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Review

Histopathological growth patterns as a candidate biomarker for immunomodulatory therapy

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ABSTRACT

The encroachment of a growing tumor upon the cells and structures of surrounding normal tissue results in a series of histopathological growth patterns (HGPs). These morphological changes can be assessed in hematoxylin-and-eosin (H&E) stained tissue sections from primary and metastatic tumors and have been characterized in a range of tissue types including liver, lung, lymph node and skin. HGPs in different tissues share certain general characteristics like the extent of angiogenesis, but also appropriate tissue-specific mechanisms which ultimately determine differences in the biology of HGP subtypes. For instance, in the well-characterized HGPs of liver metastases, the two main subtypes, replacement and desmoplastic, recapitulate two responses of the normal liver to injury: regeneration and fibrosis. HGP subtypes have distinct cytokine profiles and differing levels of lymphocytic infiltration which suggests that they are indicative of immune status in the tumor microenvironment. HGPs predict response to bevacizumab and are associated with overall survival (OS) after surgery for liver metastases in colorectal cancer (CRC). In addition, HGPs can change over time in response to therapy. With standard scoring methods being developed, HGPs represent an easily accessible, dynamic biomarker to consider when determining strategies for treatment using anti-VEGF and immunomodulatory drugs.

1. Introduction

Variations in tumor morphology and phenotype reflect genetic and epigenetic alterations which drive tumor growth, differentiation and spread. Tumor heterogeneity is present in tumors of the same type in different patients as well as between cancer cells within the same tumor. This inter- and intra-tumor heterogeneity suggests that effective treatment might vary between individuals and will rely upon tumor characterization across a range of genetic, epigenetic, phenotypic and morphological biomarkers.

Morphological variations between tumors are integrative parameters used by pathologists to guide the treatment of patients. Genetic changes associated with tumor growth and differentiation often result in observable, quantifiable physical changes in the tumor. Thus, the morphology of a tumor can be indicative of its genetic status and, by extension, prognosis and represents an immediate, accessible biomarker. Morphology-based histological grading can indeed provide an insight into the molecular subtype of a patient's tumor for many tumor types, including breast cancer [1].

We and others have shown that primary lung and liver tumors and metastases in lung and liver present with distinct HGPs [2–8] and there is some preliminary evidence that the HGP of the metastases can be predicted by the HGP of the primary tumor [9]. HGPs are yet another reflection of inter-tumor heterogeneity. HGPs are identified by light microscopy in standard H&E-stained tissue sections. In the case of liver metastases, international consensus guidelines for HGP scoring have been established [10]. Scoring according to these guidelines resulted in reproducible assessment of liver metastases' HGPs which were associated with overall survival (OS) after surgery for CRC liver metastases [10].

In this review, we use the well-defined HGPs of liver metastases to describe the key differentiating histopathological characteristics of

Abbreviations: CAF, cancer-associated fibroblast; CRC, colorectal cancer; FAP, fibroblast activation protein; H&E, hematoxylin-and-eosin; HGP, histopathological growth pattern; LSEC, liver sinusoidal endothelial cells; OS, overall survival; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau

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Table 1
Characteristics of the Histological Growth Patterns (HGPs) of liver and lung tumors.

	Angiogenic HGPs	Non-angiogenic HGPs
Nomenclature in liver	Desmoplastic	Replacement
Nomenclature in lung	Pushing	Alveolar, Interstitial & Perivascular Cuffing
Tumor architecture	- Delineated by capsule of desmoplastic stroma - Normal tissue architecture not respected	- No desmoplastic tissue rim. - Normal tissue architecture is respected
Vascularisation	Sprouting angiogenesis	Vessel co-option
Lymphocytic Infiltrate	+ + (in fibrotic capsule)	–
Contact of tumor cells with normal parenchymal cells	–	+ +

HGPs as well as their impact on the biology of these tumors. We will go on to explain the potential usefulness of integrating the assessment of HGPs into clinical management strategies when immunomodulatory treatment is considered as an option.

2. Characteristics of tumor histopathological growth patterns of liver metastases

HGPs are defined according to the specific interface between the tumor and the surrounding normal tissue. Histopathological analyses of tumors in the liver have identified differences in growth patterns and in the extent of endothelial cell proliferation and lymphocytic infiltration [3,5]. A summary of the key characteristics of the two major HGPs is presented in Table 1.

We have examined whether scoring the HGP (as defined in the consensus guidelines) from a single tissue section is as accurate as scoring the HGP from multiple samples from the same metastasis [10]. In 82% of all metastases (41 out of 50 lesions), a complete agreement was found across all samples (paraffin blocks) from the same lesions. For 6 of the remaining 9 lesions, 75–80% of agreement was found. The analytical validity of the consensus guidelines to assess the HGPs was further demonstrated by a study in which a mixed group of pathologists, clinicians and scientists were first trained and then asked to score the HGP of a set of 99 liver metastasis. Good-to-excellent correlations with the gold standard were obtained for the replacement and the desmoplastic growth pattern for the majority of the participants [10]. In addition, intra- and interobserver variability was found to be very low in our study which demonstrated the association of the HGPs with treatment response in patients with colorectal cancer liver metastases ([7], see supplementary data section of the publication). Taken together, these data demonstrate that HGPs can be assessed in a reproducible way by trained investigators.

The distinct topography of the interface between tumor and normal cells suggests that tumor interactions with parenchymal and non-parenchymal cells of the liver are specific to the HGP subtype and reflect differences in the biology of HGPs. In support of this, unpublished data of our team (in collaboration with C. Verhoef of the Dept. of Surgical Oncology, Erasmus MC Rotterdam) demonstrate that 1. even a very limited amount (5% of the interface) of non-angiogenic, replacement growth is associated with a significantly worse outcome, and, 2. that the HGPs are more accurate than the current risk scores to predict survival of patients with resected CRC liver metastases. When traditional clinical risk scores are applied, a significant proportion of clinically low-risk patients experiences rapid recurrence and cancer-related death and, vice versa, high-risk features are present in long-term survivors [11,12]. Both observations underscore the distinct biology of the different HGPs (unpublished data, manuscript submitted).

The extent of vascularization by sprouting angiogenesis is a major differentiator of HGP subtypes of liver metastases. In the replacement HGP, cancer cells exploit the normal tissue architecture by utilizing local connective tissue and blood vessels and replacing the normal epithelial layer. In this HGP, there is direct contact between cancer cells and normal cells as hepatocytes in the liver cell plates are replaced by cancer cells. Tumor vascularization is achieved by a non-angiogenic process termed vessel 'co-option' in which normal sinusoidal blood vessels are hijacked by the tumor (Fig. 1) [3,5,7].

The opposite is true for the other common HGP in the liver, the desmoplastic HGP, in which the tumor does not respect the normal tissue architecture and instead creates its own supporting stroma, in a process called 'desmoplasia'. The cancer cells are separated from liver

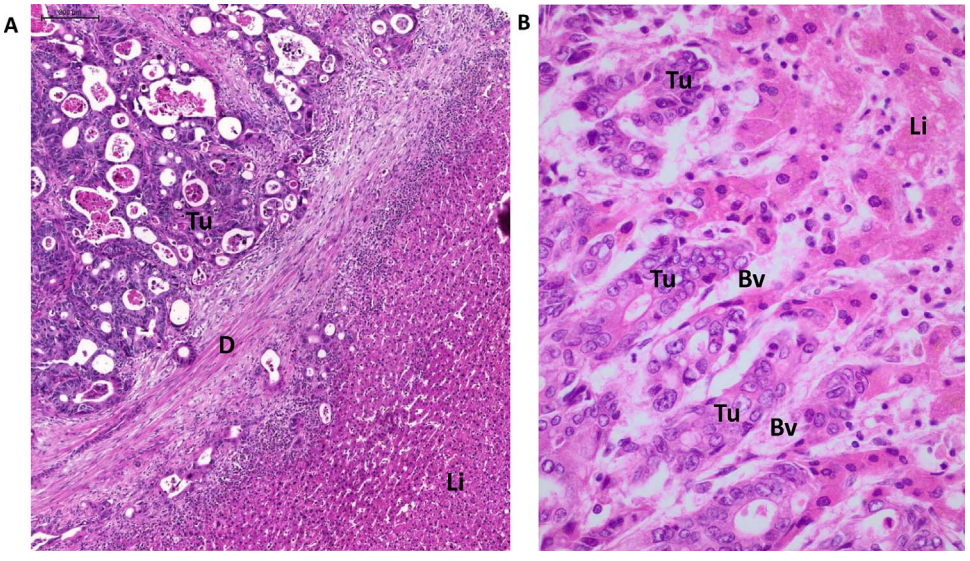

Fig. 1. Histopathological growth patterns of liver metastases.
A. Desmoplastic CRC liver metastasis: tumor (Tu, upper left corner of the image) is separated from the liver tissue (Li, lower right corner) by a rim of desmoplastic tissue (D, central part of the image, magnification ×100).
B. Replacement CRC liver metastasis: cancer cells (Tu, left side of the image) are in contact with hepatocytes (Li, right side of the image) at the tumor-liver interface. There is no desmoplastic tissue and the tumor mimics the liver tissue by replacing the hepatocytes and co-opting the sinusoidal blood vessels (BV) (magnification ×400).