Editor's Viewpoint

The Evolution of Diagnostic Pathology

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In the nineteenth century, great advances in human, veterinary and comparative pathology were made by Rudolf Virchow, when he first utilized the microscope to describe inflammatory processes, and to study disease processes at the cellular level. Virchow applied special stains to tissues to be able to identify and visualize disease processes. During that period and up to this date, pathologists still utilize similar staining techniques as those developed by Virchow. Given his innovative contributions to pathology, Rudolf Virchow is considered the "father of cellular Pathology" (5).

Virchow's contributions to cellular pathology opened a whole new world of possibilities for pathologists. Pathologists were then able to diagnose specific conditions based on cellular morphologic features. Remarkable advances in veterinary and human medicine were made, with growth and expansion of diagnostic pathology. Microscopic pathology became a specialized area, and pathologists started working closely with clinicians, which relied on diagnosis and prognosis provided by diagnostic pathologists, to make decisions on treatment for patients.

The creation of the electron microscope in the early 1930s, by Ernest Ruska, allowed pathologists to evaluate the ultrastructure of different tissues. Electron microscopy (EM) opened possibilities for pathologists to identify precisely infectious organisms in tissues, such as viral infections, alterations in cellular organelles, and cellular accumulations of different molecules, which have not been seen with histological examination (3). Up to this date, electron microscopes are still a valuable tool for diagnostic pathologists, however the high costs involved with maintaining the equipment, and sample processing, as well as the requirement for highly specialized personnel, have precluded EM from becoming more widely used in diagnostic pathology.

Remarkable advances in diagnostic pathology occurred when in the mid to late 60s, Nakane first described the use of enzyme labeled antibodies to detect antigens in tissues using light microscopy. Although similar techniques had been used for more than 20 years, those were initially developed for immunofluorescent microscopy (4). The introduction of immunohistochemistry (IHC) to detect infectious antigens in tissues and to detect different cell types, revolutionized again diagnostic pathology. It took several years for this method to become available in a diagnostic setting; however, nowadays, these have become one of the major tools utilized by diagnostic pathologists to confirm cell of origin of neoplasms, distribution of cellular protein expression, and detection of infectious agents in tissues. Likewise, the lectin histochemistry is a similar technique used to detect carbohydrate structures and subtle glycosylation changes in tissues. Lectin histochemistry can directly or indirectly (using labeled secondary antibodies) detect lectins in tissues, or by using biotinylated lectins (1).

The *in situ* hybridization (ISH) method was also developed around the late 60s. The method initially utilized DNA or RNA radiolabeled probes to detect nucleic acids within cells. The development of non radioactive probes, that were safer and allowed examination using the light microscope, has made this method more widely used by pathologists. In diagnostic pathology, ISH probes are mostly developed to target infectious agents, and to detect expression of cellular genes (2).

More recently, another assay that has been gaining attention from diagnostic pathologists is the use of tissue microarrays to identify molecular markers at nucleic acids or protein level. This technique utilizes paraffin blocks with hundreds of separate tissue cores, enabling examination of a large number of small sections from different patients at different stages of progression. Every section obtained from these paraffin blocks can then be used for immunohistochemistry, fluorescent *in situ* hybridization (FISH) and mRNA *in situ* hybridization, being a more cost-effective and less labor intensive method (7).

Modern pathologists are now getting into virtual microscopy, with whole slide scanning allowing pathologists to review digitized slides in a computer. This is being another great advance in light microscopy and diagnostic pathology, allowing pathologists to access slides anywhere in the world, with colleagues being able to see the same slide simultaneously.

We are now also entering a "molecular era", with the development of genetic and molecular tools looking for noninvasive biomarkers to diagnose diseases and specific conditions. Although some of these molecular tools, such as polymerase chain reaction (PCR), have been developed for several years, only more recently these tools have been made available in most diagnostic laboratories. These tools are becoming more widely available and increasingly cheaper, often allowing for precise diagnosis of certain diseases and conditions.

In this "molecular era" one might ask what will be the role of diagnostic pathologists in the future? The value of traditional pathology is not reduced, it is just that relying on accurate diagnosis only with traditional pathology may no longer be sufficient. It is clear that modern pathologists can no longer work alone, and those who utilize all available tools to integrate and interpret diagnosis, are more likely to succeed in providing an accurate disease diagnosis and prognosis, ultimately, improving animal health (6). Modern diagnostic pathologists should see the value of these tools and take advantage of these techniques, becoming familiarized on how to interpret the data, and use these to complement histological features one sees with light (or soon virtual) microscopy.

Since Virchow's first descriptions of cellular changes, several aspects of diagnostic pathology have changed. Diagnostic pathology has become one of the most valuable tools, if not the gold standard, for the diagnosis of diseases, being used to identify infectious agents in tissues, neoplasia, degenerative conditions, as well as other disease processes. Diagnostic pathologists should utilize available modern techniques to achieve the most accurate disease diagnosis and prognosis, and should continue to work closely with clinicians and other veterinary professionals to determine the best therapy options for their veterinary patients.

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