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Mini-review

Computational pathology: Exploring the spatial dimension of tumor ecology

Sidra Nawaz [a,b,c], Yinyin Yuan [a,b,c,*]

[a] Centre for Molecular Pathology, Institute of Cancer Research, London SM2 5NG, UK
[b] Centre for Evolution and Cancer, Institute of Cancer Research, London SM2 5NG, UK
[c] Division of Molecular Pathology, The Institute of Cancer Research, London SM2 5NG, UK

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ABSTRACT

Tumors are evolving ecosystems where cancer subclones and the microenvironment interact. This is analogous to interaction dynamics between species in their natural habitats, which is a prime area of study in ecology. Spatial statistics are frequently used in ecological studies to infer complex relations including predator–prey, resource dependency and co-evolution. Recently, the emerging field of computational pathology has enabled high-throughput spatial analysis by using image processing to identify different cell types and their locations within histological tumor samples. We discuss how these data may be analyzed with spatial statistics used in ecology to reveal patterns and advance our understanding of ecological interactions occurring among cancer cells and their microenvironment.

Introduction

The interaction between cancer and surrounding normal tissue plays a vital role in the progression of malignant disease [1–7]. Obtaining a continuous and sufficient supply of nutrients and oxygen [8] and the threat of destruction by the adaptive immune response of the host [9] are two of the major microenvironmental selection pressures faced by cancer cells. Due to genetic heterogeneity within a tumor some malignant cells are able to survive under these pressures, thus becoming 'naturally selected' [10–15]. The fitness advantages these cells have may include their ability to survive in hypoxic conditions [12,16,17], stimulate new vessel growth [18–20] and modulate the host immune response [2,21–23]. Such cells are adapted for a harsh microenvironment and have been linked with poor prognosis [17,24,25]. Pioneering research has revealed genetic changes in cancer cells during their evolution [26–28], but there is developing interest in studying this process from a novel perspective: ecology [29–31].

The synergy between cancer and normal cells is analogous to relationships between species in a given habitat, which is a prime area of study in ecology. These relationships have been systematically studied in four categories: (i) predation, where one species benefits by consuming another, (ii) mutualism, where two species interact in a way that is of benefit to both, (iii) commensalism, where one species benefits without any effect on the other, and (iv) parasitism, where one species benefits at the expense of the other [32–34]. In cancer, all four of these relationships have been observed or proposed to exist [5,30,35]. We propose that studies of cell–cell interactions in the tumor ecosystem can substantially benefit from applying these ecological concepts and accompanying analysis tools that have been developed over many decades.

Ecological studies often begin with examining the spatial distribution of species in their habitats, which is a key determinant in access to resources, predator evasion and interaction with other organisms and the environment [36–39]. In tumors, spatial mapping of cancer cells in their microenvironment can be achieved by analysis of histology samples [40–48]. However such specimen may contain hundreds of thousands of cells that would be prohibitively difficult to count by eye, and estimates may vary between observers [49]. In recent years, a new way of analyzing tumor specimen has emerged in response to this challenge. Computer vision techniques have been applied to pathology for automated identification and classification of various cell types and tumor regions [41,50–57] (Table 1), and can enable rapid mapping of their spatial locations. For example, just as large areas of land can be mapped for population density variation, a tumor sample may be processed to map changes in density of its constituent cells, as shown in Fig. 1 [58]. Such methods thus offer a new opportunity for studying interactions between cancer and normal cells.

Although the notion of ecological interactions occurring in cancer has been reviewed in great detail before [5,59], the application of computational pathology to study these interactions is a novel approach in the field of tumor microenvironment research. This review brings together three developing concepts with examples and applications: (i) ecological interactions among cancer cells and between

* Corresponding author. Tel.: +44 208 915 6632; fax: +44 207 370 5261.
E-mail address: yinyin.yuan@icr.ac.uk (Y. Yuan).

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Table 1
Computer vision tools developed for analysis of tumor histology images.

Authors, year	Description	Tissue	Stain	Accuracy	Limitations
Basavanhally *et al*., 2010 [51]	Lymphocytic infiltration detection and grading. Support vector machine classifier differentiates between samples with high and low grade infiltration.	Breast biopsy	H&E	>90%	Derived from 42 images from 12 patients. Does not provide lymphocyte locations for spatial analysis, but could be adapted for this.
Beck *et al*., 2011 [52]	C-Path: categorizes regions in a histology image to epithelial and stromal areas using 31 image-based features. Further classification of objects within these areas using morphological and contextual features. Can provide relational descriptors such as mean distance between epithelial and stromal nuclei.	Breast TMA	H&E	89%	Individual cells not detected. Classifier may need re-training before application to datasets from other institutions.
Doyle *et al*., 2012 [53]	Identification of cancerous regions in an image using a Bayesian classifier that operates at multiple resolution levels.	Prostate needle biopsy	H&E	ROC: 0.76-0.84	Patch-based rather than pixel-based classification recommended for high resolutions. Spatial data cannot currently be obtained.
Holmes *et al*., 2009 [54]	GemIdent: identification of multiple phenotypes in a microscopic image. Uses supervised machine learning algorithms for automated detection and classification of objects. Locations of objects are also reported. Not limited to a particular stain or tissue type.	Various	Various	Dependent on classifier training	User is required to train the program to enable automated identification. The algorithm is best suited to images with few colors. Detection of centroids of small and large objects to identify their location may be less reliable and require retraining of the classifier.
Lu *et al*., 2014 [55]	ASH: automated selection of hotspots of Ki67+ stain. Region-based detection of Ki67+ areas in an image using ImmunoRatio [56]. User is provided with a ranked list of 10 hotspots. These areas are labeled on the original image.	Various	Ki67	Not stated	Does not detect single cells. No comparison to Ki67 hotspot scores by a pathologist provided.
Tuominen *et al*., 2010 [56]	ImmunoRatio: ratio of positive nuclear stain to total nuclear area for an IHC marker. Each nucleus is segmented and color deconvolution applied to identify it as positive or negative.	Breast tissue sections	ER, PR, Ki67, hematoxylin counter-stain	Correlation coefficient with visual scoring = 0.98	Spatial data not provided but algorithm could be adapted for this. Web-based application may hinder high-throughput analyses.
Yuan *et al*., 2012 [57]	CRImage: identification of cancer, lymphocyte and stromal cells as well as their locations within the tissue. Support vector machine classifier uses morphological and contextual features and operates at multiple resolutions. Can be used to obtain cellularity and lymphocytic abundance scores, and cell location data enables spatial pattern analysis.	Breast whole-tissue sections	H&E	>90%	Only three types of cells detected: cancer, lymphocyte and stromal. May suffer from variability in staining and batch effects. Potential application to other tissue types but will require re-training.

This is a non-comprehensive list of some of the non-commercial methods available for automated detection of objects of interest in a tumor sample. Given also are the specific tissue and stain for which each method was developed, the accuracy of the method reported by its authors and known limitations. H&E: hematoxylin and eosin; TMA: tissue microarray; ROC: receiver operating characteristic; ER: estrogen receptor; PR: progesterone receptor.

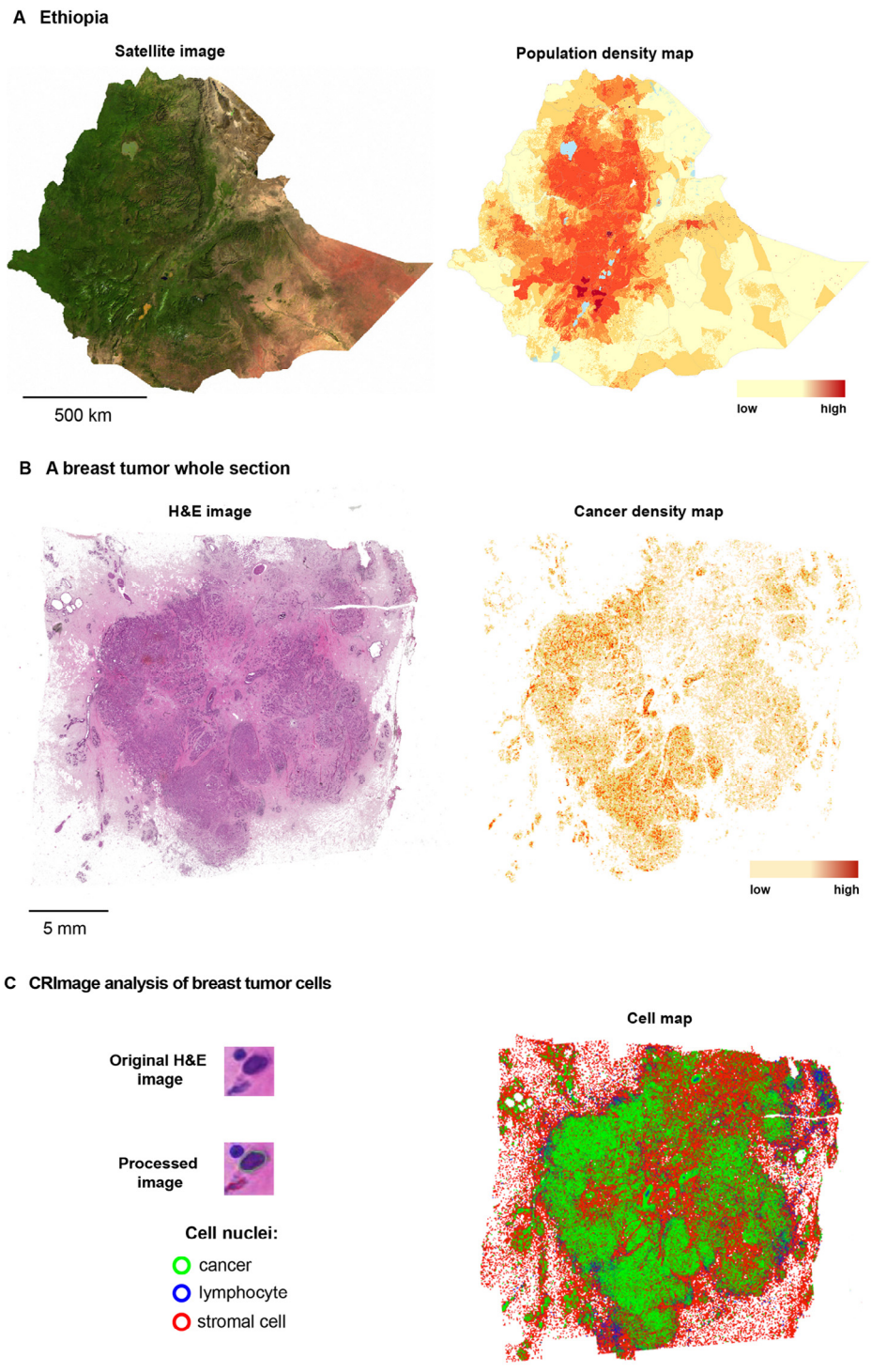

Fig. 1. Versatile spatial mapping tools can be applied to study populations at both the macro- and micro-scales. Just as demographic surveys can reveal more and less densely populated regions where, for example, a contagious disease may spread at different rates, image processing for identification and location of different cell types in a tumor can help elucidate the variation in cell density that may be used to develop new quantitative prognostic markers. (A) A satellite image of Ethiopia (left) and a corresponding population density map (right). (Left: image by Michael Adams, distributed copyright-free; right: image by Yann Brolec, adapted and distributed under a CC-BY-SA 3.0 license.) (B) A hematoxylin and eosin (H&E)-stained whole-section sample of a breast tumor (left) with a corresponding cancer cell density map (right). Cell location data were obtained from image processing of the whole-section sample using CRImage. (C) Analysis of H&E-stained breast tumor cells (left) using CRImage. The algorithm detects the darkly stained cell nuclei and classifies them into cancer, lymphocyte and stromal cell nuclei based on over a hundred quantitative features including nucleus texture and morphology. It also provides location data for all nuclei detected, which can be used in cell mapping (right). (All H&E-stained tumor images shown are distributed with permission from Natrajan et al., Breast cancer research and treatment, 2010).

cancer and surrounding healthy tissue, (ii) the use of spatial statistics methods to study them in routine ecological investigations, and (iii) computational pathology to explore the tumor microenvironment using these methods from a novel perspective.

Predation

Predation is a key component of an ecological system as it prevents a single species from becoming dominant. In tumors, certain

immune cells can locate and destroy cancer cells [60], analogous to observations of predatory behavior frequently made in ecological settings [61,62]. In ecology, spatial analyses of predator–prey relations have been used to shed new light on predatory behavior [63,64]. In one study, Ripley's K statistic [65] (Table 2) was used to evaluate the degree of uniformity in the distribution of hunting murres in two foraging zones [66]. The authors found aggregation of the birds in the two zones but a uniform distribution pattern within these zones, suggesting a shift from maximizing cooperation to minimizing competition over decreasing spatial distance as the ecological principle driving their distribution. This study epitomizes the significance of considering spatial scales in order to evaluate the dynamics of predatory behavior.

A spatial analysis of immune cell distribution within a tumor may reveal patterns indicative of the efficiency with which they can inhibit tumor growth. A recent study of ours investigated the spatial distribution of cancer and immune cells in breast tumors [67]. Cancer and immune cell co-localization was quantitatively measured using the Morisita–Horn index [68] (Table 2) following image analysis of the histological specimen. The Morisita–Horn index has been applied in studying predator–prey interactions, since it quantifies the extent of co-localization between two or more species. For example, it was used to investigate the theory of a positive association between predator body size and both mean prey body size and prey diversity [69]. In our study, a high degree of co-localization between cancer and immune cells measured by this index was found to be significantly associated with increased probability of ten-year disease-specific survival in human epidermal growth factor receptor 2-positive (Her2+) breast cancers. This suggests a likely predator–prey relation between cancer and certain immune cells in those tumors. In another study on estrogen receptor-negative breast cancer [47], we employed Getis–Ord geospatial statistics [70] (Table 2) that can pinpoint areas with significant spatial clustering or 'hotspots' of an entity. A high proportion of tumor regions containing hotspots of both cancer and immune cells was associated with high disease-specific survival in two independent patient cohorts, and provided extra prognostic information to measures of immune abundance [57]. Fig. 2 [58] displays results from a similar analysis in 180 triple-negative breast tumors from the METABRIC dataset [71]. A possible explanation is that this method is capturing, to some extent, specialized immune cells that play an anti-tumor role, and hence increased co-localization with cancer cells is linked with higher survival probability. Both of these studies underline the importance of utilizing the abundance of histology data to better understand the ecological relationships between cancer and its microenvironment.

Indeed, previous studies of immune infiltrate in cancer have revealed links between clinical outcome with immune cell presence [32,42,44–46,72–76], relative abundance [57] as well as spatial proximity of immune cells to invasive cancer cells [47,48,67,77,78] – the concept of immune contexture. These findings indicate a stronger tumor-inhibitory response of the immune system in patients with a good clinical outcome. Viewing the anti-tumor activity of the immune system from an ecological perspective as a predator–prey interaction, spatial statistics methods routinely used in ecology can be used to develop novel phenotypic prognosticators. This may in turn shed further light on the complex interactions between cancer and anti-tumor immune cells.

Mutualism

Mutualism defines a relationship between two species in which both derive benefit by interacting with each other, and plays a key part in maintaining species fitness [34]. A commonly observed example of mutualism in nature is pollinators interacting with flowering plants. A recent study employed Mantel's test [79] (Table 2) to investigate bee variation and its association with spatially-varying floral or nesting resources in an area of Mediterranean scrubland [80]. The authors discovered that smaller bee populations tended to be aggregated in space while the two most abundant bee species were segregated, and that variation in bee composition could partially be explained by the change in floral resources. Thus, a spatial analysis of bee distributions involving multiple factors revealed significant differences in how these pollinators interact with flowering plants.

Mutualistic interactions in cancer were thought to be rare as malignant cells face fierce competition from each other for the limited resources available [81]. However, one study reported cooperation between two subclones in mouse mammary tumors: a luminal HRas-wildtype subclone and a basal subclone harboring a somatic HRas mutation [82]. Both were found to be favorable for tumor growth. In another study, a mutualistic relationship between hypoxic and non-hypoxic cancer cells was found [16]. Hypoxic cells metabolized glucose to produce lactic acid which was unexpectedly found to be a prominent respiratory substrate in non-hypoxic cancer cells. Metabolizing lactic acid instead of glucose increased the availability of the latter for the hypoxic population which in turn

Table 2
Spatial statistics methods.

Method	Description	Limitations
Bayesian geospatial modeling	Allows model parameter estimation given the data and prior knowledge using a probabilistic approach (Bayes theorem) while accounting for uncertainties. Can be used for making predictions and is robust for use in large data sets.	Computationally demanding unless optimized. Assumptions made from prior knowledge are subjective.
Getis–Ord hotspot score	Identifies points of significantly high (hotspot) or low (coldspot) occurrence of an object given the global mean. Takes neighbors into account. Can be used to assess multivariate point patters. Can distinguish between high positive and low positive spatial autocorrelation.	Not suitable for identifying negative spatial autocorrelation.
Mantel test	Measures correlation between two matrices of the same rank (dimensions). Matrices typically contain distance measures between each pair of species in the sample set. Partial Mantel test can take into account a third or multiple other matrices containing confounding factors.	Unreliable for complex non-linear relationships between distances.
Morisita–Horn index	Measures the dissimilarity in species between two locations. Returns a value in the range 0 (no similarity) to 1 (complete similarity).	Sensitive to the most abundant species.
Ripley's K statistic	Global summary statistics. Measures spatial clustering or dispersion in a point pattern over small and large distances. Identifies how a distribution differs from homogeneity. Can be extended to analyze multivariate point patterns.	Cannot be used to account for continuous quantitative values associated with points, unless first discretized.

Spatial statistics offers numerous methods that can be used for analyzing ecological relationships. Listed here are those mentioned in the text, alongside their descriptions and limitations.

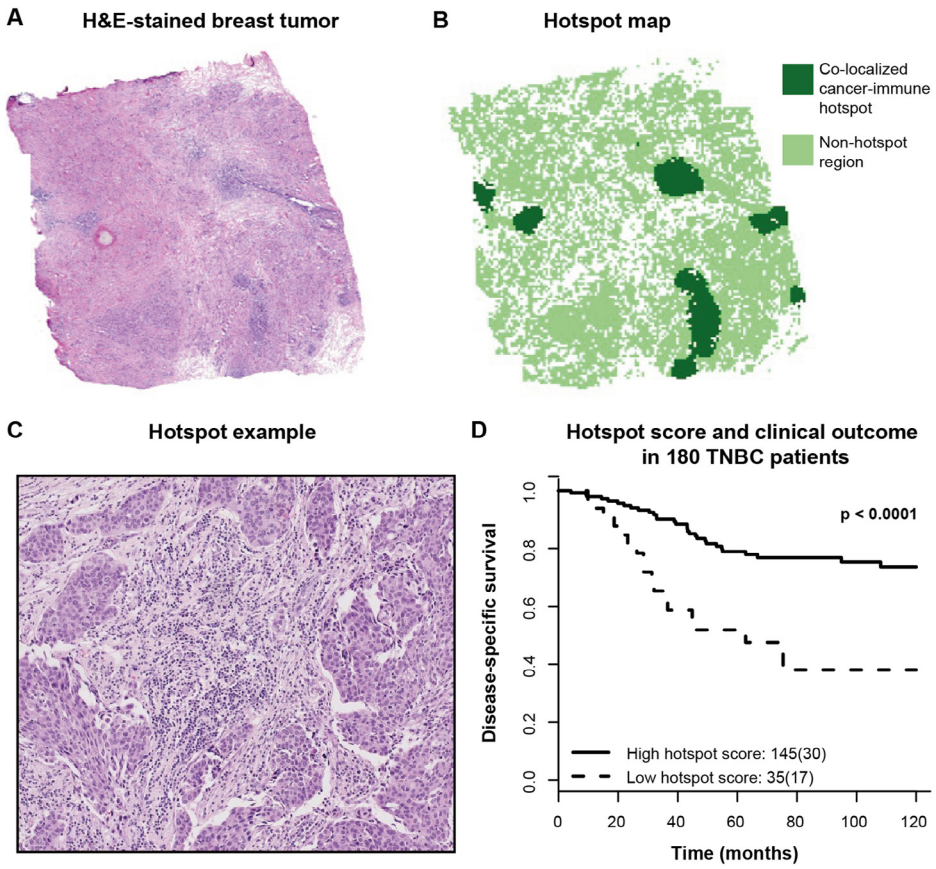

Fig. 2. Data derived from image processing of tumor sections can be used in spatial pattern analysis for discovery of new prognostic markers. Many spatial statistics methods developed for point pattern analysis can be applied to the study of tumor cells. Here, Getis–Ord hotspot analysis has been used to identify statistically significant co-localized clusters of cancer and immune cells that are prognostic in a cohort of tripe-negative breast cancer (TNBC) patients. (A) A H&E-stained breast tumor whole section. (B) Co-localized cancer-immune hotspots map for the tumor in A, constructed using cell locations obtained from CRImage and applying the Getis–Ord hotspot detection algorithm. (C) High resolution example of a hotspot region. (D) Kaplan–Meier curve illustrating ten-year disease-specific survival of 180 TNBC patients. Hotspot score is the ratio of hotspot to non-hotspot area in a sample. A high hotspot score, defined as being greater than a threshold discovered and validated in independent patient groups, correlates significantly with good prognosis. (All H&E-stained tumor images shown are distributed with permission from Natrajan et al., Breast cancer research and treatment, 2010).

generated an acidic environment that is thought to be immunosuppressive, thereby helping both populations to evade immune-mediated destruction. Other examples of cooperative behavior among cancer cells can also be found in the literature [83–85]. Thus, the relationship between cancer cells in a tumor is not always competitive; cooperative interactions may also evolve where the benefit derived from each other outweighs the need to compete.

Mutualistic relationships have also been found to occur between cancer and its microenvironment. A computational model of tumor-immune interactions proposed how signals produced by M2 macrophages may be promoting tumor viability at early stages of disease development [86]. The M2 macrophages benefit, in turn, from tumor-derived chemokines that promote the switch from M1 macrophage to the M2 phenotype [87]. The relationship between cancer cells and blood vessels can also be considered as mutualistic and analogous to that between bees and flowers. In return for obtaining essential resources from existing blood vessels, cancer cells can release angiogenic factors to promote new vascular growth. Just as floral resource distribution can partially account for the variance in bee composition within a habitat, spatial analysis of tumor specimen may reveal whether variance in resource distribution offers a survival advantage to some cells. Existing methods for vessel quantification using histology samples [88,89] could be adapted and/or combined with cell detection algorithms [57,90] to evaluate the spatial relationship between the two and its impact on patient prognosis.

Commensalism

Commensalism describes the interaction of two species whereby one gains a fitness advantage while the other neither benefits nor is harmed [34], though proving a lack of effect on the latter is often difficult. For example, urban rats typically have a commensal relationship with humans while they remain underground and feed on human waste. This relationship changes when rats cause harm to people by infesting homes, damaging property and transmitting diseases, the likelihood of which increases with urbanization. In one study, the authors sampled rat populations in an inner-city neighborhood over a year and used Getis–Ord spatial statistics to identify rat hotspots, i.e. regions where there were significantly higher numbers of rats found than one would expect given their overall spatial distribution [91]. Such studies allow urban health investigators to make more informed sampling choices in monitoring rat populations as cities expand.

In cancer, commensal relationships have been reported between tumor subclones. In one study, insulin-like growth factor II (IGF-II)-producing and non-producing cancer cells were observed to be in stable co-existence [92]. IGF-II non-producing cells gained an

advantage by obtaining IGF-II from producers without benefiting or harming them in any way. The authors also considered the diffusion range of a growth factor in a simulation to show that for greater ranges, the producer population diminished as increasing numbers of non-producers began to take advantage. Thus the establishment of equilibrium is conditional and has important implications for therapies that target growth factors.

Cancer cells also form commensal relationships with their microenvironment. For example, cancer-associated fibroblasts (CAFs) are known to support tumor growth and progression [93–95]. CXCL12, a chemokine secreted by CAFs, can stimulate angiogenesis and increased proliferation in cancer cells [6], while tumor growth factor-β signaling in CAFs is also known to modulate tumor proliferation [96]. This commensal relationship has begun to attract increasing interest in research as it is considered to play a vital role in metastasis [20]. The study of commensalism between subclones as well as the tumor and tumor-promoting traits of the microenvironment can reveal important inter-dependencies that may influence patient outcome or response to treatment. A mathematical model of cancer invasion suggests harsh microenvironmental conditions, such as hypoxia and a heterogeneous extracellular matrix, promote aggressive phenotypes with a high potential of metastasis [97]. With the aid of hypoxia or M2 macrophage-specific markers and spatial analysis of cancer cells with respect to positive and negative regions, these claims may be further substantiated by experimental evidence.

Parasitism

Parasitism differs from predation in that although a parasite harms its host, the host is not usually destroyed. A tumor can be considered as a parasite in the living organism despite not being a distinctly different species [98]. Like parasites, cancer cells undergo rapid proliferation and harm their host, by metastasizing to and destroying local and distant healthy tissue. However, since cancer cannot be transmitted or inherited, unlike many parasites, there is no selection pressure to keep the host alive. In one study, Bayesian predictive modeling [99] (Table 2) was used to identify environmental factors associated with parasites that can lead to learning difficulties in children in northwest Tanzania [100], whilst accounting for any spatial correlation that may exist between these factors. Bayesian modeling is a powerful predictive tool as it can account for spatial correlation in the data, preventing overestimation of the significance of predictors and the confidence of prediction. The results of this study demonstrate the importance of considering spatial relationships between predictors for robust predictive modeling. Such an approach may be applied in tumor analysis to study the parasitic role of cancer, and by considering environmental and spatial features, driving factors may be differentiated from confounding factors.

The reverse Warburg effect is a manifestation of the parasitic behavior of cancer [101,102]. This model proposes stimulation of fibroblasts by epithelial cancer cells to undergo aerobic glycolysis and release high-energy metabolites such as lactate. These metabolites facilitate rapid tumor proliferation as they can be metabolized in mitochondria, a more energy efficient mechanism of producing ATP than standard glycolysis. Thus, the tumor drives high-energy substrates away from healthy tissue for its own sustenance, analogous to a parasite, and unlike CAFs forming commensal relationships with cancer, fibroblasts involved in the reverse Warburg effect are often destroyed in the process via autophagy. Based on the model, it is predicted that a large stromal content of a tumor should be associated with rapid tumor growth, metastasis and a poor prognosis. To seek experimental verification, computational tools developed for quantifying stromal content in cancer histology samples can be applied for obtaining precise and reproducible measurements for comparison to clinical outcome data. In particular, spatial pattern analysis of cancer and stromal cells may add further prognostic power to such a model.

Current challenges and future outlook

There are some disparities between natural ecological settings and the tumor microenvironment that should be considered when applying ecological methods to the study of cancer. Ecological interactions in the tumor differ in some respects to those between multicellular organisms that reproduce sexually. Unlike these organisms, cancer cells do not require co-localization with each other to produce new cells. However, the high rate of accumulation of mutations due to the inherent genetic instability in cancer cells may have an effect on their ecological interactions with the microenvironment that is difficult to model. Highly proliferative cancer cell populations undergoing mitotic cell division will pass on acquired mutations to every daughter cell, hence sustaining a rapidly evolving population with a greater degree of niche heterogeneity at any one instance than is present in animal populations [81]. Detecting this heterogeneity while preserving the spatial context to construct computational models of cell ecology presents a technical challenge.

One of the drawbacks of computational analysis of histological material is the inability to distinguish between all features of interest using the same software. An image processing tool developed for hematoxylin and eosin (H&E)-stained images will not, in general, be applicable to immunohistochemistry (IHC) images; however, one may wish to incorporate the additional information provided by IHC to that obtained from H&E stains. Development of robust computer vision tools capable of analyzing images from different tissue stains could play a key role in propelling computational pathology into mainstream research and clinical use. This is especially important for the latter as variation in patient outcome despite current prognosticators remains a puzzle.

Moreover, histology on its own can be limited by the two-dimensional representation of a three-dimensional entity. Radio-imaging modalities can step in to address this problem [103]. Integrating radio-imaging data along with a variety of assays including IHC, immunofluorescence and DNA/RNA *in situ* hybridization, which can be used to reveal complex spatial patterns at protein, RNA and genetic levels [104–109], will provide additional layers of information to phenotypic characteristics obtained from H&E images. Studying the spatial structure of the tumor in this way may reveal new cancer–cancer or cancer–microenvironment interactions, such as those reported in [86,97], that exist at different spatial scales and could be exploited for patient benefit.

Summary

There is strong evidence of ecological phenomena occurring in the tumor microenvironment, and phenotypic studies of these phenomena can benefit greatly from application of spatial statistics tools routinely employed in ecological studies. Histology samples can provide an abundance of data as input for these methods due to the preserved spatial context. With the aid of computer vision in pathological research, this is becoming increasingly feasible to achieve, resulting in the emergence of new prognosticators for clinical use with potential to advance personalized therapy. Spatial pattern analysis empowered by integration of multiple layers of information could facilitate a more in-depth study of ecological interactions in the tumor and may prove to be successful in explaining the heterogeneity in clinical outcome. This could aid in identification of patients at highest risk of treatment failure who may benefit from participation in new clinical trials.

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