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Precision Molecular Pathology of Dermatologic Diseases

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Precision Molecular Pathology of Dermatologic Diseases

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Chapter 1
Introduction

Victor G. Prieto

Although special techniques have been applied for more than a century to aid the diagnosis of pathology specimens, it is only within the past 10–20 years when the field of ancillary techniques has exploded to the current levels. From the beginning of histology and pathology, morphologists have used a wide range of special techniques such as silver stains to detect the presence of axons, colloidal iron stain to detect mucin deposits in the dermis, or Steiner stain to detect spirochetes. During the 1960 and 1970s, electron microscopy allowed examination of the subcellular structures to detect, among others, the capsids of viruses, organelles associated with a particular neoplasm (Birbeck granules in Langerhans cell histiocytosis), or alteration of the basement membrane area in the different subtypes of epidermolysis bullosa. Since the 1980s immunohistochemistry has become widely used to detect antigens, with applications to neoplastic (e.g., differentiation between Paget disease and melanoma), inflammatory (differentiation among the different subtypes of cutaneous immunobullous diseases), and infectious conditions (detection of spirochetes in cutaneous lesions of syphilis). In a sense, we can consider immunohistochemistry as an early "molecular" technique since it allows the detection of specific antigens (i.e., "molecules").

In the past 10–15 years, molecular techniques such as genomic sequencing have become much more available. From an original very expensive price and long-processing times, significant advances have much reduced their turnaround time and cost and thus have made them very attractive to diagnostic applications. The range of genetic or molecular tests that can be performed on skin specimens include polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), gene arrays, routine cytogenetics, and mass spectrometry.

A very significant advance in the field of molecular techniques has been their progressive adaptation to formalin-fixed, paraffin embedded tissue specimens. As

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it is well know, due to standard tissue processing (formalin fixation, successive heating periods, and embedding in paraffin), the genetic material is partially degraded so most tests were originally developed on fresh tissue or cell suspensions, thus limiting their practical use in dermatopathology. However, by developing successive modifications, many of these tests can now be utilized on standard, formalin-fixed, paraffin embedded tissue (the material most easily available in pathology departments).

As an example of the importance of these molecular techniques, genomic analysis has allowed to confirm that the old morphologic classification of lentigo maligna, superficial spreading, and acral-lentiginous melanoma correlates with a different genetic signature. Thus, melanomas arising in skin chronically exposed to the sun (i.e., lentigo maligna melanoma) have c-kit and NRAS mutations; melanomas arising in skin intermittently exposed to the sun (i.e., superficial spreading type) typically have BRAF mutations; and melanomas arising in the acral locations or mucosae (i.e., acral-lentiginous mucosal type) most commonly show c-kit mutations. Furthermore, this analysis has not only resulted in better knowledge of the pathogenesis of cutaneous melanoma but has also provided with identification of therapeutic targets in an area surely needed of new treatments.

In summary, this book reviews the most popular and useful techniques, in our opinion, for diagnosis, prognosis, and therapeutic purposes in the field of dermatopathology. Although almost any area of dermatopathology can benefit of the use of molecular techniques, they are currently preferentially used in some conditions, and thus this book devotes one chapter each to cutaneous hematolymphoid, mesenchymal, epithelial, infectious, melanocytic, and miscellaneous lesions. We are aware that it is certainly impossible to discuss all the possible applications of these techniques to the field of dermatopathology; however, we expect that this book will serve as a tool to familiarize the readers with these techniques and help them to add these tools to the diagnostic armamentarium in pathology.

Chapter 2
Hematolymphoid Proliferations of the Skin

Carlos A. Torres-Cabala, Jonathan L. Curry, Su S. Chen and Roberto N. Miranda

Introduction

Molecular tests used by practicing pathologists are mostly performed on formalin-fixed, paraffin-embedded tissue specimens. Occasionally, when a more comprehensive molecular analysis is required, the use of fresh tissue or cell suspensions can overcome the limitations of testing on fixed tissue. The range of genetic or molecular tests that can be performed on skin specimens with lymphoid or hematopoietic disorders include, but are not limited to polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), proteomics, comparative genomic hybridization (CGH), gene arrays, and routine cytogenetics. In this review, we discuss the significance of the molecular testing most frequently used in the evaluation of clinical specimens involved by lymphoid or hematopoietic infiltrates.

Most of the molecular testing performed on cases of lymphoid infiltrates in the skin is done to identify clonality since it is generally thought that the presence of clonality supports a diagnosis of malignancy (i.e., lymphoma) and the lack of clonality excludes malignancy (i.e., reactive lymphoid hyperplasia). This approach is followed because it is considered that skin disorders with lymphoid infiltrates follow the paradigm of lymphoid disorders affecting lymph nodes, where evidence of clon-

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ality has usually been equated with malignancy. However, with further clinical specialization, increasing attention has been paid to lesions and tumors arising at extranodal sites, and differences related with specific anatomic sites have been identified, raising the concern that not all criteria applied to nodal lymphomas perfectly fit in extranodal sites. In dermatologic disorders in particular, whereas clinically typical malignant and benign lymphoid lesions exist and can be easily recognized by the clinician, there are many instances in which fully integration of clinical, pathological, and molecular findings is required to reach a definitive diagnosis. Apparent lack of concordance between these findings is a well-known, and probably not uncommon, phenomenon. Clonality may be detected in clinically indolent lesions (a "false positive," if following the paradigm of clonality being equivalent to malignancy). Conversely, clinically malignant lesions may lack evidence of clonality by molecular methods ("false negative"). Moreover, well-established pathological criteria useful for diagnosis of lymph node entities may not be applicable for their skin counterparts, such as the case of follicular lymphoma (FL), associated with t(14;18)(q32;q21) in approximately 85% of the nodal cases (and usually positive for BCL2 by immunohistochemistry) but commonly negative for BCL2 and harboring the translocation in less than 30% of primary cutaneous follicle centre lymphoma. The need for correlation of molecular findings with clinical and pathological characteristics of the lesions in dermatopathology cannot be overemphasized.

Molecular Tests Commonly Used in the Dermatopathology Practice

T-cell Receptor Gene Rearrangement Analysis

The T-cell receptor (TCR) is present within the CD3 complex that locates on the surface of T cells. Clonal rearrangement of the *TCR* gene is used to determine whether a T-cell lymphoid population is monoclonal or polyclonal [1]. The *TCR* is composed of alpha, beta, gamma, and delta chains. More than 90% of the mature T lymphocytes harbor the alpha-beta TCR [2, 3]. It is important to remember that independently from what TCR (alpha-beta or gamma-delta) is expressed, rearrangement of the gamma gene is the most frequently detected in the T cells [4]. The *TCR* gene rearrangements occur in the following chronological order: delta—alpha—gamma—beta. Since the delta receptor locus is located within the alpha gene, the delta locus is deleted most of the times when the alpha gene is rearranged [5]. PCR-based methods—the most used in daily clinical practice—can detect rearrangements of the delta, gamma, and beta chains [6]. The gamma chain gene has less variation of sequences than the delta and beta genes, thus requiring fewer specific primers for the PCR amplification [7]. Moreover, *TCR* gamma gene rearrangements are found in both gamma-delta and alpha-beta T-cell lymphomas (in the latter, a rearranged gamma allele is retained although not expressed [6]), making the *TCR* gamma gene rearrangement detection the preferred test for detection of T-cell

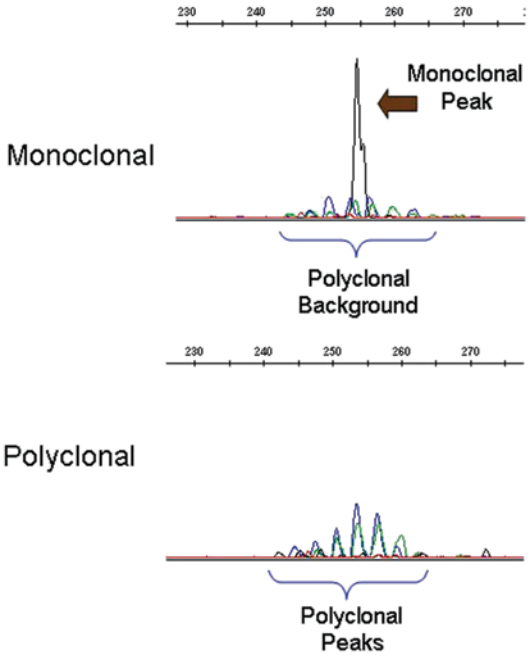

Fig. 2.1 Analysis of rearrangements of the T-cell receptor (*TCR*) beta chain by polymerase chain reaction (*PCR*). The gene scan on the top shows amplification of segments as multiple small peaks and a large predominant peak. This pattern is consistent with a monoclonal population of T-lymphocytes. In the right context, this supports a neoplastic population of T lymphocytes. The *bottom* of the Figure shows a polyclonal pattern characterized by multiple small peaks, consistent with the presence of reactive, nonneoplastic T lymphocytes. *TCR* beta chain analysis is especially useful in the identification of a monoclonal peak in a polyclonal background

clonal populations in dermatopathology. Due to its greater combinational diversity, evaluation of *TCR* beta gene rearrangement seems to be particularly useful in the detection of a monoclonal population in a background of polyclonal reactive cells [8]. Rarely, cases of T-cell lymphomas may present rearrangements of the delta chain as the only evidence of clonality [9].

The PCR products are analyzed using high-resolution capillary electrophoresis. Polyclonal proliferations render multiple peaks (more than five) whereas clonal populations are characterized by one or two peaks whose height exceeds that of the polyclonal background by a ratio of 2:1 to 3:1 [10] (Figs. 2.1 and 2.2).

The sensitivity of PCR-based tests varies depending on the type of sample and technical issues. It is accepted that the minimum percentage of detectable clonal cells by this method is around 1 % [11] and that its overall sensitivity and specificity is around 70 and 97 % for mycosis fungoides (MF), respectively [12]. False negative results may be due to low numbers of malignant T-cells or absence of *TCR* gene rearrangement in the lymphoma cells [13]. False positivity (pseudoclonality) may result from amplification of *TCR* gene rearrangement present in a few T-cells composing a sparse, reactive lymphocytic infiltrate [14]. Duplicate analyses may distinguish reactive from neoplastic proliferations since the dominant peaks detected in reactive conditions vary within the same sample while true clonal peaks are usually reproducible [15].

In recent years, great value has been given to the demonstration of the presence of identical T-cell clones at different anatomical sites as a highly specific tool in discriminating between MF and inflammatory conditions [16], the so-called stable clonal pattern [14]. However, this pattern has also been reported in some

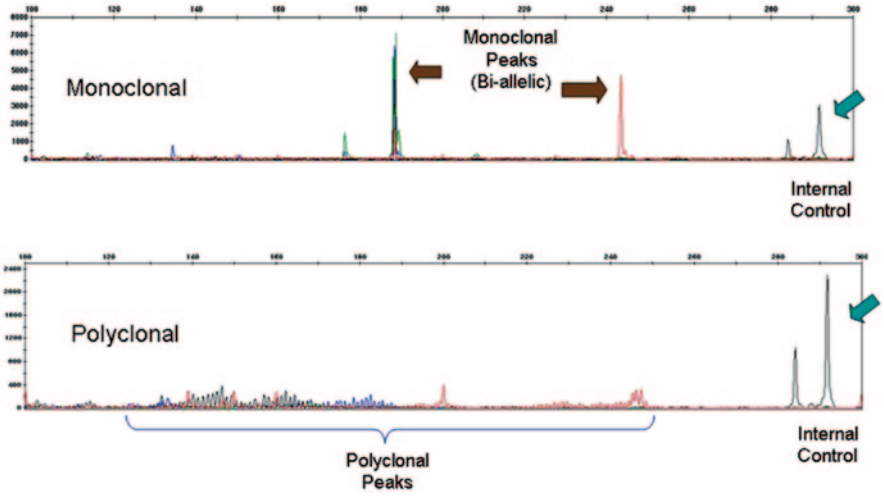

Fig. 2.2 Analysis of rearrangements of the T-cell receptor (*TCR*) gamma chain by polymerase chain reaction (*PCR*). In this case the scan on the top shows that the amplification of segments yielded multiple small peaks and two predominant peaks, consistent with a bi-allelic monoclonal population of T-lymphocytes. The *bottom* shows analysis of *TCR* gamma chain gene rearrangement in a different specimen. It reveals multiple small peaks, consistent with a polyclonal population of likely reactive, nonneoplastic T lymphocytes. As with the *TCR* beta chain gene rearrangement analysis, the correct interpretation of this result should be made in conjunction with clinical and pathological findings. The arrows indicate internal controls

inflammatory and "borderline" processes [17]. Conversely, genetically unstable subclones have been described in T-cell lymphomas [18] and clonal heterogeneity in MF lesions from distinct anatomical sites has been reported [19, 20]. The utility of comparing clones from different anatomical sizes for the diagnosis of T-cell lymphoma needs to be evaluated in the context of the clinical and histopathological findings.

Immunoglobulin Gene Rearrangement Analysis

The genes encoding immunoglobulins (Ig), the antigen receptors in B-cells, include the heavy-chain (*IGH*), kappa light chain, and lambda light chain genes. The *IGH* gene rearrangement starts with the combination of a diversity (D_H) segment with a joining (J_H) segment, which then is joined with a variable (V_H) segment to create a VDJ_H sequence. The kappa gene rearrangement then follows. Depending on the functionality of the rearranged kappa alleles, the lambda gene will be rearranged, usually after deletion of both kappa genes [21].

PCR-based methods for detection of *IGH* gene rearrangement utilize four sets of consensus primers designed to amplify three conserved framework regions within

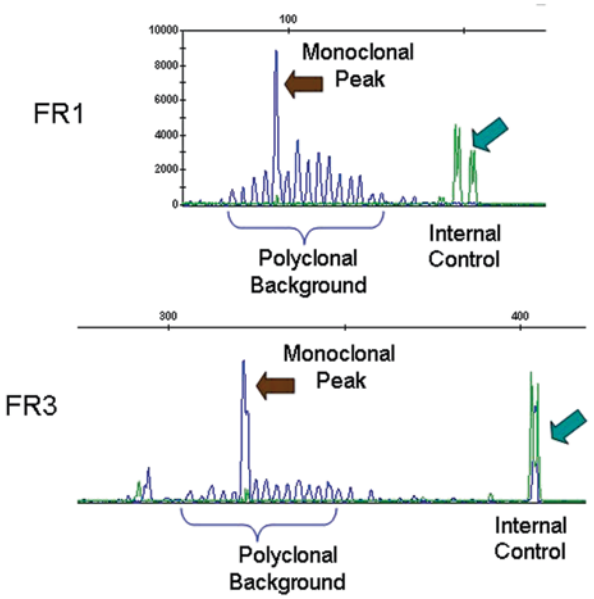

Fig. 2.3 Analysis of rearrangements of the immunoglobulin heavy chain (*IGH*) genes by polymerase chain reaction (*PCR*). Evidence of rearrangements of variable, diversity, and joining (*VDJ*) regions of IGH can be visualized by using pairs of primers at the V and J regions. Each lymphocyte in the analyzed specimen has a rearrangement that renders a segment that is amplified. In B-cell neoplastic processes, the presence of a prominent or distinct peak reflects that a significant number of cells in the specimen have the same size of amplified product. This finding constitutes a monoclonal peak and it usually correlates with a neoplastic expansion of B-cells. The use of three primers for the framework region (*FR*) of the V regions yields a higher chance of identifying a monoclonal population. The top of the figure shows the results of PCR amplification using FR1 and J region primers, while the bottom shows the amplification using FR3 and J region primers. Both analyses yielded the identification of a monoclonal peak amidst several smaller peaks, indicating that the neoplastic clone is admixed with reactive B lymphocytes. The size of the amplified segment is 98 base pairs on the top and is 320 base pairs on the bottom. Internal controls are used to confirm the size of segments and that amplification is taking place. The arrows indicate internal controls

V_H and one within J_H. From these, PCR amplification of framework region III in the V_H and the framework region in J_H segments is the most widely used test in clinical practice, due to the low molecular weight of the resulting amplicon and therefore the possibility of using formalin-fixed paraffin-embedded tissue as DNA source [22].

Same as for *TCR* gene rearrangement analysis, the peaks obtained by capillary electrophoresis are evaluated for the presence of a dominant population or populations (Figs. 2.3 and 2.4). The sensitivity of the method, although usually high, varies according to a number of factors such as the number of reactive B-cells present in the background and tissue fixation, and it can be as low as 47% in formalin-fixed paraffin-embedded tissue [23]. The rate of false negative results seems to be espe-

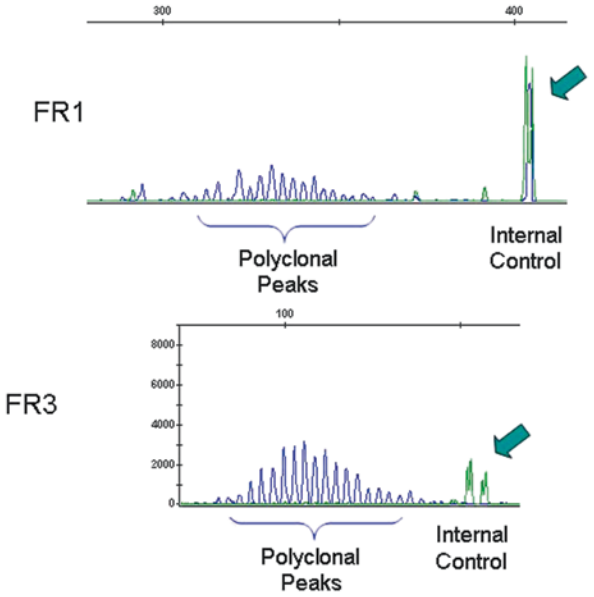

Fig. 2.4 Analysis of rearrangements of the immunoglobulin heavy chain (*IGH*) genes by polymerase chain reaction (*PCR*). In a reactive process, most lymphocytes render different size of amplified product and appear as multiple peaks, interpreted as polyclonal peaks/polyclonal background. In this case, the amplification of segments using FR1 and FR3 primers revealed a polyclonal pattern, consistent with a reactive, not neoplastic infiltrate. Similar results were obtained using FR2 primers. Correlation with clinical and pathological findings is still needed for an adequate interpretation of results of *IGH* gene rearrangement analysis. The arrows indicate internal controls.

cially high in cases of diffuse large B-cell lymphoma (DLBL) and follicular lymphoma, which have a high frequency of somatic mutations [24]. These mutations lead to sequences that are noncomplementary to the primer sequences. Such false-negative result is avoided by using multiple PCR target regions such as FR1, FR2, and FR3 segments of *IGH* gene. Another way to increase the sensitivity to detect clonal B-cell populations is by adding a test for immunoglobulin light chain (IGL) kappa or lambda gene rearrangement [25].

False positive results may be seen in cases in which the skin biopsy shows only sparse infiltrate and the PCR products amplified from the few B-cells appear as a distinct peak. Again, as for T-cell pseudoclonal cases, repeated testing may help in discriminating false clonality from true clonal B-cell expansion. Cases of cutaneous lymphoid hyperplasia have been demonstrated to be clonal for *IGH* gene rearrangement [26], and clonal *IGH* gene rearrangement has been reported in T-cell proliferations [27]. Other causes of false-positive results include immune disorders and infections showing a predominant B-cell population.

It is important to emphasize that the demonstration of monoclonal *IGH* or *TCR* gene rearrangement in a cutaneous lymphoid proliferation by itself does not make