Opinions

Pathology in the Molecular Era: Challenges and Opportunities

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Abstract: Pathology as a discipline can be traced to the 17th Century, B.C. Over the past 4,000 years, pathology has experienced four eras: the morphological, immunological, genetic, and molecular eras. Molecular pathology emerged in 1970s when Southern blot was employed to detect gene rearrangements in cancer cells. DNA sequencing and polymerase chain reaction (PCR) further revolutionized the diagnosis of human diseases. Gene profiling array and next generation sequencing are being applied in clinical diagnostics. While marveling at the advancement of new technologies, pathologists should be aware of the many challenges over the horizon. These include: 1) atypical morphology and aberrant gene expression make it more and more difficult to classify cancers; 2) genetic studies that play more and more important roles in diagnosing diseases; 3) precision medicine which renders morphology-based diagnosis less and less meaningful; 4) the reality of robust and affordable global sequencing of tumors. With development of various omics, traditionally morphology-based pathology will face even more challenges. However, these challenges can also be opportunities for pathologists, such as diagnosing diseases based on biology; classifying diseases based on therapeutic targets; getting more involved in clinical decision making; and discovering new biomarkers and therapeutic targets. Seizing these opportunities will be essential for pathology to play a central role in the 21st Century medicine.

Keywords: precision pathology, molecular era, challenges, opportunities, targeted therapy

Introduction

Pathology as a discipline can be traced back to the 17th Century B.C. when medicine was just born in ancient Egypt [1]. Throughout the history of medicine, pathology has experienced largely four eras: the morphological, immunological, genetic, and molecular eras [2]. Pathologists excel at pattern recognition; we classify diseases by comparing and summarizing distinct morphologies. We also associated certain diseases with certain pathophysiology, and thus determining proper treatment. Following pathologists' perspectives, clinicians are able to treat patients more effectively.

The microscope is one of the most important inventions in medicine. With it, we can see microorganisms that killed innumerous people around the world, identify the components of our body, differentiate benign and malignant cells in blood and tissues, and classify diseases. Although we have experienced the monoclonal antibody boom with subsequent im-

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munohistochemistry and flow cytometry, and also embraced the wonders of genetics and molecular biology, the microscope remains the required tool for a pathologist. It is the symbol of pathology; found in the logos of almost all pathology societies and groups around the world, the microscope represents the morphological era. Pathology is not only a medical science, but also a visual art. As pathologists, we embrace pictures, colors, and patterns because a new motif indicates a discovery.

Current pathological diagnosis and classification is still based on morphologic patterns with the assumption that significant correlations exists between morphology and genetics, genetics and tumor biology. Following these patterns, oncologists have designed various therapeutic regimens and carried out numerous clinical trials. However, with emerging molecular technologies and the rise of precision medicine, pathologists face a new world of challenges and opportunities. This article intends to raise awareness among pathologists of the potential setbacks to their profession and the opportunities that will arise.

Morphology as a Surrogate of Biology

Disease is caused by the loss of biological equilibrium of the body due to either intrinsic or extrinsic factors. Intrinsic factors may include genetic anomalies and lack of potential to cope with the harsh environment, while extrinsic factors include universal radiation, chemical exposure, and invasion of pathogens, etc. To treat a disease, physicians must restore the biological equilibrium by correcting the disarrayed biology of the body, as exhibited by morphologic changes. The most noted morphologic changes in pathology are "rubor, tumor, calor, dolor, and functio laesa" [Figure 1] [3]. Physicians specialized in examining morphologic changes of our body became pathologists. Pathologists observe visible biological disarrays with the naked eye and simple tools, whereas detecting the subtle morphological changes requires sophisticated instruments [4, 5]. Pathologists begin visual experiences via autopsies, develop microscopic experiences with scopes, and acquire their experiences to decode errors in human genomes with cutting-edge technologies [6]. This information is utilized by clinical therapists to treat patients carrying those errors by restoring their biological equilibrium.

Pathology Provides Targets for Treatment

Precision medicine recently became a popular concept in molecular medicine for cancer treatment [7]. Indeed, targeted therapy has already been practiced for centuries; for example, in 1549 when Wan Quan used inoculation to prevent smallpox [8], when Vasco de Gama used oranges to treat scurvy in the late 15th century [9], when Banting and Best treated a diabetic dog with insulin in 1922 [10], and when imatinib was used to treat chronic myeloid leukemia (CML) in 2000 [11]. In fact, all these therapeutic targets: smallpox virus, low vitamin C, insulin deficiency, and presence of *BCR-ABL1*, are identified by pathology laboratories; pathology remains the basis of precision medicine.

Emerging Challenges

Pathology has already experienced numerous challenges since the day it was conceived and from the day it became a discipline. Traditionally morphology-based, pathology has benefited from emerging technologies in immunology, genetics, and molecular biology. Although pathology continues to benefit from the advancement of molecular techniques, challenges arise as modern therapeutics will depend less on morphology and focus on molecular targets rather than tumor types.

Challenge 1: Atypical morphology and aberrant gene expression

Morphology is generally determined by its genetic make ups, and similar pathology most likely repre-

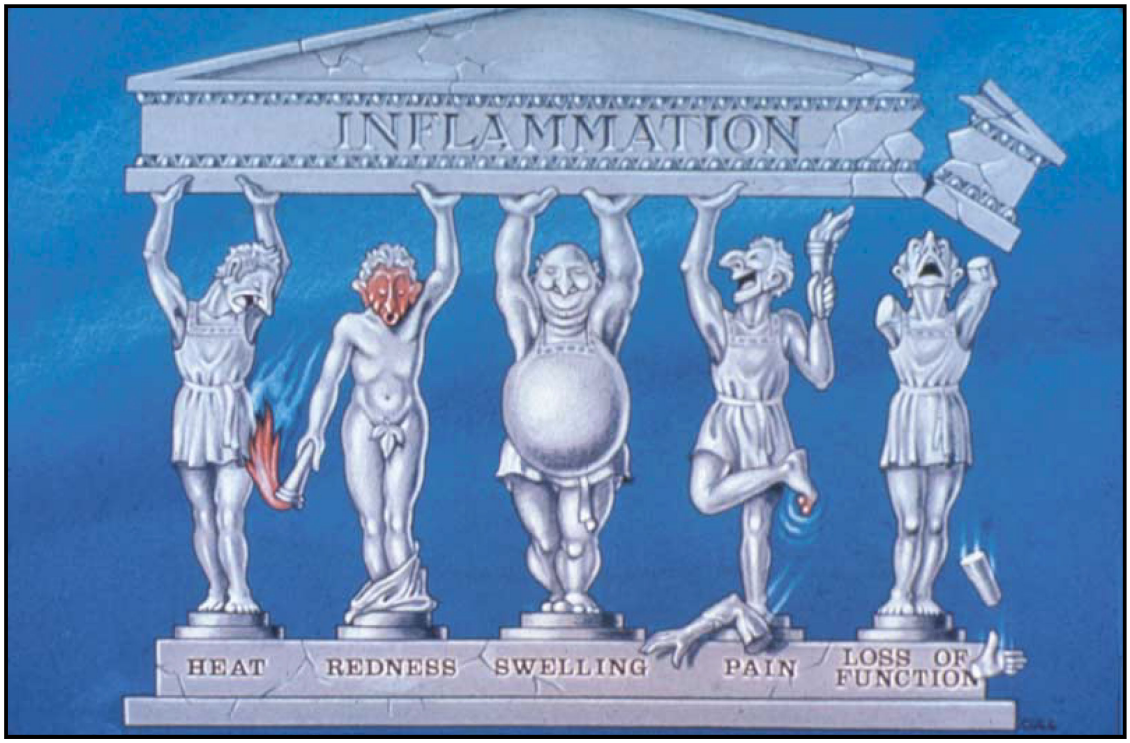

Figure 1: Cardinal signs of inflammation: heat, redness, swelling, pain and loss of function. Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Immunology] [2], copyright (2002).

sents a similar abnormality. In this regard, pathology is a good surrogate of genetic abnormality. For example, even in different organs or tissues adenocarcinoma behaves differently from squamous cell carcinoma and is thus managed differently. However, although we used to believe all the cells in a tumor arise from a single clone, recent evidence shows that a tumor is composed of heterogeneous cell populations. These cells acquire more than one and often multiple different "hits" during their evolution [12]. Therefore, their morphology may resemble several entities; because of this, the concept of "grey zone" lymphoma came into being [13].

Due to the many genetic abnormalities in tumor cells, aberrant gene expression is commonly seen in cancer cells and is even utilized for diagnosing malignancies. For example, aberrant expression of CD7 in myeloblasts indicates a neoplastic process [14], and loss of pan T-cell markers is often used to diagnose T-cell lymphomas [15]. Except for some tissue-specific markers, studies show that there is no unique marker for any tumor type and certain markers are only more commonly expressed in certain tumors. For example, cyclin D1 was once thought to be the driving force for all mantle cell lymphomas, but it is now known that it can be replaced by cyclin D2 and Sox11 in the lymphoma development [16, 17]. Even within the cyclin D1+ mantle cell lymphomas, some are more aggressive whereas others have an indolent clinical course [18]. Additionally, for decades *c-MYC* rearrangement was the signature of Burkitt lymphoma [19], but now *c-MYC* translocation or aberrant expression has been identified in many other lymphomas [20–24]. Moreover, Burkitt lymphoma can be diagnosed without *c-MYC* rearrangement at all [25–27].

Cancer is cancer indeed and they do not behave; any genes can be aberrantly expressed in cancer

cells. For example, CD3 and CD20 were considered the lineage-specific markers for T cells and B cells, respectively [28, 29], but CD3 can be expressed on B-cell lymphomas [30] and CD20 on T-cell and NK-cell lymphomas [31, 32]. Atypical morphology and aberrant gene expression led to more and more reported grey zone lymphomas [33–39], which poses a challenge to both pathologists and oncologists.

Challenge 2: Cancer Evolution and Drug Resistance

Like microorganisms, cancers evolve under the pressure of harsh environment. They survive by changing their appearances, behaviors, and responses to drugs. If time allows, almost any mutation or genetic abnormality could occur in cancer. Some mutations or genetic abnormalities may be lethal to cancer cells, so they can rarely be detected; whereas others contribute to the cancer growth advantage, and thus they become the signatures and/or Achilles's heel of cancer cells. We may still remember the 2000 Annual Meeting of the American Society of Hematology in San Francisco - almost half of the exhibit hall displayed abstracts related to imatinib studies. We were so marveled by having eventually found another wonder drug, shortly after uncovering ATRA [40]. Moreover, imatinib was not only effective for treating CML [11, 41], but also suitable for treating gastrointestinal stromal tumor (GIST) [42], and diseases with *PDGFR* and *FGFR* mutations [43]. While the narcissistic feeling was still lingering, we were surprised to hear that the cancer cells had already developed resistance to imatinib [44–47].

With cancer evolving [48], cancer diagnosis should also evolve and be at the molecular level. Although the morphology could remain the same, the driving force might have changed. For example, morphologic patterns are determined by many genes, but growth advantage could be provided by a single genetic mutation [49]. Although gene profiling has been utilized in diagnosing and classifying acute leukemias [50] and malignant lymphomas [51], the algorithm follows our tradition of pattern recognition with many identified genes being irrelevant to the cancer development. We are now facing the dilemma either to keep our tradition of pattern recognition or to change our way of thinking in order to cope with the challenges of cancer evolution.

Challenge 3: Precision Medicine Demanding Precision Pathology

With only a few exceptions, modern oncology still largely relies on toxins in treating cancers. Since most of the regimens are mutagens as well, we often cure one cancer while causing another. With whole genome sequencing being more affordable and new drugs specifically targeting certain gene products, we will be able to pinpoint the cancer specific genetic defects and to target these with new drugs. When rituximib targets CD20 of lymphoma cells, does it matter if the lymphoma is B-cell lymphoma, CD20+ T-cell lymphoma, or CD20+ classic Hodgkin lymphoma? When *c-MYC* is targeted by a small molecule for cancer treatment, who would argue whether the cancer is diffuse large B-cell lymphoma, Burkitt lymphoma, or something in between? When Her-2 is detected in lung cancer cells, will clinicians hesitate to employ Herceptin for therapy? As long as the target is identified, ibrutinib can be utilized to treat lymphoplasmacytic lymphomas and chronic lymphocytic leukemia [52, 53]. ALK+ lymphoma and lung cancer can be treated similarly with ALK inhibitors [54]. Anti-CD30 is effective for treating both ALCL and classic Hodgkin lymphoma [55]. When morphologic, immunophenotypic or even genetic patterns are gradually replaced by specific therapeutic targets, the crisis really comes: microscope will be collectable and the past glory of traditional pathology will fade.

Opportunities in the Horizon

Does it sound like crying wolf? But it is the reality that pathology practice constantly evolves. However, challenges often go hands in hands with opportuni-

Table 1: Proposed information in the final diagnosis of cancer

1. Diagnosis according to WHO classification (morphological/immunological/genetic)
2. Therapeutic targets (*BCR-ABL*, CD20, CD30, CD52, c-Kit, *EGFR*, Her-2, *MYD88*, etc.)
3. Pathway markers (*PI3K*, *mTOR*, *JAK2*, *BRAF*, *Erk*, *NF-kB*, etc.)

ties. Indeed, since pathology utilizes morphology as a surrogate of biology, why not diagnose diseases directly from biology?

Opportunity 1. Pathway- and network-based diagnosis

Cell growth and differentiation depend on its intrinsic genetic programming and extrinsic environment. Signals are sensed by the cell via receptors and conveyed to the nucleus through various pathways. Interactions between different pathways via functional nodes form a global functional network [49], which largely dictates the cell's fate. Since morphology and immunophenotype are not always surrogates of cancer biology [56–58], we should utilize the available molecular means to identify pathways of the diseases and make the diagnosis accordingly (Table 1).

Several signal transduction pathways have been identified to be active in almost all the cancers [59, 60]. They include the PI3K/Akt/mTOR/p70S6K, Ras/Raf/MEK/Erk, PD-1/PD-L1, and Notch-1 pathways [61–64]. Identification of these pathways will not only help us diagnose cancers, but also provide therapeutic targets to treat them.

Opportunity 2. Classifying diseases based on therapeutic targets

Therapeutic regimens are employed and clinical trials carried out based on classification of diseases. Classification is useful to clinicians only when it could guide the therapy for diseases and management of patients. When a neoplasm is classified as classic Hodgkin lymphoma, it indicates that this lymphoma will likely be managed with ABVD therapeutic regimen (with/without radiation) and the patient will be followed up per the protocol for Hodgkin lymphoma. When a diffuse large B-cell lymphoma is diagnosed, the patient will be treated with R-CHOP, with addition of etoposide if the lymphoma has a high proliferation rate. Since most of the current regimens are toxins, many patients will develop a secondary malignancy such as myelodysplasia after the chemotherapy. Because cancer is cancer indeed and cancers have common properties, therapies targeting the prominent pathways and the specific molecular targets rather than the histologic type will be more efficient and less toxic. Therefore,

1) Treatments should be tailored to the specific "hits" of the cancer;

2) Cancers with similar active pathways should be treated with similar regimens;

3) Therapy (agents/dosages) should be tailored to each individual based on the bioavailability and metabolism.

Opportunity 3. More involved in clinical decision making

Pathologists traditionally play supporting roles for clinicians because they do not directly interact with patients. However, since pathologists usually make the final diagnoses and provide therapeutic targets (see above), they should be involved more in the clinical decision making processes - with precision pathology, they help the clinicians choose the correct therapies; with pharmacogenomics, they guide the clinicians with accurate dosages; with prognostic markers, they predict the clinical outcomes and recommend the patient follow ups. Pathologists should be more proactive in helping the clinicians manage the patients.

Opportunity 4. Discovery of new biomarkers and therapeutic targets

With the firsthand specimens from patients, pathologists are able to employ immunohistochemical and molecular assays to identify or discover new biomarkers and therapeutic targets. As all the cancers have similar properties, such as genetic abnormalities, high proliferation rate, growth advantage, anti-apoptotic mechanism, and acquired new abnormalities that shunt/leak signals through the pathways or networks [49]. When an abnormality is identified in one cancer, it may also be present in a seemingly unrelated cancer. For example, Her-2 was commonly detected in breast cancer [65], it can also be detected in several other cancers [66]. Thus, pathologists should try to identify all the possible therapeutic targets for clinicians in the diagnosis of cancers.

Since pathologists have an easy access to patient specimens, they have an upper hand in the discovery of new biomarkers and potential therapeutic targets. With more and more knowledge on therapeutic targets, pathologists will be able to provide better diagnostic service.

Summary

Despite all these challenges in the molecular era of pathology, if pathologists could take advantage of precision medicine and new technological advances and seize the above opportunities, we will play more and more important roles in decision making for patient management.

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