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Pathology to Enhance Precision Medicine in Oncology: Lessons From Landscape Ecology

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Abstract

A major goal of modern medicine is increasing patient specificity so that the right treatment is administered to the right patient at the right time with the right dose. While current cancer studies have largely focused on identification of genetic or epigenetic properties of tumor cells, emerging evidence has clearly demonstrated substantial genetic heterogeneity between tumors in the same patient and within subclones of a single tumor. Thus, molecular analysis from populations of cells (either a whole tumor or small biopsy of that tumor) is, at best, an incomplete representation of the underlying biology. These observations indicate a significant need to define intratumoral evolutionary dynamics that yield the observed spatial variations in cellular properties. It is generally accepted that genetic heterogeneity among cancer cells is a manifestation of intratumoral evolution, and this is typically viewed as a consequence of random mutations generated by genomic instability within the cancer cells. We suggest that this represents an incomplete view of Darwinian dynamics, which typically are governed by phenotypic variations in response to spatial and temporal heterogeneity in environmental selection forces. We propose that pathologic feature analysis can provide precise information regarding regional variations in environmental selection forces and phenotypic adaptations. These observations can be integrated using quantitative, spatially explicit methods developed in landscape ecology to interrogate heterogeneous biological processes in tumors within individual patients. The ability to investigate tumor heterogeneity has been shown to inform physicians regarding critical aspects of cancer progression including invasion, metastasis, drug resistance, and disease relapse.

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Personalized medicine aims to use patient-specific metrics to provide an optimal cancer therapy customized for each individual patient.¹⁻³ Massive biobanks of patient tissues provide extensive libraries of genetic data that can be evaluated against targeted therapies.⁴ However, it is becoming clear that discriminating and cataloging genomic libraries of patient samples falls short, due in part to intratumoral heterogeneity. Personalized cancer treatments will require more than just matching a patient's tumor genomics with that of a central library.

Detailed molecular data from multiple regions in the same tumor reveal striking variations. Distinct populations of tumor cells displaying different biomarkers and gene signatures appear to coexist.⁵ This invites a greater understanding of tumor heterogeneity at molecular, cellular, and tissue temporal and spatial scales.^{6,7} Unfortunately, current proteomic and genomic methods fail to wholly address heterogeneity. Current techniques rely on single sample that homogenizes into large numbers of undoubtedly variable cells. It is likely that even these “averaged” data will differ from region to region within the same tumor, and certainly between tumor sites in the same patient.⁸⁻¹⁰ Batching and averaging information from millions of cells is likely limiting for developing personalized cancer treatments.

We propose extending pathology to identify, classify, and quantify cell to cell, region to region, and tumor to tumor heterogeneities. Such pathology metrics can supplement current efforts toward personalized medicine. We propose analyzing histologic samples by using the theories, tools, and experiences of landscape ecology.

Landscape ecology measures, analyzes, and studies the spatial and temporal heterogeneities of natural ecosystems.¹¹ Since the pioneering work of Carl Troll in 1939, landscape ecologists have used maps, vegetation, and geologic surveys, photographic images and, most recently, satellite imaging to study the interactions between organisms with their environments. While maps are not the only tools of landscape ecology, these data acquisition methods empower investigators to study spatially explicit biological interactions. Together with information about organisms and the patterns of the organism's environment, investigators can interrogate habitat change, conservation, and other ecological interactions. We propose that many of these same principles and techniques can be developed and applied to create an emerging field of “landscape pathology.” While there are many definitions of “landscape,” we are using the definition of landscape from Turner,¹² which states that “a landscape is an area that is spatially heterogeneous in at least one factor of interest.” Thus, we define landscape pathology as a proposed discipline to apply quantitative, spatially explicit methods from landscape ecology to define the heterogeneous biological processes of cancer cells (the “organism”) in histologic samples (the “habitat”). Using landscape pathology methods can help investigators gain a more precise understanding of local selection forces and, in turn, adaptations within subpopulations of cancer cells in a tumor, which may be clinically important in understanding disease progression, treatment response, and relapse.

Figures 1A and B provide an example of this concept. Here, hematoxylin and eosin–stained histology slides, the standard for primary diagnoses in cancer, may be compared with traditional landscapes commonly viewed as maps or satellite imagery. Regional classifications, spatially explicit analyses, and quantifiable metrics common in ecology can add to the pathologist's clinical toolbox.^{13,14} These classifications and analyses will provide pathologists with the ability to quantify morphologic heterogeneity within a tissue section with exquisite precision. This in turn will empower pathologists with the ability to rapidly identify areas of necrosis, high rates of proliferation, high incidences of inflammatory response, regions of high or low nuclear pleomorphism, and similar clinically pertinent morphologic features.

The Clinical Problem

Intratumoral heterogeneity manifests in at least 3 general ways:

1. *Mixtures of normal and malignant cellular populations within tissue.* Pathologists typically describe this variation in qualitative terms. The pathologists will, for example, recognize benign cells such as fibroblasts, lymphocytic, and epithelial cells. They will also identify inflammation, necrosis, hyperplasia, preneoplastic disease, benign tumors, and malignant cancers.¹⁵
2. *Variations in the microenvironment.* Blood flow in tumors typically results in temporal and spatial variations in concentrations of growth factors, substrate, and metabolite concentrations. These in turn manifest as regions of necrosis and variable cell density. Each of these variations selects for the adaptive evolution of local tumor populations which can be spatially evaluated in a quantitative manner including, but not limited to spatial evaluation of clustering populations or proliferative phenotypes.¹⁶
3. *Genetic heterogeneity.* Increased mutation rate owing to intracellular properties such as DNA repair or genotoxic environmental factors such as hypoxia appear to continuously generate new mutant cells. Cells carrying these mutations, in turn, proliferate if the phenotypic expression of that mutation confers an increased fitness. An example is the perpetuation of the mutator phenotype.¹⁷

In current practice, pathologists attempt to overcome this heterogeneity by selecting regions of tissues for genetic analysis that minimizes normal or necrotic cells. This is commonly performed using a slide marker to draw directly on the glass slide in an effort to select regions of high tumor cellularity or conversely, to scratch out large regions that are not of interest including normal margins or necrosis. In this way the technique currently “homogenizes” the samples and averages any downstream information that may be useful in evaluating intratumoral heterogeneity.¹⁷

We propose that current methods for personalized cancer therapy—treating target lesions as a single heterogenous genetic sample—are not wholly adequate. This is primarily because evolutionary strategies or adaptations often involve several phenotypic changes that in turn can be achieved through an even larger number of genetic pathways. That is, evolution directly acts on cellular phenotypes and not genotypes. In fact, Darwinian dynamics, which

can be described as dynamics of systems which drive fitness by natural selection, are manifested in phenotypic changes.¹⁸ Second, clear evidence indicates that extensive genetic heterogeneity exists within cancer cells in the same tumor.⁵ Averaging or lumping tumor heterogeneity into single metrics or qualities may mask key aspects of the tumor's progression and state, and unwittingly “throw away” valuable information on the heterogeneity itself and what it indicates.¹⁹ We suggest embracing the information content of spatially explicit considerations of cellular, micro-environmental, and regional heterogeneities. The ability to investigate tumor heterogeneity has been shown to inform physicians regarding critical aspects of cancer progression including invasion, metastasis, drug resistance, and disease relapse.^{20–23}

It is important to understand why intratumoral heterogeneity is clinically important. Clinicians have deep experience with disease relapse, changes in treatment effectiveness, and hormone status or other clinically relevant deviations in the greater cancer cell population. We propose that the described changes in the patient's overall disease state are due in significant part to cancer cell populations changing as different subpopulations of cancer cells evolve toward increased fitness by natural selection. Intratumoral heterogeneous subpopulations of different cellular phenotypes in a single tumor make these Darwinian dynamics possible, which in turn makes treating cancer a moving target.

Cancers are complex but not hopelessly so. Tumors can be understood by characterizing and embracing their underlying ecological and evolutionary dynamics, including both spatial and temporal variability. Fortunately, pathologists have been observing and quantifying variations within the morphology of cancer cells for well over a century and are well positioned to visually evaluate heterogeneity across histologic samples.²⁴ We propose that the challenges are 3-fold: identification of cellular heterogeneity must become (1) regionally explicit, (2) quantitative and reproducible, and (3) high throughput.

Until recently, these challenges would be insurmountable due to the time consuming and subjective manual screening of tumor heterogeneity.²⁵ However, with the advent of whole-slide imaging technology and recent improvements in pattern recognition software, it is possible to computationally evaluate millions of cells in minutes and hundreds of patients in hours to days.²⁶ Whole-slide imaging allows high resolution and high throughput image acquisition for every cell within a given tissue sample.²⁷ There are a number of advantages of these technologies including low costs, high throughputs, quantitative results, and rapid evaluation of tissue samples, which are already routinely produced in every hospital within the United States and the majority of similar centers around the world.¹³

While current imaging technology and methods are necessary, it is not sufficient to visually investigate spatial distribution of cells and microenvironmental properties in patient samples. We propose that the discipline of landscape ecology, with its associated tools and theories, can be used to evaluate the relationship between pattern and process in pathology. While pathology is equipped to identify patterns in tumors, landscape ecology provides the tools to evaluate these patterns and understand the underlying biological relationships.^{28–30} Together, quantifiable metrics of digital pathology and landscape ecology can contribute to

personalized medicine. Thus, “landscape pathology” has the potential to provide new information regarding patients' intratumoral heterogeneity.

Benefits of Landscape Ecology In Pathology

Landscape ecologists and pathologists work on a spectrum of scales which spans from 10^6 to 10^{-6} m, yet the substrates on which both groups work is identical. In modern studies, these are most frequently digital images, whether from a satellite or a microscope lens. Whole-slide microscope images are the maps with which landscape pathologists will work. Distinctive heterogenous regions can be identified along with their relative contribution to the total tumor volume as well as their interactions with each other. This is done by using segmentation and classification methods¹⁴ to identify distinct regions in the tumor and to allow examination of their boundaries.

The first consideration for segmentation and classification in tumors may be the criteria with which regions will be identified. In landscape ecology, “patches of the land cover region” can be wetlands, clusters of trees, or any relatively homogenous area of interest. Habitat patches are identified in a number of different ways including reflectance data, time series of vegetation activity estimated from the reflectance data, estimates of surface roughness/vertical structure from RADAR or LiDAR sensors, or surface temperature from thermal remote sensing, to name a few.^{31–33} These variables, which a landscape ecologist may use, might then be chosen to classify habitat using a number of pixel-based or object-based methods.^{34, 35} Pixel-based and object-based classification methods are also common in the current standards of digital pathology analysis.^{36, 37} In fact, pathologists now have a plethora of commercially available image analysis tools to segment objects (ie, delineate patches) and classify spatially explicit regions (ie, identify habitats) by examining vascular density, relative cell viability, and necrosis or immunohistochemical evidence of regional oxygen concentration such as HIF-1 α expression.³⁸

As demonstrated in Figures 1C and D, histologic pattern recognition utilizes random forest classification to identify color and texture variation in both image types through a commercially available image analysis platform (Definiens, Munich, Germany). By teaching the algorithm to recognize homogenous regions, a computer can reliably identify “patches” in a robust and repeatable way. Individual physical or molecular features derived from the images can be used to catalog morphologically homogenous cell populations and relationships between cells or regions and establish testable hypotheses to further interrogate processes of the system.³⁹ Similar to the patch-matrix (also sometimes referred to as patch mosaic) paradigm in landscape ecology practices, histologic pattern recognition also creates and thus defines a border between tissue regions.⁴⁰ Of course, alternatives in landscape ecology to evaluate spatial heterogeneity such as gradient paradigms are also used.⁴¹

Qualitatively, pathologists could then begin to evaluate the regions of interest with these or other classification methods of patches. With quantitative image analysis and statistical methods, pathologists can quantify precise metrics of the regions.⁴² Initially these metrics might include area, intensity, roundness, and other physical features.⁴³ However, pathology can learn from landscape ecology that patches can form mosaics.⁴⁴ They can demonstrate

edge effects and they can be clustered.^{45, 46} These features of tissue architecture can also be quantitatively measured and used to evaluate the disease at a mesoscopic scale (200µm to 2µm). For example, the architecture of prostate glands and their orientation have been shown to be informative of patient prognosis⁴⁷ and retained architecture in breast cancer tissues has been shown to inhibit malignant progression.⁴⁸

Multiple regions or patches typically exist in a landscape. We expect that similar variations will be found in most clinical cancers using the tools for landscape pathology.^{49, 50} In turn, this will allow the intratumoral heterogeneity to be characterized, quantified, and ultimately compared. A way in which the heterogeneity may be characterized can include habitats of vascular regions^{51–53} or regions of increased lymphocytic response.⁵⁴ Each quantitative method cited includes clinically meaningful prognostic or predictive value.

Landscape Ecology Applications In Pathology

Evaluation of the consequences of having multiple sub-populations of cells in specific patterns in a tumor will require learning from landscape ecology, which focuses on the feedback loops between patterns and processes. Patterns observed in pathology include points of blood vessels or ectopic lymph nodes, expression levels of biomarkers such as Her2Nue, or regions of high proliferation. Each of these patterns has diagnostic and prognostic value to the pathologist. Here we describe 4 analyses and their utility to pathology.

The first is point patterns, which consist of point locations distributed in 2-dimensional space. Landscape ecologists would characterize these as random, regular, or clustered. In pathology, point pattern analysis would allow quantification of the spatial distribution of some cellular or tissue feature (ie, nuclei) in the tumor. Metrics such as Ripley's K function can be used to compare one region of the tumor to another.^{55,56} Specifically, Ripley's K is a statistical metric for quantifying deviations from spatial randomness and has been used in mammography as a classification method.⁵⁷ Point patterns in a histologic section have been shown to be important at the histologic scale in interrogating aspects of the environment including lymphocytic invasion, cancer-associated fibroblastic localization, or the distance from vasculature to cancer cell populations.^{58, 59} This information could help oncologists most carefully predict response to specific therapies.

Second, regional variations in necrosis, ectopic lymph nodes, or other intratumoral features can be described as values of the number of cells, size of necrotic regions, or distribution of lymphocytes in space. In this example, measures of spatial autocorrelation, such as Moran's I,^{60, 61} can reveal the scale and degree of dependency among observations. This is, for example, important to be useful to quantify dispersal (migration) of specific cell populations by evaluating the pH of the microenvironment.⁶² Specifically, the location of cancer cells can be quantified to better understand if cancer cells are moving together in regions in which acid-mediated invasion provides spaces of increased selective advantage or if invasion is more correlated with random Brownian motion.

These metrics may also be useful in predicting response to specific treatments such as hypoxia-activated prodrugs or predicting prognosis of the patient's own immune response.⁵⁴

Specifically, the spatial correlation of cancer cell populations is useful in understanding the overall heterogeneous organization of cancer cells. That is to say, cancer is not randomly oriented or unorganized, but rather is a function of selective Darwinian dynamics of an adaptive landscape which may be measured and investigated. Furthermore, quantification of spatial relationships among tumor cell clusters of networked populations in relation to these environmental responses can be used to reveal prognostic indicators such as increased nuclear pleomorphism in regions of increased vascularity.^{63, 64}

Thirdly, distance measures are frequently used tools to collect quantitative data in histologic images.⁶⁵ For example, number of interactions with neighbors in a nearest neighbor analysis to discern morphologic or other similarities between nearby individuals can provide information about the size and connectivity of pockets of cells with distinct morphologies. The spatial identification of vessels and ectopic lymph nodes may have near-term clinical implications as key pathologic findings and progression projections.⁶⁶

Finally, it is likely that landscape pathology, as in landscape ecology, will require mathematical models for simplifying and interrogating complex ecological and evolutionary systems.⁶⁷ Ecologists and pathologists alike use models to expand testing beyond the time, expense, and often feasibility of experimental designs.^{68, 69} Mathematical models have also had great impact in cancer research in recent years.^{70–72} Spatial models in particular have a clear role in interrogating pathologic samples.⁷³ One such model not yet translated to medicine deals with ecological niche modeling.^{74, 75} Here niches are defined as the geographic areas necessary for a species to survive. This approach uses matching of individual traits in a species to available resources on the landscape. Furthermore, the niche may limit the distribution of invasive species to a particular region of the landscape.⁷⁶ These models are often also referred to as bioclimatic envelop models and species distribution models. Here we draw striking parallels to cancer, which grows asymmetrically due to what has been proposed as localized niches of heterogeneous microenvironmental resources.^{53, 77} Also interesting is the direct link of the ecological niche model to spatial distribution of resources within regions of a system.^{78, 79} These models have been highly successful in predicting invasive species spread into new regions of the globe.⁸⁰ These concepts may be mapped in landscape pathology images in 3 dimensions as a topographical representation of an histologic image where the third dimension is a measure of multiplexed features of tissues or cells including glucose levels, pH, physical space, and oxygen concentrations much like a number of variables including elevation, rainfall, food sources, and other considerations might be used in niche models (Figs. 1G, H).

Summary and Future Directions

In summary, we propose that pathologists have the opportunity to define the Darwinian dynamics within cancers through application of methods and principles of landscape ecology. Using automated image analysis techniques much more precise information can be investigated regarding intratumoral heterogeneity. Furthermore, spatial heterogeneity in these tissue “habitats” can be measured and used to define both prognosis and optimal therapeutic strategies. The latter will require transition from targeted therapies based on genomic analysis of small tumor samples to environmentally and phenotypically defined

targets based on comprehensive knowledge of the spatial variations throughout the tumor. This is facilitated by automated, high throughput image analysis technologies to identify variations in the physical or molecular metrics of cells and environmental properties.

This approach will require quantitative, reproducible, and comprehensive analysis of cancer as an ecological system. Evaluation of these data as prognostic and predictive biomarkers will require significant effort. However, we propose that this approach to understanding and quantifying intratumoral heterogeneity is necessary to achieve current goals of personalized cancer therapy.³⁵

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Figure 1.

Hematoxylin and eosin (H&E)-stained histology slides may be compared with traditional landscapes. (A) A standard histologic H&E image of an invasive tumor compared with (B) a standard satellite image of the Blue Ridge Mountain range in Shenandoah, Virginia. (C) Regional tissue classification of viable tumor (red), mild necrosis (purple), necrosis (blue), and other nontarget tissue (green); (D) compared with random forest classification in variant habitats which seems to correlate most with increasing elevation from green to blue to red to purple. (E) Feature data are displayed in the Z dimension to create a topographical map of the H&E relative to the morphologic feature being evaluated (red: blue color layer ratio); (F) 3D topographic representation of the Shenandoah habitat site demonstrating heterogeneous elevation. (G) Topographical heatmap is used to rapidly identify regions with like feature characteristics for this H&E image. Top scale bars (A, C, E) represent 300µm and the top insert map scale bar is 3 mm; bottom scale bars (B, D, F) represent 3000m and the bottom insert scale bar is 100 km. Credit Google Earth for all satellite imagery. The white arrow indicates North. The red asterisk is the location of Washington, DC.