in primary CBCL. Comparative genomic hybridization and FISH have detected imbalances in chromosomes 7 and 18, while microarray-based CGH and PCR have identified the presence of genes that are frequently rearranged or amplified in human sarcomas, such as SASH1/CDK4 (12q13.3) and the human proto-oncogene BCL-2. Other gene abnormalities noted in primary CBCL include the inactivation of tumor-suppressor genes p15 (INK4b) and p16 (INK4a).

The loss of these genes may provide some insight into the progression of the disease. In addition to the detection of a variety of genes, PCR can also be used to determine if chromosomal translocations are present in primary CBCL. For example, although a t(14;18) chromosomal translocation of the BCL-2 gene has been associated with nodal follicular lymphomas, PCR analysis has demonstrated that this translocation is not very common in primary CBCL. The pathogenesis and prognosis of cutaneous lymphoproliferative disorders will continue to be investigated as molecular technology advances.

### T-Cell Receptor Gene and Immunoglobulin Gene Rearrangements

Detection of T-cell receptor (TCR) and immunoglobulin (Ig) rearrangements is becoming increasingly common for identification and classification of a variety of dermatologic malignancies, including CTCL and CBCL. Detection of TCR and Ig molecules is based on fundamental rearrangements that occur during development and maturation of B cells and T cells. Immunoglobulins determine B-cell clonality and are fundamentally characterized by 2 identical heavy and light chains. Rearrangements of the constant (C), variable (V), diversity (D), and joining (J) gene segments occur in the heavy chain, while rearrangements of the C-V-J regions are responsible for formation of the light chain. Diversity is created in each Ig by the number of genes at each locus (ie, germline diversity), rearrangements of gene segments, and somatic hypermutations. As a consequence of this enormous amount of diversity, a unique Ig for each B cell and its clone is created.

As in Ig, a similar process occurs in TCR rearrangements: TCRγ gene rearrangements, most commonly detected by PCR, are the result of rearrangements of a small number of V-J gene segments that are independent of the V-J regions involved in the formation of immunoglobulins. A large amount of diversity is also created in TCR rearrangements, not only as a result of combining different V-J gene segments but also as a result of nucleotides that fill in gains between the joining V and J segments created by loss of original nucleotides. However, in the process of TCR rearrangement, no somatic hypermutations occur as in Ig.

Previous detection of TCR gene rearrangements have relied heavily on Southern blotting; however, new advances in molecular diagnostics are resulting in the replacement of Southern blotting with PCR, which has several advantages over Southern blotting: (1) increased efficiency and speed (hours vs days); (2) less genetic material required; (3) increased cost-efficiency; and (4) higher degree of sensitivity. The disadvantages of PCR include the possibility of false-positive and false-negative results. False positives may result from the presence of clonal populations in diseases not considered to be lymphomas (eg, lymphomatoid papulosis) and cross contamination. False negatives may also occur as a result of deletion or lack of rearrangement of a particular gene.
in lymphoma. Despite these potential disadvantages, the overall higher sensitivity of this assay has made it a common diagnostic tool for dermatologic malignancies.

The usefulness of PCR in immunologic studies results from its ability to identify Iggs or TCR gene rearrangements in B cells and T cells, respectively. Polymerase chain reaction can be used to detect the β, δ, or γ chains in T-cell receptors and the Ig heavy chain (IgH) in B cells. Because the TCRγ chain gene has fewer V and J gene segments than the β or δ chains, fewer primers are needed for PCR amplification. As a result, most PCR assays rely on detection of the TCRγ chain rather than the β or δ chains. Following PCR amplification, different techniques may be used to analyze the TCRγ chain or IgH, including polyacrylamide gel electrophoresis, single-strand conformation polymorphism analysis, denaturing-gradient, or temperature-gradient electrophoresis. Fluorescent fragment analysis or heteroduplex analysis can also be used for analysis of rearrangements.70

Accurate determination of B-cell or T-cell clonality is extremely important because it can help distinguish a malignant lymphoma from a benign lymphoid infiltration.71 Presence of clonality typically implies the presence of a malignancy, and PCR analysis can help verify the phenotype of lymphocytic cells. As a result, PCR is increasingly becoming the mainstay for the diagnosis of CBCL and CTCL.

Polymerase chain reaction detection of TCR gene rearrangements is quite useful for the diagnosis of CTCL, especially when the findings of histologic analysis are inconclusive. In addition to confirming histologically diagnosed CTCL, PCR can be used in the early stages of CTCL when diagnosis is not absolute.71-73 This use of PCR also helps distinguish CTCL from other diseases with similar clinical or histologic presentations.74 Gene rearrangement detection with PCR in histologically atypical cutaneous T-cell lymphoid infiltrates has a sensitivity of approximately 94% and a specificity of 91%.75 Presence of TCR rearrangements may also have a correlation with disease outcome. The presence of PCR-detected clonal T cells in patients with SS has been correlated with poor prognosis.44 Presence of clonality typically implies the presence of a malignancy, and PCR analysis can help verify the phenotype of lymphocytic cells. As a result, PCR is increasingly becoming the mainstay for the diagnosis of CBCL and CTCL.

The use of PCR in LT and LT has helped classify this low-grade malignancy as a CTCL through the detection of T-cell clones.77,78 Identification of such T-cell clones provides a method not only for classifying malignancies but also for identifying the sequence of a tumor-derived TCRγ gene rearrangement in early lesions with a low tumor clone density, thereby facilitating molecular staging.79

Aside from its uses in CTCL and LT, PCR has allowed for amplification of the V and J region of the IgH gene for the determination of B-cell clonality.80,81 The use of PCR to assess B-cell clonality in primary CBCL is highly sensitive, but false positives can occur as a result of the scarcity of reactive B cells that may be present in the skin.82 However, this potential pitfall may be circumvented with combined use of both PCR and immunopathologic analysis: identification of B cells and/or plasma cells by immunopathologic analysis is unlikely to produce false-positive results with PCR detection of IgH.82 Thus, its uses for accurate identification of and differentiation between clinically similar counterparts make PCR an extremely valuable tool, especially for determining patient treatment options and disease prognosis.

The use of PCR for the detection of rearrangements will likely have an impact on the classification of cutaneous lymphomas. The establishment of B-cell or T-cell clonality has led to the categorization of other lymphoproliferative disorders (eg, parapsoriasis and lymphomatoid papulosis) that are not classified as CTCL or primary CBCL. Detection of gene rearrangements has also led to clarification of B-cell and T-cell lymphomas in that T-cell–rich B-cell lymphomas can now be identified.

In summary, molecular diagnostic assays such as PCR have an important role in the diagnosis of cutaneous lymphomas. In addition, these assays can be used to monitor progression of the disease and response to treatment in patients with cutaneous lymphomas. Although these assays now enjoy widespread use, it should be noted that PCR is not used alone for the diagnosis of cutaneous lymphomas but is more commonly correlated with clinicohistopathologic characteristics of the disease for the most accurate diagnosis.

Kaposi Sarcoma

Kaposi sarcoma is a malignant tumor of endothelial cells that presents with multiple vascular tumors usually occurring in the skin. There are many different types of KS, including classic KS, transplantation-associated KS, and AKS (KS associated with AIDS). All clinical forms of KS share the same structural characteristics and clinical presentation, but progression of the disease varies among the different types.83 Classic KS is typically a benign disease affecting elderly men of Italian, Jewish, or Mediterranean lineage. In these patients it presents as a slow-growing sarcoma, often progressing over many years. Eventually, the lower legs may swell, blood flow may be compromised, and the disease may even spread to other organs.84

Kaposi sarcoma also occurs in immunosuppressed individuals such as transplant recipients undergoing immunosuppressive therapy and patients with human immunodeficiency virus (HIV). Up to one third of patients with AIDS who are not receiving highly active antiretroviral therapy have KS, and it may be a presenting symptom in some patients. More aggressive than other types of KS, AKS spreads more quickly and often to many parts of the body.84

The association between KS and human herpesvirus (HHV) 8 has been established in AKS and in other forms of KS. Studies of viral genes with RT-PCR have elucidated the relationship between expression of the HHV-8 homologue of the G protein–coupled receptor (vGCR) and the viral transcriptional transactivator HIV-1 Tat.85 The tumorigenic and angiogenic effects of vGCR, activated by HIV-1 Tat, may contribute to AKS pathogenesis. Such studies will increase understanding of how HHV-8 and HIV interact to produce the unique disease entity, AKS.

Molecular diagnostics have not only confirmed the association between HHV-8 and KS but have been used to quantify the HHV-8 DNA. Measuring HHV-8 viral loads.
may play an important role in monitoring disease progression and treatment response in patients with KS. This qualitative assessment of HHV-8 viral load has been correlated with HIV viral load and CD4 counts in patients receiving highly active antiretroviral therapy. Patients with low viral loads, as detected by PCR, are more likely to be asymptomatic than those with high viral loads. Such PCR assays are performed with a variety of primers targeted at specific regions of the HHV-8 genome such as the ORF-K1 region. With improved technology, this qualitative detection is becoming easier and more cost-effective, which will facilitate its use in clinical laboratories.

The use of PCR in clinical settings has particular importance in KS diagnosis in that the detection of HHV-8 RNA in a vascular neoplasm by PCR helps differentiate KS from other vascular lesions. Direct PCR DNA sequencing of the ORF-K1 membrane protein gene and the ORF-K15 genes of HHV-8 has also allowed for the identification of 7 distinct subtypes of HHV-8. Analysis of these genomes and the comparison of the classic and AKS genomes has provided us with a better understanding of the origin and spread of this virus.

The understanding of KS pathology is also being enhanced through the use of other molecular diagnostic techniques. Although the disease is not commonly associated with cytogenetic abnormalities, FISH has been used to determine if a series of chromosomal aberrations (eg, 8q and 1q trisomy) may play a role in disease pathogenesis. In addition, with PCR, enhanced telomerase activity has been demonstrated in KS, which may explain the tumor’s resilience and proliferation. Investigations into such enzymes may help delineate whether KS is truly neoplastic in origin rather than a vascular hyperplasia. Aside from telomerase activity, activation of oncogenes such as fibroblast growth factor 4 (FGF4) and INT2 in chromosome 11 has been detected in KS. DNA microarrays have also been used in KS to detect the presence of the c-Kit proto-oncogene. Activation of such oncogenes by HHV-8 may facilitate the progression of the disease.

**Molecular Diagnostics and Melanoma**

Melanoma, a malignant neoplasm of melanocytes, accounts for only 4% of all skin cancers. However, this malignancy causes 79% of skin cancer–related deaths, and its incidence is increasing. Because melanoma is a curable disease if caught in its early stages, molecular diagnostic techniques that permit early diagnosis would be extremely desirable. Molecular techniques could also contribute to the understanding of melanoma and help determine its staging and prognosis.

The ability to detect tyrosinase transcripts in circulating melanoma cells was first described by Smith et al over a decade ago. Since then, many studies have been conducted to evaluate the validity of this assay. Many studies have demonstrated that tyrosinase is usually not detected in the peripheral blood of healthy individuals. However, the ability of RT-PCR to detect tyrosinase and other melanoma markers in the peripheral blood of patients with malignant melanoma has been shown to be highly variable. Previous studies report positive RT-PCR findings in 18% of patients with stage I disease, 28% for stage II, 30% for stage III, and 45% for stage IV disease. This large variation in RT-PCR sensitivity (0%-100%) may be owing to 1 or more of the following: (1) tumor heterogeneity; (2) intermittent shedding of tumor cells in the blood; and (3) methodologic differences in mRNA extraction or complementary DNA synthesis in the different studies. This may explain the low prevalence of RT-PCR positive findings in patients with stage IV disease.

Study results are also conflicting as to the prognostic value of these tests. Some studies have indicated that the presence of circulating melanoma cells in the peripheral blood correlates with tumor thickness, clinical stage, and overall disease survival. In addition, RT-PCR detection of circulating melanoma cells may also have some value in monitoring response to treatment and immunotherapy. Despite the promising applications of this technique, other studies have indicated that the clinical value of RT-PCR in detecting circulating melanoma cells is minimal to none.

The staging for malignant melanoma relies on the detection of malignant cells by sentinel lymph node (SLN) biopsy. Conventional techniques typically involve pathologic examination using hematoxylin–eosin staining and immunohistochemical analysis. Although these techniques confer a dependable degree of accuracy, new molecular diagnostic techniques such as RT-PCR are becoming increasingly accepted methods for the detection of malignant cells. Reverse transcriptase PCR has been shown to offer a greater sensitivity than both hematoxylin–eosin staining and immunohistochemical analysis: it can detect 1 tumor cell among 10³ normal cells. It is most frequently used for the amplification of mRNA from tyrosinase, an enzyme expressed by melanocytes that catalyzes the conversion of tyrosine into dopa and dopaquinone. Tyrosinase is a marker of melanocyte differentiation; therefore, it can be used to detect metastasis of malignant melanocytes. However, because other nonmelanocytic cells such as Schwann cells and nevus cells in lymph nodes can express this enzyme, the detection of multiple melanocytic markers (ie, MAGE-3, MART-1, GAGE, MUC-18, and p97) may aid in the more accurate identification of metastatic cells. Amplification of these melanoma markers can be performed from samples obtained from peripheral blood or SLN biopsy specimens.

Because of its increased sensitivity over hematoxylin–eosin staining and immunohistochemical analysis, RT-PCR is being used more frequently to detect occult metastasis in SLNs. The detection of melanoma markers in SLNs has a relationship with melanoma prognosis: higher rates of positive RT-PCR findings are associated with melanoma recurrence in regional lymph nodes and decreased rates of overall survival. In conjunction with detection of various biomarkers, detection of apoptotic-related genes such as the survivin gene, has been related to poor prognosis and decreased survival. Studies have found a correlation between the presence of such micrometastasis and Breslow tumor thickness. For example, it has been demonstrated that tumors less than...
1.5 mm thick are RT-PCR positive for survivin transcripts 26% to 62% of the time; those that are greater than 1.5 mm but less than 4 mm thick are positive 42% to 68% of the time; and finally, tumors greater than 4 mm in thickness are RT-PCR positive 77% to 85% of the time.125,127,129 The ability to detect transcripts associated with micro-metastasis using RT-PCR could have strong implications on the classification and staging of tumors. As a result, a more comprehensive Sunbelt Melanoma Trial is under way to determine clinical relevance of RT-PCR in the detection of melanoma markers.130

Along with their uses for clinical staging and detection of metastases, molecular techniques can detect gene mutations in patients with malignant melanoma. Polymerase chain reaction has been used to detect different interleukin (IL) 10 promoter polymorphisms associated with increased susceptibility or resistance to melanoma as well as certain inflammatory diseases, viral infections, cancers, and transplant rejection. Ethnic differences in the IL-10 gene have also been studied. Different single-nucleotide polymorphisms detected in the regulatory region of the gene coding for IL-10 resulted in the production of "high"-expression genotypes and "low"-expression genotypes.132 Detection of mutations in the p53 tumor suppressor gene and inactivation of the Ras association domain family 1 (RASSF1) gene have also been accomplished through PCR.133,134 Polymerase chain reaction is also being used to identify tumor-associated antigens such as MAGE-A6, MAGE-A4a, MAGE-A10, CT7/MAGE-C1, GAGE-3-8 group, RAGE-1, -3, and antigens such as MAGE-A6, MAGE-A4a, MAGE-A10, reaction is also being used to identify tumor-associated some abnormalities associated with melanoma.143

Arrangements account for more than 70% of the chromosomal abnormalities on chromosomes 1, 6, 7, and 9.142 These rearrangements include the 1p36 subtelomeric region, have been noted in individuals with nodular and metastatic melanomas.143 Because measurement of telomeres in some cancers is of diagnostic value, quantitative FISH is being used to evaluate telomeres and their possible use for the diagnosis of melanocytic lesions.145 With the aid of FISH, the KiSS-1 gene, a human malignant melanoma metastasis-suppressor gene, has been localized to chromosome 1q32.146 In uveal melanoma, FISH and CGH are used to detect abnormalities in chromosome 3 and 8, which have been shown to correlate with patient prognosis and survival.147,148 Fluorescence in situ hybridization is also being used to understand the interaction between immunotherapy and gene expression. Deletions of the h2m gene on chromosome 15 have been identified as possible barriers to patients receiving immunotherapy.149 These applications of molecular diagnostics are helping to identify the underlying genetic pathogenesis of metastatic disease.

New melanoma markers used in RT-PCR detection of the disease are being continually identified with the aid of DNA microarray technology. DermArray DNA microarrays (IntegriDerm Inc, Birmingham, Ala) have also identified other genes known to be involved in melanogenesis, such as silver/Pmel17, melan-A/MART-1, tyrosinase, and TRP-2/DCT (dopachrome tautomerase).150,151 Numerous other markers have been identified:

- Tyrosinase-related protein 1 (TRP-1)/DHICA oxidase
- RAB7
- Cyclin-dependent kinase 6
- Solute carrier family 3 member 2
- CD44 antigen
- Protein tyrosine phosphatase type IVA, member 2
- RANTES
- Tyrosinase
- Coagulation factor VIII–associated
- Nidogen-2
- Melan-A/MART-1
- TRP-2/DCT (dopachrome tautomerase)
- Silver/Pmel17

In conjunction with their uses in the identification of biomarkers of melanoma, DNA microarrays are helping unravel the mystery of the molecular pathogenesis of melanoma. The genetic pathogenesis and underlying basis for the aggressive nature of the disease is currently poorly understood, but this relatively new technology is helping to identify various gene expressions possibly responsible for its aggressive nature. High-penetration susceptibility genes such as CDKN2A and CDK4 (the INK4a/ARF locus on chromosome 9p21) have already been identified in melanoma.152 These 2 genes are involved in the cell-cycle regulatory pathway, and their mutations appear to confer susceptibility to melanoma in high-risk families.153 DNA microarray analyses of melanoma gene expression have correlated the increased expression of other genes such as the WNT5A and RHOC gene with higher cell motility and invasiveness.154,155 The desensitization of the receptor for WNT5A, Frizzled 3, has also been correlated with inhibition of protein kinase C pathways, which are pathways associated with cytoskeletal organization and invasion.1,1155 Direct correlation between WNT5A and cell motility suggests that this ligand may be used in the future as a potential target for cessation of melanoma progression.1 The RHOC gene, a mem-

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ber of the Rho GTPase family, activates another receptor in the protein kinase C pathway, thereby enhancing metastasis of cells.\textsuperscript{156}

Another gene that has been associated with progression of melanoma is the microphthalmia transcription factor gene (Mitf). Mitf in melanocytes is involved in the transcription of tyrosinase and tyrosinase-related protein 1, and it regulates the BCL-2 gene, a proto-oncogene that appears to be necessary for melanoma survival.\textsuperscript{157} The pathway involving the Mitf and BCL-2 genes is currently being used as a potential target for the treatment of chemoresistant malignant melanoma.\textsuperscript{158} Using oligonucleotide arrays, researchers have found that thrombospondin 2, an extracellular matrix molecule, is strongly correlated with melanoma metastasis but not with primary tumors.\textsuperscript{159} As a result of its continued use, DNA microarray technology is enabling a better understanding of genes possibly responsible for the aggressive nature of melanoma. In the near future, DNA microarrays will have substantial impact on the elucidation of the pathogenesis and treatment of melanoma.

**SCC and BCC**

Squamous cell carcinoma and BCC are common skin cancers known to be induced by UV radiation. However, little was known about the molecular pathogenesis of these cancers until the recent use of molecular diagnostics. Gorlin syndrome, which is an autosomal dominant disease characterized by multiple BCCs at an early age, has helped elucidate the molecular mystery of BCC. Mutations of the patched (PTCH), p53, and BCL-2 genes have been demonstrated in Gorlin syndrome and in sporadic BCC.\textsuperscript{160,161} PTCH and p53 genes are major targets for UV radiation in BCC, and inactivation of these genes manifests in cellular proliferation.\textsuperscript{162} Studies have shown that 30% to 40% of BCCs show mutations in the PTCH gene, and 50% to 56% of BCCs show p53 mutations.\textsuperscript{162,163} Under immunohistochemical analysis, p63 expression has been found in BCCs as well as SCCs, indicating that this p53 homologue on chromosome 3q27 may play a role in the development and differentiation of these cancers.\textsuperscript{164} Other genes such as the Janus protein tyrosine kinase 2 (Jak3), microsomal glutathione S-transferase 1 (GST 12), teratocarcinoma-derived growth factor cripto, glutaredoxin, and monocyte chemoattractant protein 1 (MCP-1) have been identified in BCCs using complementary DNA microarrays and RT-PCR.\textsuperscript{165} These genes have previously been associated with other tumors, and DNA microarrays have now linked their presence to BCCs. In addition to identifying these specific genes, molecular techniques have also allowed for targeting of certain genes for the treatment of BCC. It has been discovered that interferon α induces Fas expression and apoptosis in hedgehog pathway–activated BCC, which points toward a possible nonsurgical therapeutic alternative for patients who are poor surgical candidates.\textsuperscript{166}

Mutations of the p53 gene associated with UV radiation have also been associated with SCC.\textsuperscript{167} The p53 gene is involved in inducing cell apoptosis, which involves the expression of a variety of genes responsible for the appropriate regulation of cell cycles. A p53 polymorphism at codon 72 has been strongly associated with skin cancers in epidermodysplasia verruciformis, which is an autosomal recessive hereditary disorder characterized by chronic infection with human papillomavirus and causing disseminated eruptions of wartlike lesions. These lesions have the potential to transform into SCCs. Molecular studies have shown that patients with the malignant form of epidermodysplasia verruciformis were homozygous for arginine (Arg/Arg) at codon 72 of the p53 gene, while none of the patients with the benign form had this polymorphism.\textsuperscript{168}

Analysis with CGH has also found UV-B–specific mutations of the Ha-ras gene, gains of 8q, and loss of 4p in SCC. Via RT-PCR, other genes have also been studied in SCC including Rhoc and EMMPRIN.\textsuperscript{169} Rhoc has been linked to an invasive phenotype in tumor cells, and EMMPRIN is involved in the invasion and metastasis of tumor cells via elevated levels of matrix metalloproteins.\textsuperscript{170} Other genes that have been found via DNA microarrays and RT-PCR to be biomarkers for SCC in vivo and in vitro are fibronectin 1, annexin A5, glyceroldehyde 3-phosphate dehydrogenase (G3PDH), zinc-finger protein 254, and Huntingtin-associated protein.\textsuperscript{171} Identification of genes through molecular diagnostics allows for supplemental methods of diagnosis of the disease. Additionally, the identified genes are potential targets for pharmaceutical therapies, which expands the usefulness of this technique.

**CONCLUSIONS**

Molecular diagnostic techniques are identifying molecular markers that may elucidate the pathophysiology of dermatologic diseases. Tumor markers will likely play an important role in the identification, staging, and treatment of malignancies. Promising tumor markers for future clinical diagnostic tests include tyrosinase in conjunction with a panel of specific tumor antigens for melanoma and T-cell receptor monoclonality for CTCL. The advantage of the detection of molecular markers is that malignancies can potentially be identified at an earlier stage, which would result in improved long-term survival of patients and/or improved quality of life.\textsuperscript{172}

The enormous potential for developing molecular methods to identify these tumor markers is offset to some degree by limitations of the techniques. The time and expense involved in properly identifying a potential tumor marker are considerable. Before a potential marker is incorporated into a clinical test, its biology must be understood; an assay system that is simple, specific, and reproducible must be developed; and the results of these assays must be placed in the context of existing pathologic and molecular data along with solid patient statistics to determine its individual viability. Many tumor markers are not specific enough to allow for unambiguous diagnosis of a disease state based solely on the presence of the marker. Shortcuts in the development of assays or clinical testing may generate confusing and conflicting results if time is not taken to integrate these rapidly developing molecular techniques into their proper biological context.\textsuperscript{172}

Despite these limitations, however, many molecular diagnostic assays have been used to investigate the patho-
physiology of a variety of dermatological malignancies. Melanoma, SCC, BCC, CTCL, and LyP have all been investigated using molecular technology. As the significance of the genetic bases of these diseases is determined, new and better treatment options may emerge.

Molecular assays are also being used to evaluate the role of specific gene expression in the pathogenesis and treatment of cutaneous inflammatory disorders such as atopic dermatitis and psoriasis. The detection of unique gene expression patterns in these disease states may facilitate more precise targeting of biological medications and lead to increased efficacy with fewer adverse effects. Elucidating possible genetic markers involved with inflammatory disorders such as psoriasis may enhance understanding of the interaction that occurs between genotype and environment to produce the disease state. Eventually, identification of contributing environmental factors and specific genetic susceptibilities for inflammatory skin disorders may allow targeted avoidance counseling for at-risk patients.

The utility of these assays in the clinical setting is currently limited because many of them lack approval from the US Food and Drug Administration, which requires that products be safe, effective, and reproducibly manufactured. In addition, the use of the assay should have a favorable outcome on the management and treatment of the malignancy. As such, the American Society of Clinical Oncology recommends that testing for genetic mutations be done only when “the tests can be adequately interpreted; and the results will influence the medical management of the patient.” As the technology behind these molecular techniques evolves, clinical trials will be developed and evaluated. Although currently these tests have little influence on medical treatment and management of dermatologic malignancies, they will likely have a role in the future clinical setting as an aid to both the diagnosis and treatment of dermatologic diseases.

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