The Mosaic Within: Unraveling Intratumor Heterogeneity in Colorectal Cancer

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INTRODUCTION

Colorectal cancer (CRC) remains a challenging disease to treat and has a high mortality rate, despite a slight decrease in its incidence in developed countries due to preventive and screening programs. Efforts based on whole tumor profiling demonstrated that the CRC molecular subtypes were associated with specific tumor morphological patterns representing tumor subregions. Our hypothesis is that the molecular characteristics of the entire tumor are influenced by its morphological heterogeneity, which in turn can have a substantial effect on the existing molecular predictors. We examined six distinct morphological patterns, or morphotypes, which included complex tubular, desmoplastic, mucinous, papillary, serrated, and solid/trabecular structures to gain a deeper understanding of the relationships between gene expression and tumor morphology.

BACKGROUND

Colorectal cancer (CRC) is a highly heterogeneous disease that poses challenges for proper treatment selection and developing targeted therapies. Differences both between and within tumors of the same cancer type make it difficult to categorize and classify CRC. The current TNM staging system is the gold standard for diagnosis and prognosis (1). However, molecular taxonomies categorizing tumors into subgroups sharing common molecular traits have emerged, with consensus molecular subtypes (CMS) representing their common denominator (2).

When analyzing gene expression in tissue sections, the most abundant cell types tend to drive the results while less abundant ones are overlooked. This is why selecting representative regions enriched in tumoral cells is necessary for profiling solid tumors. While newer technologies allow for finer cell selection, they are not yet widely used in clinical practice (3,4).

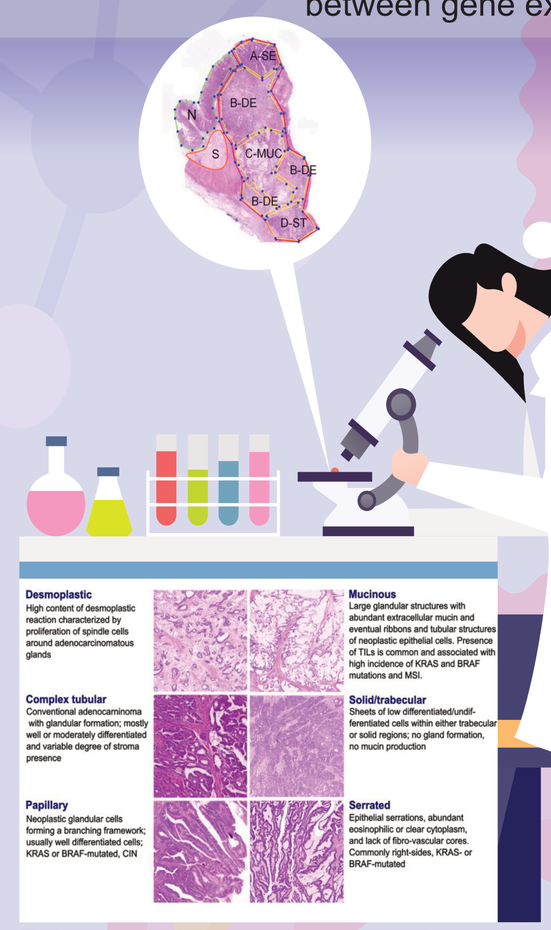


Fig 1. The six CRC morphological patterns of interest (morphotypes). UP: example of an original annotation used for macrodissection and RNA extraction. DOWN: examples of morphotypes – complex tubular (CT), desmoplastic (DE), mucinous (MU), papillary (PP), serrated (SE), and solid/trabecular (TB).

The importance of a morphological perspective on molecular classification has been recognized, with six morphological patterns identified as strongly associated with molecular subtypes. Interestingly, a data-driven image-based classifier resulted in selecting similar morphological patterns.

METHODS AND SAMPLES

111 samples of colon cancers were examined. Morphological regions (fig.1) of interest were marked in scanned whole slide images for RNA extraction. Morphological regions of interest, representing complex tubular (CT), desmoplastic (DE), mucinous (MU), papillary (PP), serrated (SE) and solid/trabecular (TB) morphologies (some cases had several morphotypes profiled: Fig 2.) were digitally marked in scanned whole slide images (at 20x magnification) and macrodissected for RNA extraction.

Additionally, from several slides, tumor-adjacent normal (NR) and tumor-associated stroma (ST). For n=28 cases, whole-tumor regions were macrodissected from the histology section immediately adjacent to the section used for morphological regions. RNA samples were hybridized on Clariom™ D microarrays and data preprocessed using standard Bioconductor packages.

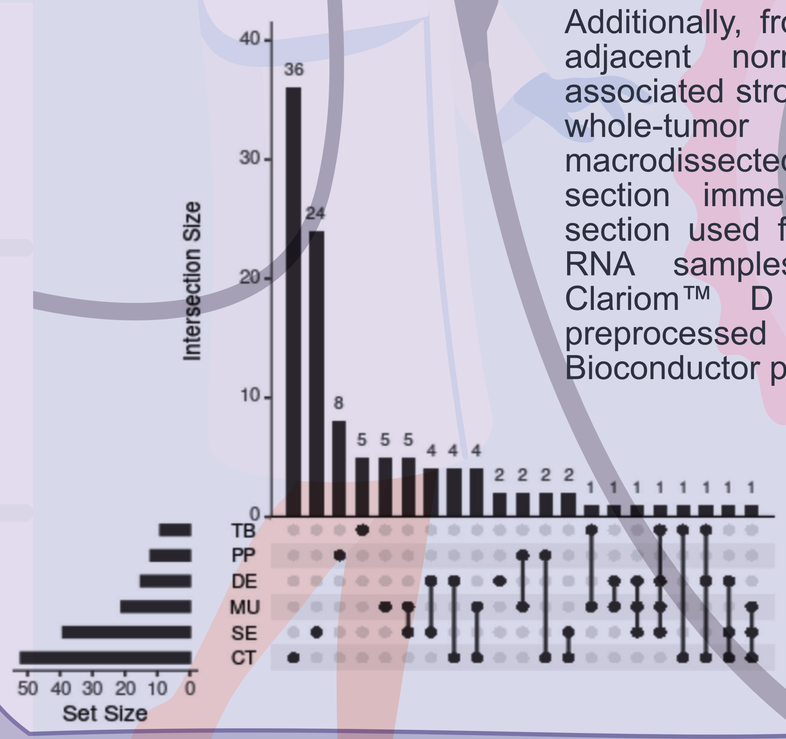
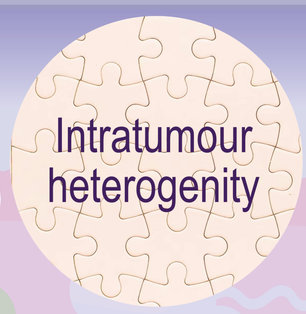


Fig 2. Morphotype distribution per case (unique tumor) and intersections (some cases had several morphotypes profiled)



RESULTS AND DISCUSSION

Biological Process	Pathways
Inflammation	Complement (mediates immune cell recruitment), IL-2, IL-6, TGF-β, Inflammatory response
Neoangiogenesis	IL6/JAK/STAT3, hypoxia, TNF-α., KRAS
Metastatic potential	EMT, coagulation ,TGF-β, NFkB, and Notch, apical junction
Development	Notch, hedgehog, Wnt
Catabolism	Peroxisome, fatty acid metabolism, oxidative phosphorylation, glycolysis, protein secretion
Apoptoses	TNF-α., allograft rejection,
Cell cycle disruption	unfolded protein response, Mtorc1, G2M checkpoint, Myc targets
Proliferation	P53, Mtorc1, Myc, G2M checkpoint, mitotic spindle, notch signaling, protein secretion
Oxidative stress	Fatty acid, oxidative phosphorylation, glycolysis, UV response

Table 1. This table shows the possible effect pathways associated with different biological processes

The results show a whole landscape of changes at gene and pathway levels, with morphotypes residing on a continuum space of molecular descriptors.

The analysis of molecular pathways in CT and TB morphologies showed that both had activated pathways involved in proliferation processes, while TB also shared features with MU and DE morphologies, including active TGF-β signaling, apoptosis, and an active immune system response (fig.3, table 1.). SE and PP morphologies are considered indicative of a distinct oncologic pathway known as the "serrated pathway," as they share similar features such as well to moderately differentiated, low stromal content, and preserved crypt structure. Further molecular analysis revealed that SE and PP exhibited similar activation of hallmark pathways, such as down-regulation of EMT, IL2/STAT5, IL6/STAT3, and KRAS signaling, while up-regulating MYC targets. In addition, SE and PP displayed unique hallmark pathways, such as androgen response, heme metabolism, and IL6/STAT3, which were silenced and statistically significant.

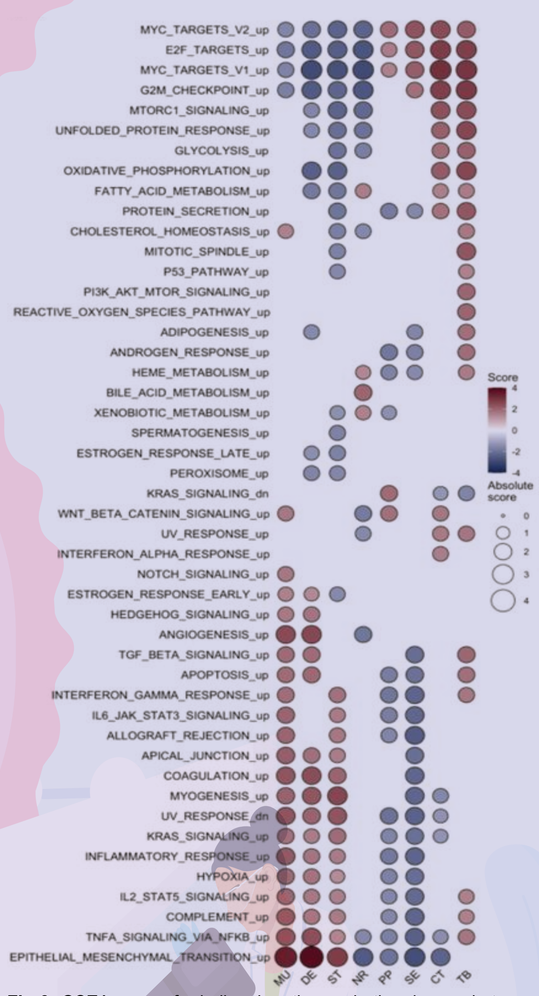


Fig 3. GSEA scores for hallmark pathways in the six morphotypes and two non-tumoral regions. Only pathways with statistically significant scores are shown.

CONCLUSION

- These findings shed light on the intratumoral heterogeneity of colorectal cancer and may have important implications for the development of personalized treatment strategies.
- Tumors require multiple sampling locations to obtain a more accurateesult.

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