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REVIEW

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Digital pathology in personalized cancer therapy

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Abstract: The development of small molecule inhibitors of growth factor receptors, and the discovery of somatic mutations of the thyrosine kinase domain, have resulted in new paradigms for cancer therapy. Digital microscopy is an important tool for surgical pathologists. The achievements in the digital pathology field have modified the workflow of pathomorphology labs, enhanced the pathologist's role in diagnostics, and increased their contribution to personalized targeted medicine. Digital image analysis is now available in a variety of platforms to improve quantification performance of diagnostic pathology. We here describe the state of digital microscopy as it applies to the field of quantitative immunohistochemistry of biomarkers related to the clinical personalized targeted therapy of breast cancer, non-small lung cancer and colorectal cancer: HER-2, EGFR, KRAS and BRAF genes. The information is derived from the experience of the authors and a review of the literature. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 4, pp. 570–578*)

Key words: digital pathology, immunohistochemistry, IHC biomarkers, breast cancer, NSCLC, personalized targeted therapy, EGFR, HER-2, telepathology systems, quantitative analysis

Introduction

Two key technologies are transforming pathology: digital pathology and personalized medicine. Advances in pathology and diagnostic technology, in conjunction with advances in genetics and genomics, are promoting the development of personalized medicine.

In recent years, digital imaging has been applied to many medical fields**.** Due to technological improvements of hardware and software, digital microscopy has become an important diagnostic tool in surgical pathology. Digital microscopy creates a digital version of whole glass slides (WSI, Whole Slide Image), which can be dynamically viewed, navigated and magnified on a monitor across a computer network. Digital slides can be integrated into existing hospital databases and accessed via intranet or the internet for teaching, primary diagnosis, teleconsultation and quality assurance [1]. For many pathologists, knowledge of digital microscopy relates to telepathology. The introduction of high resolution and automated whole slide imaging allows pathologists to share images rapidly with remote locations. Telepathology is the first area to experience the widespread use of digital systems. Telepathology and WSI are already impacting pathology practices. Digital images allow the performance of digital algorithms for analysis and quantification [1–7]. Software for digital image analysis has long been used in the research setting to quantify morphometric features or the intensity of staining. Digital image analysis is now available in a variety of platforms to improve the quantification performance of diagnostic pathology [1, 5–7].

Personalized medicine refers to the specific delivery of healthcare (either preventive or therapeutic) according to single or multiple genomic or molecular biomarker(s). The US Congress has attempted to define personalized medicine as: "the application of genomic and molecular data to better target the delivery of healthcare, facilitate the discovery and clinical testing of new products, and help determine a person's predisposition to a particular disease or condition" [8].

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The objective of personalized medicine is to identify patients at risk of illness according to their genomic profile, and to provide the proper drug at the right dose for the right patient at the right time. Essential components of personalized medicine for cancer patients may include the presence of disease-specific gene(s) or gene profiles that control cancer growth, the standardization of biomarkers for identification of such a molecular target, and the application of an effective therapy against it [9].

Immunohistochemistry (IHC) is a frontline assay for identifying these molecules and revealing translated proteins in normal and diseased cells. IHC as a diagnostic tool in surgical pathology has replaced other ancillary studies, and is widely applied in tumor diagnostics, sometimes with specific IHC antibodies for a particular type of cancer, and sometimes in differential diagnosis which requires a panel of antibodies. It has also recently been gaining ground in prognostic and predictive markers for a number of different tumors to predict response to targeted therapy. Screening for biomarkers related to patients' response to molecular targeted therapy upgrades the pathologist's responsibility in therapy decisions.

Pathologists in their diagnostic processes rely on the patient's clinical data, results of imaging, morphology, and various ancillary techniques such as immunohistochemistry, cytogenetics, and molecular diagnostics. IHC has been most commonly used as a research tool in biomedical research. A three-fold increase in immunohistochemical studies over the last 20 years indicates the growing popularity of IHC tests [10].

Quantification of immunostaining is a frequently used technique in pathology. Manual quantification of immunohistochemically stained tissue biomarkers is laborious and subjective, and is prone to inter- and intraobserver variability. Various discrete scoring systems, based on evaluation of the intensity and extent of immunostaining, have been proposed to improve reproducibility. Software for digital images analysis has long been used in the research setting to quantify morphometric features or intensity of staining. New automated procedures implemented for quantitative digital image analysis of various immunohistochemical markers are more objective and faster, and provide accurate and sensitive measurement of protein expression within tissue specimens [1, 4, 6, 11]. However, the use of automated image analysis technology for tissue biomarker quantification by itself can introduce non-biologic variables that potentially could bias the analysis. The threshold and cut-off values for image acquisition and data extraction from the acquired images are critical for measuring staining intensity. Automated quantitative imaging systems are sensitive to technical variables that can influence the intensity of the immunostaining (e.g. calibration compression). Strict standardization of the overall assay, using standard operating procedures and appropriate quantifiable reference controls, are a fundamental prerequisite to achieve the accurate and reliable quantification of protein biomarkers in tissues [1, 5, 11, 12].

Most currently used automated image analysis algorithms are designed to brightfield IHC in systems based on virtual slides. The available IHC algorithms are nuclear, membrane, cytoplasmic, rare event. There are a number of computer-based programs designed specifically for the quantitative analysis of IHC such as: BLISS and IHC score from Bacus Laboratories, Inc (Lombard, IL, USA); ACIS from Clarient, Inc (San Juan Capistrano, CA, USA); iVision and GenoMx from BioGenex (San Ramon, CA, USA); ScanScope from Aperio Technologies (Vista, CA, USA); Ariol SL-50 from Applied Imaging Corporation (San Jose, CA, USA); LSC Laser Scanning Cytometer from CompuCyte Corporation (Cambridge, MA, USA); and SlidePath's Tissue Image Analysis and AQUA from HistoRx Inc (New Haven, CT, USA) [4, 13]. Some of the image analysis systems have additional options for fluorescent imaging, Fluorescence In Situ Hybridization (FISH), and Chromogenic In Situ Hybridization (CISH). There are other available solutions for image analysis in IHC, which are not based on whole slide imaging of pathology slides (e.g. Ventana Image Analysis System (VIAS). Several commercial image analysis applications for IHC quantification have proved that automatic quantification provides more reliable and more uniform results than manual evaluation, and have received clearance from the US Food and Drug Administration (FDA) [5, 6].

The role of IHC data sets and analysis in diagnostic pathology is being challenged by other quantitative methods such as DNA microarray and qRT-PCR in cancer detection, classification and predicting cancer treatment response [14].

Tissue Micro Arrays (TMA) applications are another important tool for molecular analysis to help identify new diagnostic and prognostic markers and targets in human cancers. The key benefit underlying TMA technology is the ability to assay hundreds of patient tissues arrayed on a single microscope slide. TMAs are ideal for efficient screening of prospective biomarkers by a variety of different mechanisms including IHC, FISH and RNA *in situ* hybridization. Most studied biomarker assays on TMAs use immunohistochemical techniques. The TMA slides can be 'whole-slide' imaged. This allows results of experiments to be shared with other investigators. Signifi-

cant work has been done to standardize data capture to facilitate the subsequent exchange of information. TMA histospots allow the assessment of tumor heterogeneity and provide a new opportunity for developing automated methods for TMA analysis. There are ongoing efforts to generate software tools for automated analysis of TMA localization data. The advances made in automated quantitative analysis of IHC-TMAs play an important role in defining predictive biomarkers for future biospecific therapies [15–18]. Several experiences of TMA analyses using virtual slides have been reported. Currently available TMA scoring image analysis systems include: 3DHistech Mirax TMA, Alphelys Spot Browser® 3, Aperio TMALab™ II, Bacus TMAscore, Dako ACIS® IIITissue Micro-Array, Definiens TissueMap, and SlidePath OpTMA [1, 4].

Multispectral imaging is becoming important in immunohistochemistry analysis. This technique of multiplexing capacity allows immixing of three or more chromogens in multicolor IHC [19].

Personalized targeted therapy

Personalized medicine is an initiative to treat each patient as an individual. In recent years, targeted therapies for a variety of malignant tumors have achieved clinically significant response rates and have enabled oncologists to develop individual-based therapy strategies for patients. It has been demonstrated on a large scale that the personalized medicine approach is associated with improved clinical outcomes [20–25]. The rationale for such therapeutic approaches is the identification of the targeted molecule in the tumor of the patient to be treated. Especially for solid malignant tumors, a growing list of target molecules is now routinely estimated by IHC staining in biopsy specimens and surgically removed tumor material, generating important data for therapeutic decisions and prognostically relevant information [25].

The identification of patients who may benefit from targeted approaches demands accurate assays for the biomarkers determining optimal treatment. Currently proposed biomarkers help in early cancer detection or selecting cancer patients for treatment. They have also built a base in diagnostics and predicting biological pathways of cancers. There are 286 known tumor suppressing genes, and 33 known oncogenes, many of which are being actively targeted by drug companies. However, there are only five well-documented cancer drugs targeting the genes: HER-2/neu, EGFR (Epidermal Growth Factor Receptors), KRAS, BRAF (proto-oncogene B-Raf), and the adenomatous polyposis coli (APC) gene.

The biomarkers can be detected by immunohistochemical methods, quantitative proteomic methods, and methods such as quantitative real-time polymerase chain reaction (qRT-PCR) [26]. The promotion of molecular and individualized medicine is based on the improvement and miniaturization of methods of proteomics and genomics in the search for biomarkers of disease onset, progression and treatment response [27]. In the pathology labs, the main techniques used to analyze the tumor tissue for specific genetic/molecular aberrations are: *Polymerase Chain Reaction (PCR)-based sequencing for the molecular composition, *immunohistochemistry for revealing cells translated proteins (a frontline assay), and FISH for genetic composition.

Immunohistochemistry is the commonest screening strategy for evaluating most studied biomarkers in the pathology labs, e.g. for Her-2, estrogen receptors (ER) and progesterone receptors (PR), EGFR. IHC has encountered problems of reproducibility and lack of standardization, resulting in poor concordance between laboratories. Different labs use different IHC reagents and kits with different staining characteristics; they also have slightly different IHC staining processes, different scoring schemes for nuclear stains, and different cut-off thresholds. The reproducibility of IHC stains is important for consistent algorithm performance. For this reason, it is recommended to use FDA-approved IHC kits and to employ appropriate morphological studies and controls as specified in the instructions for the IHC kits [21–23, 28–30]. Clinical assays to assess cancer biomarkers include IHC status for the detection of protein over-expression, and/or gene amplification by fluorescence *in situ* hybridization, with validated clinical trials having gained FDA approval. To help pathologists, specific algorithms for cell membrane immunoreactions, mainly of HER2 protein detection, and for nuclear signal detection, especially of ER/PR, have been developed for the automated classification of breast cancers and predicting their potential response to immuno- or hormone therapy, respectively. Various integrated scanning and image analysis systems are available for automated examination of immunohistochemistry and FISH signals; some are based on virtual slides or WSI, offering options of remote IHC analysis [1–7, 18, 19]. Many studies have focused on variables related to the automated image analysis in immunohistochemistry and virtual microscopy [12, 18, 31–34]. Several software developers have applied for and received FDA approval for digital-assisted analysis of HER2 staining and other stains including estrogen receptor and progesterone receptor [5, 6].

Human Epidermal Growth Factor Receptor 2 (HER-2)

The HER family of transmembrane receptors consists of four related members: Her-1 (also known as epidermal growth factor receptor (EGFR), HER-2, HER-3, and HER-4. They consist of an extracellular ligand binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. HER2 overexpression by gene amplification occurs in approximately 20% of breast cancers and is associated with aggressive biology and poor prognosis of resected and metastatic tumors. HER2 status has been shown to be predictive for response to certain chemotherapeutic agents. Overexpression of HER1 is found in about 40% of breast cancers and also portends a poor prognosis in breast cancer. Overexpression of either HER1 or HER2 is associated with relative resistance to endocrine therapy [35, 36].

The assessment of HER2 in invasive breast cancer has been mandatory for treatment decisions since the advent of targeted therapy with the recombinant humanized IgG monoclonal antibody trastuzumab (Herceptin®, Genentech, Inc., South San Francisco, CA, USA; Hoffmann-La Roche Ltd., Basel, Switzerland) and the small molecule dual HER1/HER2 tyrosine kinase inhibitor lapatinib (Tykerb, GlaxoSmith-Kline, Philadelphia, PA, USA) [22, 28, 30, 37]. Screening of newly diagnosed breast carcinomas is mostly performed by IHC. A typical immunohistochemical panel provided in the pathological assessment of breast cancer includes HER-2 and ER/PR stains performed by IHC validated assays [19, 21].

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), in their joint guidelines for the laboratory evaluation of HER-2 status, describe in detail the methodological requirements for IHC and FISH analyses [23, 26, 30]. An objective, reproducible technique for HER2 and ER/PR interpretation is particularly valuable, since the result leads to an important treatment decision. HER-2 is a membrane stain evaluated by its intensity and completeness of the membrane staining according to the recommended scoring method. Pathologists may use a semi-quantitative or automated method to determine protein expression [5, 31–34].

Breast cancer specimens with equivocal immunohistochemistry undergo validation using a Her-2 gene amplification method called Fluorescence In Situ Hybridization. FISH testing is a far more quantitative and reproducible method, as it directly measures the number of copies of the HER2 gene. However, FISH testing is associated with greater cost and takes more time than an analysis employing immunohistochemistry [38]. Newer methodologies for establishing Her-2 status including RT-PCR and CISH have not yet been validated.

The IHC Image Analysis algorithms for automated assessment of HER-e and ER/PR (with FDA Clearance: Aperio and ACIS III) can be used as an aid to pathologists for the assessment of stained breast tissue slides [5, 6]. Additionally, the Aperio system offers an option for remote IHC quantitative analysis of HER-2 and/or ER/PR in virtual slides or WSIs [5].

Estrogen receptors and progesterone receptors

Today, immunohistochemistry is accepted as the standard evaluation method for determining ER and PgR status. The increased sensitivity depends mainly on the pretreatment and detection systems. CAP/ASCO have developed and published joint guidelines aimed at improving the accuracy of immunohistochemical ER/PgR testing in breast cancer [21]. These guidelines describe validation procedures for ER and PgR IHC assays that are used to predict response to tamoxifen and aromatase inhibitors (predictive markers). IHC validation, additional quality control and assurance measures are important new requirements of the ASCO–CAP guidelines. As with all IHC studies of therapeutic targets, accurate and quantitative assessments of the results are critical. Several major factors can dramatically affect the apparent ER and PR status of the breast cancer as determined by IHC, including: tissue fixation, choice of anti-ER or anti-PR antibody, manual vs. automated staining platforms, and the determination of thresholds for reporting positive results. Improving the quality of IHC assays on a global scale requires that all laboratories implement standardized and validated assays recommended by CAP/ASCO for ER and PR testing [21, 29]. Some studies have suggested that although IHC performed on TMAs can closely approximate the results obtained by evaluating much larger tissue sections, they are still unlikely to account for all the heterogeneity, and TMAs are not used in routine clinical practice. It is unrealistic to expect that even perfect tests for ER and PR alone, regardless of the technology, will be sufficiently powerful to accurately predict the response of all patients, because the biology is so complex. New, more powerful predictors are needed, and are being investigated. They will most likely be based on multiple biomarkers [39].

Automated microscopy and computerized processing have provided increased accuracy in quantification and standardization. A number of groups have published data on the automated assessment of the ER/PR in breast cancer [34, 40–43] describing an ex-

cellent correlation between manual and automated analysis. Various automated image analysis systems offer a possibility of rapid and objective scoring of immunostained nuclei (IHC Nuclear Image Analysis algorithms), but only a few have received FDA approval [5, 6]. Although IHC test methods have improved with the widespread adoption of automated staining platforms, the laboratories with assays cleared or approved by the FDA must verify the performance specifications stated by the manufacturer.

Ki-67 — a cell proliferated associated nuclear antigen, is another prognostic marker supplemented the breast cancer characteristics. The Ki-67 status is reported as a percentage of invasive carcinoma cells exhibiting positive nuclear staining: less than 10% (favorable prognosis); more than 20% (unfavorable prognosis); and 10% to 20% (borderline category). The Ki-67 growth fraction is significantly related to the grade in most tumors, being the highest in grade 3 invasive carcinomas. ER- and PR-negative tumors tend to have a high Ki-67 proliferation index. The microscopic quantitation of Ki-67 Labelling Index (Ki-67 LI) by the IHC method is available via commercial computerised algorithms based on digital microscopy or WSIs.

Epidermal growth factor receptor (EGFR; HER1, erbB-1)

Epidermal growth factor receptors are among the most studied cancer biomarkers because of their oncogenic activity in diverse tumor types. EGFR is highly expressed in a variety of solid malignant tumors and its expression has been correlated with disease progression and poor survival. EGFR recently has attracted clinical attention because of the development of targeted therapies. There are two classes of anti-EGFR agents — monoclonal anti-EGFR antibodies and small-molecule EGFR TKIs — currently used in EGFR-targeted therapy [24]. Therapies based on monoclonal antibodies (MAbs) such as IMC-C225 (cetuximab) have been tested in clinical trials on patients with head and neck cancer, colon cancer, pancreatic cancer, and non-small-cell lung cancer (NSCLC) [25].

EGFR in NSCLC

Identification of the relevant molecular subtypes of this heterogeneous disease and selecting patients for the appropriate targeting agents is critical in the personalized therapy of NSCLC. Two classes of anti-EGF receptor (EGFR) agents, monoclonal anti-EGFR antibodies and small-molecule EGFR tyrosine kinase inhibitors, have been used for the treatment of NSCLC (24). The clinical guidelines for NSCLC have been changed to incorporate genetic testing for EGFR mutations to identify patients who would benefit from targeted oral therapy with erlotinib or gefitinib instead of chemotherapy. EGFR inhibitors such as erlotinib (Tarceva, Genentech/OSI Pharmaceuticals) are now considered the best treatment option for patients with NSCLC whose tumors harbor EGFR mutations. It has been documented that EGFR mutations are related to lung cancer histology. Lung adenocarcinomas frequently possess EGFR mutations and frequently exhibit increased EGFR copy number [44]. In the United States, approximately 15% of patients with adenocarcinoma of the lung harbor activating EGFR mutations. The majority of these mutations are in exons 19 and 21 of the EGFR gene [22]. EGFR mutations are present in adenocarcinomas in about 10% of Western patients and in about 50% of Asian patients, most frequently in women, non-smokers, and non-mucinous tumors [45, 46]. Large-cell carcinoma harbors EGFR mutations very rarely. EGFR mutations have been found particularly frequently in adenocarcinomas of the papillary subtype [47]. It appears that EGFR mutations occur much less frequently in squamous cell carcinoma than in adenocarcinoma, with a reported incidence of 0–14% [45, 48]. In the setting of recurrent and metastatic disease of lung cancer, EGFR testing is a recommendation for three histological types: adenocarcinoma, large cell carcinoma, and NSCLC not otherwise specified. Erlotinib is recommended as the first-line systemic therapy in patients with these three relevant histological subtypes and a positive EGFR mutation. Due to the ongoing revolution in the treatment of lung cancer, pathomorphological reporting has another value. The microscopic evaluation of the histologic subtype of NSCLC has become important in tumor selection for EGFR testing.

With the advent of targeted therapies, there has been an increasing interest in IHC based EGFR screening methods using paraffin-embedded tumor specimens to select cancer patients eligible for treatment with cetuximab [25, 49–51]. The interpretation of immunohistochemical results relies on subjective judgment. With EGFR, distinct membranous staining of tumor cells is considered as a positive immunoreaction. Various IHC scores have been used for manual evaluation of EGFR immunoexpression [49, 50]. Only limited studies have analyzed the criteria needed to define a positive immunoreaction for EGFR [51]. Although digital pathology offers an option of computerized quantitative analysis for EGFR expression by IHC algorithm for membrane staining [4–6], the results of the clinical implications of EGFR

overexpression have been inconclusive thus far. Immunohistochemistry-based assays measuring EGFR expression cannot reliably predict the response to EGFR TKI therapy. Clinical studies have been unable to demonstrate a distinct correlation between EGFR expression and the likelihood of response to EGFR inhibition with targeted antibodies [52, 53]. Since the use of EGFR overexpression as a prognostic marker has largely been unproductive, considerable efforts have been made to develop antibodies that react specifically with the mutant form of EGFR. The detection of mutant EGFR by these two antibodies has been performed by Western blotting, immunofluorescence and IHC [54].

In the published provisional clinical opinion of ASCO on mutation testing for advanced NSCLC — at the time of publication, there was no FDA-cleared test for the EGFR mutation. Fluorescent *in situ* hybridization and immunohistochemistry testing for EGFR at the present time are not recommended for the purposes of treatment decisions because they do not reproducibly predict outcome [22]. The most commonly used methods to detect mutations are direct sequencing and real-time PCR.

KRAS oncogene

The KRAS oncogene (the cellular homolog of the Kirsten rat sarcoma virus gene) is a critical gene in the development of a variety of cancers. Mutations in the KRAS gene are common in many types of cancer, including pancreatic cancer (~65%), colorectal adenomas (30%), colorectal cancer (30% to 50%), lung cancer (~20%) and ovarian cancer (~15%) [55, 56]. KRAS mutation status has great value in assisting therapy decisions. Mutations in critical areas of the KRAS gene, such as codons 12 and 13, are a negative predictor of response to anti-EGF receptor antibodies in colorectal cancer, and similarly are indicators of resistance to small-molecule tyrosine kinase inhibitors in NSCLC patients. KRAS mutation status is determined by many methods, including allele-specific PCR, real-time PCR methods and nucleic acid sequencing techniques in tumor samples. Patients with colorectal cancer and with KRAS mutations do not respond to treatment with vectibix (Amgen) or cetuximab (Erbitux, Bristol-Myers Squibb) — the antibodies that inhibit EGFR [57, 58]. The pathologist's role is to assess tumor histology and KRAS mutation status prior to the therapeutic decision concerning the use of an EGFR inhibitor [59]. Also, ASCO supports KRAS gene mutation testing before the initiation of EGFR inhibitors in patients with metastatic colorectal cancer [60].

Adenomatous Polyposis Coli (APC) gene

The APC suppressor gene is mutated in most colorectal cancers [61]. The protein made by the APC gene plays a critical role in several cellular processes that determine whether a cell may develop into a tumor. Approximately 3–5% of colon cancers are associated with high-risk, inherited colon cancer syndromes. Familial Adenomatous Polyposis (FAP) is an autosomal-dominant syndrome that accounts for < 1% of all colorectal cancers. More than 90% of families affected by FAP, or an FAP variant, have a mutation in APC, which encodes the tumor suppressor APC — a key molecule in several intracellular pathways [61–63]. Genetic testing for FAP typically involves DNA sequencing, often preceded by complementary methods which correctly identify up to 95% of mutations [63].

BRAF gene

BRAF is a serine/threonine protein kinase, encoded on chromosome 7q34, that activates the MAP kinase/ /ERK-signaling pathway. More than 30 mutations of the BRAF gene associated with human cancers have been identified. The frequency of BRAF mutations varies widely in human cancers from more than 80% in melanomas and nevi, to as little as 0–18% in other tumors, such as 1–3% in lung cancers and 5% in colorectal cancer. Approximately 42% of melanomas harbor activating BRAF mutations. The commonest mutations are V600E and V600K. The development of targeted therapies for malignant melanoma has occurred rapidly, from the discovery of the target to drug development. We have been able to give sufficient drugs to block mutated BRAF, resulting in clinical benefit for a majority of patients treated at higher doses. PLX4032 is important because it represents an advance in targeting BRAF; it suppresses the activated oncogenic pathway by inhibiting the ERK kinase cascade. High objective response rates were observed in the phase I clinical trial of PLX4032 in a cohort of melanoma patients selected for tumors with the V600E mutation. Ongoing phase II and phase III clinical trials are limited to those patients with BRAF V600E mutations [64–66]. Recently, the results of a phase III randomized clinical trial comparing BRAF inhibitor vemurafenib with chemotherapeutic agent dacarbazine in patients with previously untreated metastatic melanoma with the BRAF V600E mutation was reported [67]. Although many patients have tumor regression, the majority of patients treated with BRAF inhibitors develop secondary resistance and subsequent disease progression. Thus these de-

velopments in melanoma not only validate the concept of personalized cancer therapy, but also highlight the need to serially characterize tumors in order to understand the mechanisms leading to acquired resistance and to develop rational combinatorial therapies [67].

Conclusions

Personalized medicine represents the future due to better understanding of the unique genetic characteristics of tumors and their metabolic pathways. The great achievements noted in developing new drugs against cancers with a specific genetic composition can efficiently interrupt pathways of some organ-specific tumor cells. Molecular profiling and driver mutations of the cancers need more accurate diagnostic and molecular tests in the pathology labs. The use of powerful computer technology and digital microscopy has the potential to upgrade the pathologist's contribution in the successful development of personalized targeted therapy.

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Conflict of interests

The authors declare that they have no conflict of interests.

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