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The impact of molecular pathology in oncology: The clinician's perspective

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Since Virchow established the foundation of "Cellular pathology" our understanding of cancer has dramatically and fundamentally changed. We now know that cancer is a complex disease originating from genetic defects and chromosomal alterations in unstable, rapidly dividing cells. As a result normal cellular behavior is gradually lost and the cells acquire a malignant phenotype [5]. These advances in cancer genetics have established the basis for the concept of "molecular pathology" in oncology. But how can molecular pathology be translated into routine clinical practice and what are the clinician's expectations and needs?

From the clinical point of view the primary diagnosis of a malignant tumor still relies on histopathological features. Accordingly, tumor typing, grading and staging is critical for the accurate diagnosis and appropriate clinical management of patients with solid tumors. Clinical pathology directly influences the choice of therapy and is often crucial in deciding whether mutilating surgical procedures or chemotherapies with significant side effects are justified. Therefore, it is vital for the patient and his fate that the pathology report is precise and answers the clinician's questions. Hopefully, both basic scientists and clinicians recognize the impact of the pathologist's diagnosis.

The following questions need to be answered or have to be taken into consideration before treatment commences:

(1) Is it a cancer or a premalignant lesion?
(2) What is the exact tumor stage according to the TNM classification?
(3) What is the best primary therapy?
(4) Are adjuvant or neoadjuvant treatments indicated?
(5) Are there any predictive markers for tumor response to radiation or (radio-)chemotherapy?
(6) Does this patient show an increased risk of recurrence or metastasis?
(7) What is the individual prognosis?

These issues are highly important for several reasons:

First, if a premalignant lesion like a Barrett's esophagus is occasionally diagnosed, the clinician is confronted with a serious problem. Should the patient be treated with hazardous interventions according to the worst-case scenario or should he just be controlled regularly? To date it cannot be predicted whether a premalignant lesion will ever progress into an invasive carcinoma. Therefore, new parameters have to be identified characterizing cases that are likely to progress and exhibit the characteristics of malignant tumors. One such example is the study by Sudbo et al. which showed that aneuploid oral leukoplakias have a very high propensity for progression into malignant tumors whereas diploid lesions very rarely do so [19]. One would hope that such findings can be translated to routine diagnostic standards in the clinical setting.

Second, it is well known that patients diagnosed with early-stage solid tumors have better chances for a curative treatment (high intervention efficiency and improved survival) than patients with advanced-stage tumors, which are more often treated with a palliative intention (low intervention efficiency). Pancreatic cancer and cancer of the biliary tract are examples of tumors that are usually diagnosed too late for a curative therapy. At the moment, neither clinicians' knowledge

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nor advanced medical imaging tools like MRI or computed tomography are really useful for detecting these cancers at early stages.

Third, an exact tumor staging is mandatory because it influences the decision whether a cancer patient will be treated with surgery alone, or if adjuvant or neoadjuvant treatment modalities will be applied. For example, colon cancers are only treated with postoperative adjuvant chemotherapy if they exhibit lymph node involvement. However, most chemotherapeutic agents are cytotoxic and harm normal cells. Unfortunately, it cannot be foreseen if the individual patient would benefit from this treatment. This poses a considerable clinical dilemma because patients with *a priori* resistant tumor cells could be spared exposure to this treatment modality, which is associated with substantial side effects. Markers predicting response to adjuvant and neoadjuvant therapies would have significant clinical utility as they could be used to improve the quality of life of cancer patients.

Fourth, there is a wide diversity in the clinical outcome between patients of the same histological tumor entity and clinical stage receiving identical treatments. This demonstrates that grading and staging are not completely sufficient to describe the clinical behavior of malignant tumors. Seemingly similar histopathological characteristics may actually mask a heterogeneous group of biologically and clinically different tumor entities that have to be identified in order to individualize and improve the management of cancer patients.

Defining the best treatment modality for the individual cancer patient is critical and indispensable for disease management. For many solid tumors, the definition of "best" is currently evolving and controversial. For example, postoperative radiochemotherapy is standardized for the treatment of locally advanced rectal cancers. However, specialized medical centers are actually applying various combinations of preoperative radiation or radiochemotherapy. Whether the preoperative therapy should consist of radiochemotherapy or radiation alone, and whether the radiation should be short-time or long-time are currently debated issues. Additionally, the chemotherapeutics are constantly modified and improved, and even the surgical techniques are being redefined [13].

There is much hope that molecular pathology will overcome at least some of these clinical dilemmas in the next decade. Recent technological advances have already equipped researchers with more accurate tools to probe the biological basis of cancer, and it is generally assumed that these methodologies will play a key role in deciphering the molecular fingerprint of cancer. For example, comparative genomic hybridization (CGH), spectral karyotyping (SKY), expression profiling by microarrays, tissue arrays and two-dimensional gel electrophoresis (2D-PAGE) have been successfully used to explore the genome, transcriptome and proteome of cancer cells [1,4,7,9,10,14,17]. Other technologies like single nucleotide polymorphism (SNP) arrays, comparative genomic hybridization arrays, protein arrays and new developments in mass spectrometry like matrix-assisted laser desorption/ionization (MALDI) or surface-enhanced laser-desorption ionization time-of-flight (SELDI-TOF) will provide new insights into molecular medicine [12,15,16,21]. In contrast to the conventional gene-by-gene or protein-by-protein approach these parallel analysis platforms increase assay throughput while reducing time and costs because high numbers of targets can be simultaneously studied. For example, gene chips can be used to evaluate expression of more than 30,000 transcripts in one experiment. In the next step, tissue arrays can be applied to validate the leads by analyzing hundreds of tumors at a time using probes for DNA, RNA or proteins. This allows linking the molecular changes to "histopathological and cellular tumor features". Another example of research avenues stimulated by technological advances is the large-scale use of mass spectrometry to study protein–protein interactions in the tumor-host-microenvironment [11].

However, the application of these high-throughput platforms are not necessarily straightforward:

First of all, careful study design, documentation and execution are extremely important. Otherwise huge data sets with little clinical relevance may be generated. For example, many different statistical approaches such as supervised and unsupervised techniques can be used to analyze microarray data. However, universally accepted procedures remain to be defined. Each tool has unique characteristics and study results may vary depending on the selected method and applied parameters. Although standardization of microarray data is emerging and guidelines such as the MIAMI criteria [2] have been developed, many expression profiling studies still suffer from insufficient documentation to allow comparison and interpretation of data from different laboratories. Many authors do not provide detailed information about the time between sample collection and processing, the protocols for freezing or fixation are inadequately described, and sample quality control or purity may not be discussed. Additionally, patient selection is often inade-

quately described including just the total number of patients without breakdown into important subgroups. For example, different tumor stages and material from patients who received different therapies in different hospitals may be mixed. Such discrepancies in study material will certainly impact the results. Sometimes a (statistically) small number of conscientiously selected patients can provide better information than a huge mixture of patients. Another critical step is the use of RNA amplification. Some research groups apply linear amplification methods, some PCR-based technologies. A related practical dilemma involves sample preparation. Should the tumor cells be microdissected in order to separate them from the host cells knowing that laser capture microdissection is labor-intensive, time-consuming and requires a pathologist with expert knowledge? Or should the mixture of tumor cells and host stromal cells be used for analysis? To summarize, standardization, automation, cost-effectiveness, simple and generally accepted software tools for statistical analysis are urgently required before high-throughput assays are ready for clinical use.

What do clinicians expect from future trends of molecular pathology?

It is mandatory to intensify the crosstalk between basic scientists, pathologists and clinicians. This is necessary because the translation of basic research findings into clinical practice – from bench to bedside – is still emerging. The typical drug discovery and development cycle takes about 10–14 years, and the first drugs developed based on molecular understanding are now entering clinical practice. To ensure that these new therapies are appropriately used it is critical to couple them with accompanying molecular pathology diagnostics. Although there are encouraging examples like the use of Trastuzumab (Herceptin®) for treatment of HER-2/*neu*-positive metastatic breast cancer [3,18] or the c-kit inhibitor Imatinib (STI571, Glivec®) for gastrointestinal stromal tumors [8,20], the routine clinical application outside specialized centers remains to be problematic. First of all, distinct methods with different specificity and sensitivity are used to detect these aberrations. For example, techniques to identify or measure HER-2/*neu* include IHC and FISH (most widely used), but also Southern blot, Northern blot, PCR, CISH and ELISA [6]. Additionally, many hospitals are lacking the financial resources to establish new diagnostic tools or therapy strategies in the clinical practice. As long as not even specialized centers can afford these future trends, the potential benefits of "genetic medicine" or "chip diagnostic" will probably never arrive at the doorsteps of cancer patients. Subsequently, scientists and clinicians must be encouraged to work closely with each other in order to establish new strategies and design trials that could result in innovative diagnostic and therapeutic tools. Additionally, novel infrastructures have to be developed that allow researchers from different departments to cooperate successfully. From our point of view, only interdisciplinary centers will help to overcome these drawbacks.

Hopefully, in the not too distant future it will be possible (1) to detect cancer before symptoms indicating invasive and metastatic disease appear, (2) to improve the currently used staging classification, (3) to measure tumor aggressiveness and (4) to predict tumor response to treatment and clinical outcome. Customized diagnostic devices like laboratory-on-a-chip technology may be used to detect global biomarkers in biological fluids such as serum or urine. Instead of a handful of markers and few therapeutic options focusing on one pathway, entire sets of biomarkers and associated therapeutic modalities may be available for particular clinical situations in order to improve the individual patient's management.

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