Review

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Histo-genomics: digital pathology at the forefront of precision medicine

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Abstract: The toughest challenge OMICs face is that they provide extremely high molecular resolution but poor spatial information. Understanding the cellular/histological context of the overwhelming genetic data is critical for a full understanding of the clinical behavior of a malignant tumor. Digital pathology can add an extra layer of information to help visualize in a spatial and microenvironmental context the molecular information of cancer. Thus, histo-genomics provide a unique chance for data integration. In the era of a precision medicine, a four-dimensional (4D) (temporal/spatial) analysis of cancer aided by digital pathology can be a critical step to understand the evolution/progression of different cancers and consequently tailor individual treatment plans. For instance, the integration of molecular biomarkers expression into a three-dimensional (3D) image of a digitally scanned tumor can offer a better understanding of its subtype, behavior, host immune response and prognosis. Using advanced digital image analysis, a larger spectrum of parameters can be analyzed as potential predictors of clinical behavior. Correlation between morphological features and host immune response

can be also performed with therapeutic implications. Radio-histomics, or the interface of radiological images and histology is another emerging exciting field which encompasses the integration of radiological imaging with digital pathological images, genomics, and clinical data to portray a more holistic approach to understanding and treating disease. These advances in digital slide scanning are not without technical challenges, which will be addressed carefully in this review with quick peek at its future.

Keywords: digital pathology; image analysis; omics; pathology informatics; precision medicine; virtual slides.

Introduction

Malignant neoplasms are known for their heterogeneity and evolutionary progression. The oncogenesis model described by Nowell in 1976 [1] demonstrated that when tumors accumulate mutations over time they advance to more aggressive cancers and potentially metastasize. Cancer evolution was explained further by Hanahan and Weinberg [2] who outlined the principal biological features of carcinogenesis that involve altered metabolic properties, higher mutational rates, and the capability to invade tissues and metastasize. This evolutionary model of carcinogenesis can efficiently explain the heterogeneity and multi-clonality of many cancers [3]. Different microenvironmental cues including host immune response, hypoxia, drugs and nutrient diffusion rate result in selective pressures for the development of cancer over time. To further elaborate, in the background of cancer cell high mutational rate, different heterogeneous cancer clones accumulate more mutations, become more aggressive, evade host defense systems and metastasize [4–8]. These features of evolution and heterogeneity set cancers as ever-moving targets, thus presenting diagnostic, prognostic and even therapeutic challenges [9]. Also, chemo- and immune-resistance, due to microenvironmental natural selection and the presence of other intrinsic factors like cancer stem cells [10], represent significant obstacles to effective cancer therapeutics [11].

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Similar to cancer cell evolution, our approaches to face the cancer challenge also evolved over time. In the 1970s until early 2000s, researchers and clinician scientists focused on one gene or one biological signaling pathway at a time. However, after the era of human genome project [12], we are currently moving fast into the age of molecular profiling of cancer genomics [13], bioinformatics [14], precision medicine [15] and digital imaging that generate a wealth of “raw data” through high-throughput experimental and analytical platforms. These new advances in research tools though needed, are very complex and expanding, which sometimes makes them difficult to interpret, envision or properly utilize [16, 17]. The hope for better understanding and clinical utilization of these new tools comes through the integration of different data types [18, 19]. Pathologists, in particular, are the best fit for the job of data integration and understanding due to their unique position as a link between the bench and bedside [12–14]. This is evidenced in their emerging role in analyzing resected tumors for ancillary therapeutic predictive markers such as PD-1 [20–22].

The emergent field of digital pathology enabled the pathologists to actively contribute to better understanding of cancer pathogenesis through histo-genomics; which is simply the interface between morphology and genomics. Digital pathology can add an extra layer of information that may help us visualize, interrelate and understand the wealth of genomic, transcriptomic and proteomic data that lack spatial information [23]. Thus, digital pathology can shed more light on cancer clinical behavior, immune response and genomic data. The ultimate goal is a better quality of life for cancer patients through dissecting cancer biology, and eventually providing better therapy, which requires visualizing the evolution/progression of a malignant tumor in 4D (temporal/spatial) via histological/genomic/bioinformatics data integration.

The potential clinical utility of advanced image analysis

Histologic evaluation of tissues is the basis of anatomical pathology practice yet is famous for its inter- and intra-observer variations [24]. The subjective evaluation of histologic specimens, as currently practiced, cannot possibly capture the detailed wealth of information hidden in the heterogeneous lesional tissue in many diseases, including cancer [25]. These limitations triggered the concept of objectively quantifying morphological properties in histopathological images to aid clinical decision-making

[26]. This required high-quality digital images. Older versions of slide scanning techniques demonstrated sluggish image acquisition, expensive data storing and slower networks, which in turn hindered file transfer and remote viewing. Further advancements in computer hardware and software have made slide scanning, acquiring whole slide images, digitizing of pictures and their online transfer faster and more efficient. In addition, improvements in image analysis algorithms enabled large whole slide image archives to be accessible for the extraction of quantitative and objective imaging features [26–28]. Currently, digital pathology can provide a targeted analysis of different cell components in tumor specimens and assist in tumor classification [26, 28]. In addition, image analysis algorithms can accurately define different features from the morphology of the nucleus to the characterization of composite tissues by applying different zonation and determining tissue interfaces [29–38].

A larger number of parameters can be analyzed in a scanned digital slide/image. A partial list is shown in Table 1. An important initial step in image analysis is the segmentation of different areas of tissue interfaces [39, 40]. Segmentation involves software algorithms to differentiate between higher-order structures like interfaces between stroma/epithelium, tumor/normal tissue or areas of microvascular invasion. These always require complex

Table 1: Examples of parameters that can be analyzed in scanned slides.

Architectural parameters:
– Stroma/epithelium interface
– Tumor/non-tumor interface
– Immune cell infiltrate
– Necrosis: localization and percent
– Vascular distribution and microvascular proliferation
Cytological Features:
– Nuclear size
– Nuclear color
– Nuclear texture
– Nuclear contour
– Nuclear hyperchromaticity
– Nuclear membrane circularity
– Extent of nuclear chromatin clumping
– Nucleolar prominence
– Nuclear/cytoplasmic ratio
– Nuclear membrane irregularity
– Tumor vascularity
Stromal features:
– Stromal Cellularity
– Interstitial immune response
– Analysis of specific stromal marker expression by immunohistochemistry

techniques to describe textures or patterns in statistical terms. Currently, applying these algorithms needs sophisticated computer software hence it is hard to apply to large databases of scanned images. Other types of segmentation are less demanding, such as segmentation/delineation of cell nuclei. Different techniques are used such as color, texture thresholds, the nuclear shape and other variations [29, 37]. For example, nuclear morphometry studies can efficiently depict parameters including the size of the nucleus, its circularity, nuclear hyperchromasia, and the extent of chromatin clustering [41]. Further analysis of defined features can be used for clustering and classification of tumors by machine-learning algorithms. A representative model for the image analysis procedure is demonstrated in Figure 1 [42].

To decide which character/parameter is important for diagnostic, prognostic or therapeutic purposes, two main approaches are generally used. One is the supervised or model-based approach and the other is the unsupervised or model-free approach. The model-based approaches incorporate prior knowledge of pathologists into the analyses. They employ quantitative measures of pathologically-identified morphologic models to investigate their molecular/clinical correlates. The model-free approaches do not require previously known features. Instead, they use clustering analyses to detect different histological structures and their association with cancer behavior that may reveal new features never been known to human interpretations. Further illustration of the

potential use of these approaches is discussed in the following sections.

Correlation between morphological features and clinical behavior

The morphologic features such as shape, spatial arrangement, and texture of cell nuclei are known to help in determining the histological classification and grading subtypes of different tumors (as in the case of papillary thyroid cancer [43]). These features can be surrogate indices of tumor behavior or prognosis. A classic example is a prognostic value of nucleolar grading in renal cell carcinoma [44]. Advanced digital imaging was able to take this to a new level. A good example of this is central nervous system (CNS) primary tumors [45–50]. For instance, a model-free clustering of nuclear features in digitalized slides of different glioblastomas (GBMs) revealed significant morphological correlations to patient survival. GBMs with elongated nuclei were associated with a shorter patient overall survival [25].

Another interesting example was found in breast cancer, where features that describe the morphology of the interface between the tumor and surrounding stroma was able to predict overall survival, and this was independent of other clinicopathologic and molecular factors in two independent cohorts [39]. In another study,

Figure 1: A representative protocol for image analysis of scanned immunohistochemistry slides.

(A) Original scanned image (hematoxylin and DAB). (B) Image after background correction and segmentation of cell area (green outline). Separation of hematoxylin (C) and DAB stains (D) using color deconvolution. (E) Separation of stroma (green) from epithelium (purple) using texture analysis. Reused from [42] following the guidelines of the publishing journal.

Table 2: A list of potential applications for digital pathology analysis.

Potential application	Representative studies
Predicting clinical behavior:	
– Glioblastoma	Cooper et al. [25]
– Breast cancer	Beck et al. [39]
– Prostate cancer	Doyle et al. [52]
– Oropharyngeal cancer	Lewis et al. [56]
– Gastric cancer	Kolin et al. [42]
Immune response:	
– Breast cancer	Yuan [58]
– Gastric cancer	Feichtenbeiner et al. [59]
– Colorectal cancer	Galon et al. [60]
Genetically subtyping cancers	
– Glioblastoma	Chang et al. [61]
– Breast cancer	Yuan et al. [62]
3D reconstruction of solid tumors for margin assessment	
– Breast cancer	Clarke et al. [63]
Radio-histomics	
– Prostate cancer	Langer et al. [64]

image analysis of digital slides from triple-negative breast cancers specimens provided a prognostic model [51]. Digital pathology applications also made remarkable progress in finding grade differentiating features such as mitotic counts in prostate cancer [52–55] and identifying indolent versus aggressive p16-positive oropharyngeal squamous cell carcinomas [56].

Digital image analysis was also used to correlate marker immunostaining with clinical behavior. Recent studies assessed the prognostic significance of human tissue kallikrein-related peptidases expression via image analysis of immunohistochemically stained digitalized slides in gastric cancer [42, 57]. A list of potential applications of digital pathology is shown in Table 2.

Correlation between morphological features and host immune response

The role played by tumor microenvironment during cancer evolution and the development of treatment resistance has long been acknowledged and thoroughly reviewed [56, 65, 66]. Digital pathology has been used successfully for the assessment of various microenvironmental cues, including the presence of infiltrating immune cells [67, 68], cancer-associated fibroblasts [69, 70] and stromal vasculature [71–73]. Particularly, the location of immune cells within a tumor microenvironment was found to be critical for progression in many cancers such as colorectal cancer [60, 74, 75]. Also, a number of gene expression

profiles revealed marked molecular heterogeneity within the immune cell infiltrate [76–79]. In breast cancer, different subclasses of lymphocytes can confer different pro- or anti-tumor functions [74, 78, 80].

Attempts to analyze immunohistochemically labeled lymphocytes in scanned/digitalized sections were performed [62, 81, 82]. In scanned digital slides from breast cancer patients, spatial statistics investigate were used to study different clustering patterns of B- and T-cells in tumor-draining and healthy lymph nodes [83]. The results revealed a higher lymphocyte clustering in lymph nodes draining tumors compared to healthy lymph nodes, with more clustering of T-cell more than B-cells, suggesting a differential role in host immune response. Digital pathology was also used to identify and quantify functionally active T-cells and natural killer cells in breast cancer [84]; and to identify the role of regulatory T cells (FOXP3⁺CD3⁺) in follicular lymphoma which was, contrary to conventional wisdom, associated with good outcome [85]. Using image analysis techniques, a functional distance range of 30–110 μm between the cytotoxic and regulatory T cells showed a correlation with significant improvement in 10-year survival in 50 patients who have been diagnosed with gastric cancer [59].

Moreover, advances were made to measure distances between infiltrating lymphocytes and individual cancer cells to facilitate organized investigation of cancer-lymphocyte interactions in large patient cohorts [58]. A considerable number of studies have concluded that the location of immune cells in relation to malignant cells is of clinical value in different cancer types [60, 86–89]. A higher concentration of CD8⁺ T-cells in remote stromal locations away from cancer cells independently predicted breast cancer-specific survival [88]. A higher concentration of CD3⁺ cells at the invasive margin of colorectal cancers significantly correlated with disease-free survival [60]. Also, higher CXCR5⁺ immune cell density at the invasive edge of different tumors correlated with bad outcome, contrary to a better good outcome if present central in the tumor [90]. Another study revealed that the presence of more dendritic cell aggregates in breast cancer correlated with a better prognosis [91].

Combination of genetic studies and digital pathology correlate with tumor clinical behavior

Malignant neoplasms as discussed before are extremely heterogeneous. Therefore, the expression of certain

genes can differ significantly among different cell types in various regions within the same tumor. While OMICs provide high molecular resolution, they lack any spatial information. This lack of cellular/histological context can lead to bias/inconsistency of molecular data because of sampling issues. Conversely, histopathological features, in many instances, do not correlate accurately with the clinical behavior of tumors. Thus, integrating the histomorphological context to genetic data may enable for a better understanding of the clinical behavior of malignant tumors.

For example, one study developed computational pipelines to integrate bioinformatics and digital pathology, aiming to subtype glioblastoma and renal cell carcinoma into biologically-relevant groups using TCGA data [61]. Another study used imaging and morphological parameters to identify three glioblastoma clusters characterized by different molecular events in histological areas enriched with specific microenvironmental cells [46]. A third study identified morphological patterns associated with survival outcome in triple-negative breast cancer [51]. In the latter study, nuclear features, regardless of their cell types, were quantitatively measured and correlated with gene expression profiling of the same set of triple-negative tumors. Some of these nuclear features, such as nuclear size, were found to be an independent prognostic value. Another breast cancer study found a strong correlation between analysis of nuclear morphology and copy number mutations [62]. Using manual annotation of 177 digitized frozen section images, Cooper et al. [25], defined the boundaries between necrosis and angiogenesis in 99 GBM tumors. Further analysis of the latter study revealed a correlation between the common defining histological features of GBM such as palisading necrosis and perivascular hyperplasia and the hypoxic upregulation of mesenchymal genes. Multiple other studies used TCGA resource to discover novel biological sub-classifications for a broad set of cancers [92–98].

Integrating 3D digital pathology and molecular genomics

The 3D imaging is not empirically novel in medicine as it has been implemented by non-invasive radiological imaging, which provided useful temporal and 3D images to neoplastic information for heterogeneous primary and metastatic tumors [99, 100]. Histopathology, however, can offer a robust capability, not available to radiology, which is to map phenotypic and genetic aberrations at

the cellular resolution. Traditionally, pathologic diagnosis using thin-cut sections mounted on glass slides made almost all tissue-based studies, including digitally scanned slides, limited to a two-dimensional (2D) space. Thus, image analysis of 2D microscopy was, until recently, subject to the same limitations of spatial information loss. The new advances in 3D microscopy allowed investigators to study disease-related biological structures in full authentic *in situ* 3D environments, which provided a wealth of information [101, 102].

In attempts to apply the analytical power of digital pathology in a 3D format, Clarke et al. [63] described a novel methodology of “3D pathology volumetric technique” to accurately measure tumor volumes in breast lumpectomies by preparing and processing whole mount slides and digitalizing them according to published methods [103, 104]. They used a pixel spacing of 2 μm for better optimization of tumor detectability and computational feasibility [105]. Their 3D pathology volumetric method managed to improve the 3D visualizations of different tumor foci with better accuracy, than the conventional grossing techniques, of locating *in situ* breast carcinomas. A more recent study by the same group proposed an image-processing framework to incorporate the 3D digital pathology morphology with cell-specific molecular and biomarker features [106]. This incorporation of molecular biomarkers into the 3D image analytics offered additional advantages for the investigation of tumor clinical behavior at different spatial frames and a specific 3D micro-environmental context to the molecular alterations and signaling events.

Radio-histomics: potential clinical significance

Radio-histomics or the interface of radiology and histology is an emerging field. The integration of radiological imaging with digital pathological images, genomics and clinical data can provide a more holistic approach to understating and treating cancers. In a recent study, different parameters of MR imaging of prostatic tissues correlated significantly with certain histological features that differentiated malignant from normal tissue [64]. In addition to the merge of radio-histomics, digital pathology can benefit from the advanced image analysis technique used in radiology. A number of promising studies have already investigated the relation between the features of quantitative radiology imaging, genomic profiles and clinical outcomes [107–110]. These quantitative

Figure 2: The integration of clinical parameters with digital pathology image analysis, omics data and 3D radiological image analysis can provide a more comprehensive view that can be used for tumor subtyping, assessment of prognosis and predicting response to treatment.

radiology-imaging techniques can be of value if used within the realm of digital pathology. Further elaboration of the integration between digital pathology and other disciplines are illustrated in Figure 2.

Challenges, potential solutions and future directions

The advances in digital slide scanning are not without technical challenges. Scanning a slide has to account for processing artifacts, staining variations between different slides, tissue section thickness and others. These present a considerable hurdle to embrace image analysis technology in clinical settings. However, these challenges are driving the evolution of digital pathology by inventing new methods to address them [35, 111–115]. Different algorithms have been developed to normalize pixel data and standardize color attributes in whole slide image database [37, 38, 112]. Other algorithmic approaches have been created to fix image defects due to tissue folding [35, 111, 113]. Currently, commercially available high-quality digital

pathology solutions such as PerkinElmer (USA), TissueGnostics (Austria) and Definiens (Germany) are available. Thus far, a number of these methods were used to study tumor pathological characteristics in cancer specimens, leading to significant findings [39, 68, 84, 116, 117].

Other challenges that face digital pathology include its integration with other disciplines such as the molecular and bioinformatics disciplines. For example, one challenge for such integration is the hard choice between using frozen tissue or formalin-fixed and paraffin-embedded (FFPE) tissue. The quality of nucleic acids for molecular studies is generally better in frozen tissues. Thus, frozen tissues are often preferred for molecular profiling, while FFPE tissues are often used for pathological image analysis tools [39, 52, 53, 55, 56]. The future of addressing such a challenge remains open to discussion.

Another barrier to the progress of digital pathology is the lack of sharing and distribution of algorithms and picture processing applications beyond the reach of image analysis experts to the wider research society. Rendering free software and algorithms for defining specific features publicly accessible will promote the advancement in this field. Some shared platforms are currently available such

as Pathology Analytic Imaging Standards (PAIS), which was created to support the standardization of image analysis algorithms and image features [118, 119]. Resources of web-based interfaces and online tools to support the visualization and analysis of pathology image data are becoming more readily available such as The Cancer Digital Slide Archive (CDSA, <http://cancer.digitalslidearchive.net/>) [120]. Another challenge for digital pathology is its need for independent validation of different findings, and the need to develop a standardized approach for assessment of correlations between morphology and molecular data. This is of particular importance to identify future genetic signatures, biomarkers and therapeutic targets that can be applied clinically.

Conclusions

The future advances in digital pathology are potentially limitless. Digital pathology represents not only a promising tool for disease diagnosis, but also for high-throughput and quantitative data extraction and analysis for multipurpose biomarker discovery for translational research on a wide spectrum of diseases [46, 121]. Thus, digital pathology can be considered critical to the future of precision medicine when treatment can be tailored to individual tumors in individual patients. While this new field holds great promise for better understanding of disease pathogenesis, progression and response to treatment, the scale of large imaging data can be overwhelming and technically challenging [41, 122]. However, with the advancement of computational hardware, software, online storages and the futuristic ideas of incorporating molecular and 3D information, it is almost impossible to predict the outcome except for its positivity.

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