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REVIEW ARTICLE

The influence of basic research in Pathology: the paradigm of cancer

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Introduction

The word *Pathology* originates from the greek words *pathos* (suffering) and *logos* (study) and, as its name implies, it is a discipline devoted to the study of the cause, the pathogenesis, the morphological changes and functional derangement in cells, tissues and organs that underlie disease [1]. Anatomic Pathology has originated in Europe 245 years ago and it has come a long way since the time that Morgagni encouraged the postmortem search for the cause and nature of disease (Figure 1) [2]. In the 1870s, in the University of Berlin, C. Ruge and J. Veit introduced surgical biopsy, with the application of the microscope as an essential diagnostic tool that allowed precise diagnosis and more effective treatment [3]. During the years that followed, the histological techniques have been continuously improving and pathologists have been incorporating a variety of methods in their every-day practice, making diagnosis more refined and definite. In the last decades this improvement has been complemented by cytologic and molecular methods, expanding the orizons of Pathology from gross specimens to DNA. So, today, pathologists are able to make diagnoses by examining a whole organ, a fragment of tissue, or even a few cells. They integrate data from gross and microscopic examination, cytological and molecular methods so that they can face common diseases such as cancer and inflammation.

Key words: basic research, breast cancer, colorectal cancer, pathology

Advances in cancer biology

Cancer is indeed a common disease. In the developed world roughly one in three people in their lifetime will suffer from some type of cancer and approximately one fourth of them will die from the disease. Advances in facing cancer range widely, from basic research designed to understand the molecular causes of cancer, through the application of this knowledge for the patient's benefit. Both basic and clinical research, the latter being dependent on the former, are now developing at a fast pace [4].

Based on the generated plethora of data, there is no doubt now that tumorigenesis is a multistep process and that each step reflects specific genetic alterations that drive the progressive transformation of normal cells to highly malignant cells. Hanakan and Weinberg [5] have characterized 6 essential biological alterations that govern malignant growth, the hallmarks of cancer: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. The particular challenge in cancer is to discover the molecular abnormalities that dictate each specific malignant growth, to use this information in order to understand the process of malignancy, and, based on these data, to develop more rational and effective approaches for diagnosis and treatment.

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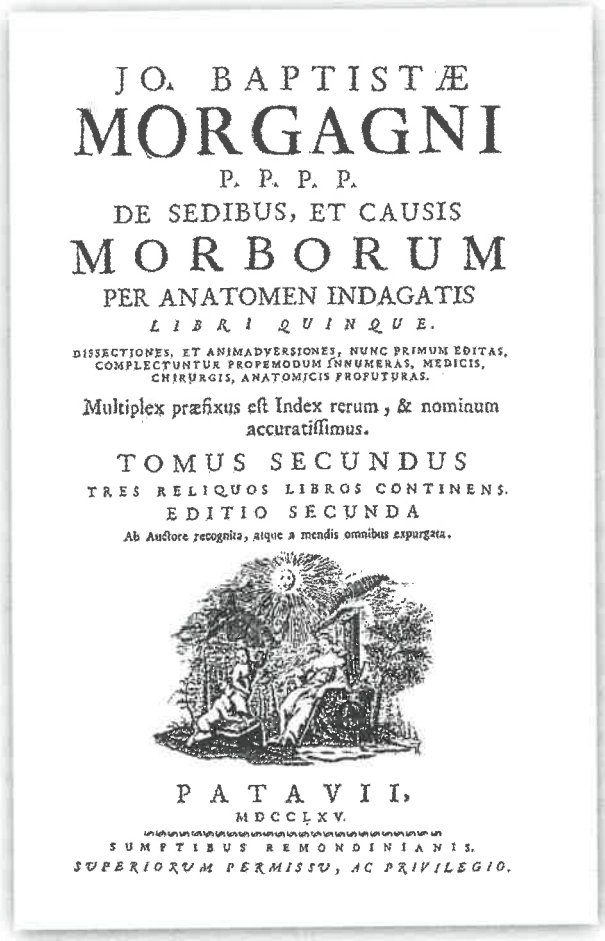

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Figure 1.

We are living in one of the most exciting periods in research, the postgenomic era. Only half a century has passed since the discovery of the structure of DNA by Watson and Crick [6] and we are already able not only to detect one gene per experiment, but also to monitor multiple genes, or even the whole genome of a single cell, and access the picture of the interactions among thousands of genes simultaneously. We are utilizing the microarray technology. DNA microarrays, microscopic chips of specific DNA fragments, can be used to assess gene expression, single nucleotide polymorphisms and genomic deletions within a specific cell population, or even one cell [7,8]. The generated data can be used to accurately diagnose tumors as well as to estimate their prognosis and predict their response to therapy. Gene expression is only a piece in the complex puzzle since, in order to understand the specific intracellular events involved in carcinogenesis, one should identify the involved critical messenger RNAs (Transcriptomics) and the proteins (Proteomics). As new markers are identified, their significance in a wide range of tumor samples can be evaluated with high efficiency and throughput with the use of tissue microarrays. Tissue microarrays are suited for all analyses that are performed *in situ*, namely immunohistochemistry, fluorescence *in situ* hybridization (FISH) and RNA *in situ* hybridization (RNA-ISH) [9]. This technology provides maximal use of limited tissue resources and has become one of the most promising tools in cancer research, leading to a new era of clinically significant cancer diagnoses.

It is estimated that there are 40,000 to 70,000 genes in the human genome encoding 250,000 to 1 million proteins, whose synthesis is regulated by 10,000 transcription factors [10]. The complexity of the regulatory circuits involved in non-neoplastic human cell homeostasis is obvious. The complexity increases in transformed cells, and this is reflected by the presence of more than 100 distinct types of cancer and many more subtypes within specific organs [5]. The challenge is to apply the acquired rich and complex body of information in order to understand cancer biology and exploit the growing knowledge for the benefit of mankind.

It is important to realize that the application of molecular biology techniques, which allows more precision in diagnosis and possibly in prognosis, is not simply a matter of labelling the disease. The major challenge that we are facing today is to control cancer by population-based screening. Most screening procedures aim at finding incidental asymptomatic cancers. Screening can also aim at finding genetic predisposition factors associated with high risk for cancer development or at identifying individuals who can benefit most from specific prevention strategies [11]. Along these lines it is conceivable that a better understanding of the stepwise progression to malignancy can affect the health policy.

Sporadic colorectal carcinoma; a multistep process of neoplastic progression

The pathogenesis of colorectal carcinoma has been elucidated more than any other tumor in recent years, partially due to its increased frequency and to the relatively easy access by endoscopy. Every stage of its development has been identified and the progressive accumulation of genetic changes at the molecular level has been shown to parallel the clinical and histopathologic progression defined as "adenoma-carcinoma sequence" [12,13].

Colon adenocarcinoma evolves through a series of morphologically identifiable stages from normal ep-

ithelium, to epithelial hyperplasia followed by formation of adenomas that progressively enlarge and ultimately undergo malignant transformation [1]. These morphological changes, readily identifiable in routine histologic sections correlate with specific molecular events: loss or mutation of the Adenomatous Polyposis Coli (*APC*) tumor suppressor gene, activation of *ras* and ultimately loss of Deleted in Colorectal Cancer (*DCC*) and *p53* genes [14]. The events are shown in Figure 2, however their illustrated sequence of occurrence does not necessarily apply to all tumors since the order of mutations is less important than their total accumulation.

The *p53* tumor suppressor gene is the most frequently altered gene in human cancers [15]. Besides its definite role in pathogenesis, several studies have addressed the prognostic relevance of alterations in the *p53* gene in colorectal cancer. Such studies are retrospective or prospective and utilize immunohistochemistry, single-strand conformation polymorphism (SSCP), reverse transcriptase polymerase chain reaction (RT-PCR) or sequencing techniques [12] in pathology material. Unfortunately the results are controversial.

k-ras activating mutations occur relatively early in the 'adenoma-carcinoma' sequence [16]. The reported data on the prognostic significance of *k-ras* mutations are not consistent. Such studies are retrospective or prospective and utilize immunohistochemistry, fluorescence *in situ* hybridization (FISH), SSCP, restriction fragment length polymorphism (RFLP), temporal temperature gradient gel electrophoresis (TTGE) or sequencing techniques in pathology material [12].

The *DCC* gene encodes for a transmembrane cell-surface molecule with homology to adhesion molecules [17]. Although it was initially believed to act as a tumor suppressor in colorectal carcinoma [18] recent data with DCC knock-out mice and expression studies give rise to doubts about this role [19,20]. However, there is evidence that chromosomal loss of 18q21 (loss of heterozygosity; LOH) may have a prognostic significance in colorectal carcinomas

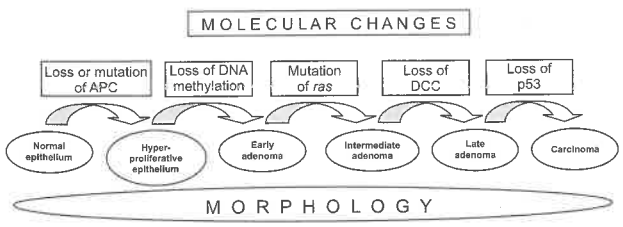


Figure 2. The "adenoma-carcinoma" sequence in colorectal cancer.

[21]. The actual target of 18q allelic losses might be other genes that have similar location with *DCC*, such as *DCP4*, *bcl-2* and *MADR2* [12].

Microsatellite instability, namely instability of small repetitive DNA sequences scattered throughout the human genome, which is associated with mutations in mismatch repair genes, characterizes hereditary, nonpolyposis colorectal carcinomas (HNPCC) [22]. Studies have shown that 12-35% of sporadic colorectal carcinomas have also acquired defects in the DNA repair system (mismatch-repair deficiency) that result in a more rapid accumulation of genetic deficiencies of the implicated tumor suppressor genes and oncogenes [23-25]. Moreover, there appears to be a trend of improved overall survival in patients with microsatellite unstable tumors.

In conclusion, regarding sporadic colon cancer, molecular studies on the biological alterations of genes casually involved in its pathogenesis, might also provide significant information on the prognosis of this neoplasm. Alterations of the identified molecular biological factors can be determined in the archival pathology material with the use of newly developed molecular techniques. It is conceivable that not a single event but a combination of certain alterations will be of prognostic significance. For identification of the different patterns of alterations microarray technology is necessary.

Breast cancer biology; recent advances

Besides colorectal carcinoma, breast cancer is another neoplasm in which developments in biology over the last decades have brought molecular medicine closer to the clinic. Breast cancer is clinically heterogeneous, with varying natural course and response to treatment. Such heterogeneous behavior probably reflects the genetic complexity of individual tumors, which have many genetic and epigenetic alterations influencing the expression of several key proteins involved in tumor development, growth, invasion and metastasis [26].

Individualization in breast cancer treatment has started approximately two decades ago with the discovery of the first estrogen receptor, ERα [27,28]. Estrogen receptors (ER) are members of the nuclear receptor superfamily of ligand-activated transcription factors and mediate the effects of estrogens on target tissues. ERα is now well characterized and is an established predictive marker in breast carcinoma. Its detection in breast carcinomas (mostly by immunohistochemical methods) influences decisions on whether to give or not therapy with anti-estrogens. The cloning of a second estrogen receptor, ERβ, 9 years ago,

has prompted re-evaluation of the role of ERs in breast carcinogenesis [29]. Since the first reports on ERβ, intense investigation has focused on its detection in normal and neoplastic mammary gland and its possible prognostic value. Most studies have focused on ERβ mRNA, using PCR-based techniques or *in situ* hybridization [30]. Although researchers agree that ERβ mRNA is expressed in breast cancer, there is a controversy on whether it is related with other known prognostic markers or with prognosis. The general sense is though that increased ERβ mRNA is associated with poor responsiveness to endocrine therapy and worse prognosis. The analysis of ERβ protein has become possible only recently, with the development of polyclonal and monoclonal antibodies and only a handful of studies have been published. Researchers agree that more archival and prospective studies are needed before definite conclusions can be drawn on the putative role of ERβ in breast carcinogenesis and its possible role as a prognostic marker in breast carcinomas [30]. Skliris et al. in a very recent paper present data which suggest that loss of ERβ expression is one of the hallmarks of mammary carcinogenesis and that it may be a reversible process involving methylation [31].

A number of genetic events have been implicated in breast carcinogenesis, such as mutations in oncogenes and tumor suppressor genes. The most frequent sites of gene mutations in breast cancer are in the *p53* gene. Twenty to 40% of tumors have such a mutation, often accompanied by LOH of the wild-type allele [32]. Data on *p53* mutation analysis from human breast carcinomas, transgenic animal models and experimental cell studies indicate that *p53* plays an important role in mammary carcinogenesis. Moreover, studies utilizing microdissected neoplastic tissue have shown that *p53* mutations can occur early in breast carcinogenesis, being detected in 30-40% of high grade ductal carcinoma *in situ* (DCIS) [33]. Based on immunohistochemical data, the prognostic and predictive value of *p53* overexpression appears weak [34]. However, data from sequencing techniques show a strong prognostic significance of *p53* mutations [35]. It has also been proposed that certain mutations are associated with even poorer prognosis.

Furthermore, studies in advanced breast carcinoma have identified specific *p53* mutations with significant clinical implications. Based on these data it has been proposed that *p53* analysis of the primary breast tumor is helpful in predicting the response to DNA-damaging drugs as well as tamoxifen in patients with metastatic disease [32].

The *Her2/neu* is an oncogene that encodes a 185-kDa transmembrane tyrosine kinase. It is the best example of utilization of laboratory-based data for the benefit of the patient, since it has been successfully used as a target for specific therapy. Overexpression of Her2/neu protein arises from amplification of the *Her2/neu* gene [36]. Her2/neu overexpression has been shown in 20-40% of human breast carcinomas and is associated with poor prognosis [37]. The vast majority of *Her2/neu* studies have been performed using immunohistochemistry in archival pathology material. The introduction of new molecular laboratory techniques, such as FISH, allowed assessment of the level of *Her2/neu* gene amplification in histologic sections [38]. More recently, a novel technology to detect DNA probes (chromogenic *in situ* hybridization; CISH), has been developed and its standardization is on the way [39].

Breast cancer, with its increased incidence and the lack of specific markers to predict clinical course, has been the perfect candidate for exploring the possible usefulness of microarrays. Data on breast neoplasms have demonstrated the ability of microarray-based expression profiling to predict chemotherapy responses and disease-free and overall survival from profiles generated from breast cancer surgical specimens [40-42]. Aggregate patterns of gene expression (metagenes) have been identified that are capable of predicting outcomes in individual patients with an accuracy of 90% [26,43]. Another revolutionary hypothesis that emerges from these studies is that the natural history of breast carcinoma is largely encoded in the primary tumor cells and possibly determined by initial mechanisms of transformation [26].

In summary, the significant progress on microarrays has enabled pathologists to characterize breast cancers more accurately and correlate them with clinical outcome or chemotherapy responsiveness [44]. In addition, the identification of specific new genes could lead to the development of new therapeutic strategies. It is important to note that modern techniques can be applied to large surgical specimens as well as to breast core needle biopsy samples or fine needle aspirates.

Epilogue

Modern Pathology has started to incorporate a rapidly increasing number of molecular biology techniques in order to answer more and more clinical questions regarding not only diagnosis but also the individual biological potential and prognosis. The new laboratory techniques in Pathology are demanding, thus it is important to pay close attention to the maintenance

of quality controls in Pathology laboratories. Specialization, collaboration between groups of Pathologists and constant scientific input from specialized scientists are necessary for not only accurate diagnosis but also for the observation and identification of new molecular events within the cancer cell that could lead to novel therapeutic approaches.

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