IMPROVE CANCER TREATMENT:

Intra-tumoral metabolic plasticity effect of colorectal cancer tumors on immunotherapies dynamics

SUMMARY

Similar to genetic heterogeneity, the metabolic phenotypes of cancers are highly heterogeneous and this heterogeneity results from diverse signals in the tumor microenvironment. Hence, overcoming metabolic plasticity is an important goal of modern cancer immunotherapeutics. Here, we described an integrated protocol to discern the metabolic and protein changes including accumulation of metabolites and their relationship with the tumor microenvironment (TME) in colorectal tumors (CRCs) during the course of cancer progression. Using Mass Spectrometry Imaging and GeoMx platforms, this work elucidates the interactions between metabolic pathways and function of immune cells and provides novel rationales for designing the next-generation cancer metabolism drugs.

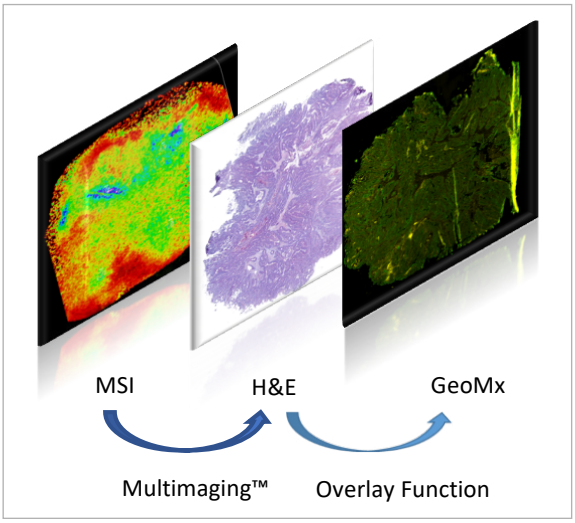
APPROACH

When diagnosed at early stages, colon cancer is associated with good overall survival rates, but these numbers rapidly decline in stage III or stage IV metastatic disease. The high heterogeneity of colon cancer contributes to differences in therapy response and, consequently, survival.

In an era of individualized treatment, scientists are striving to map colon cancer heterogeneity and to determine which factors can function as better prognostic and predictive markers for this disease. Immunotherapies offer a potentially effective treatment to these patients; however, the therapeutic efficacy is limited to a small number of population. Thus, it is urgent to look for alternative combinatory therapies by analyzing interactions between metabolism and immune phenotypes. We thus aim to establish potential therapeutic targets for new metabolism-based anticancer drugs.

In the present study, we characterized intra-tumoral metabolic phenotypic heterogeneity in CRC and how these metabolic changes impeded the function of immune cells. We used a spatial multimodal approach previously described [1] (Fig.1) which allows in-situ multi-modal molecular architecture analysis and thus captured CRC phenotypic heterogeneity. By analyzing interactions between metabolism and immune tumor microenvironment (TME), we aimed to establish opportunities for next-generation immunotherapies that could be further improved using combination approaches that simultaneously inhibit metabolic pathways.

FIGURE 1. Spatial multi-omics workflow



INTRA-TUMORAL METABOLIC PHENOTYPES CHARACTERIZATION

Our patient cohort consisted of five (5) resected frozen CRC tumors at different pathological stages (pTs) from pT1 to pT3. We analyzed lactate and pyruvate distributions in these patients by QMSI (Fig.2).

Regardless of cancer progression, the lactate and pyruvate mostly accumulated in the different tumor regions and showed an heterogenous distribution along the tumor content, thus creating two distinct metabolic phenotypes within the tumor (Fig.3).

The ROIs for immune markers analysis were delineated based on the detected metabolic phenotypes. The drawn ROIs for further deep-molecular analysis corresponded to the tumor regions with different lactate and pyruvate accumulation (Fig.3).

Phenotypes:

- High pyruvate/low lactate
- Low pyruvate/high lactate

FIGURE 2. Distribution of lactate and pyruvate in CRC tumor

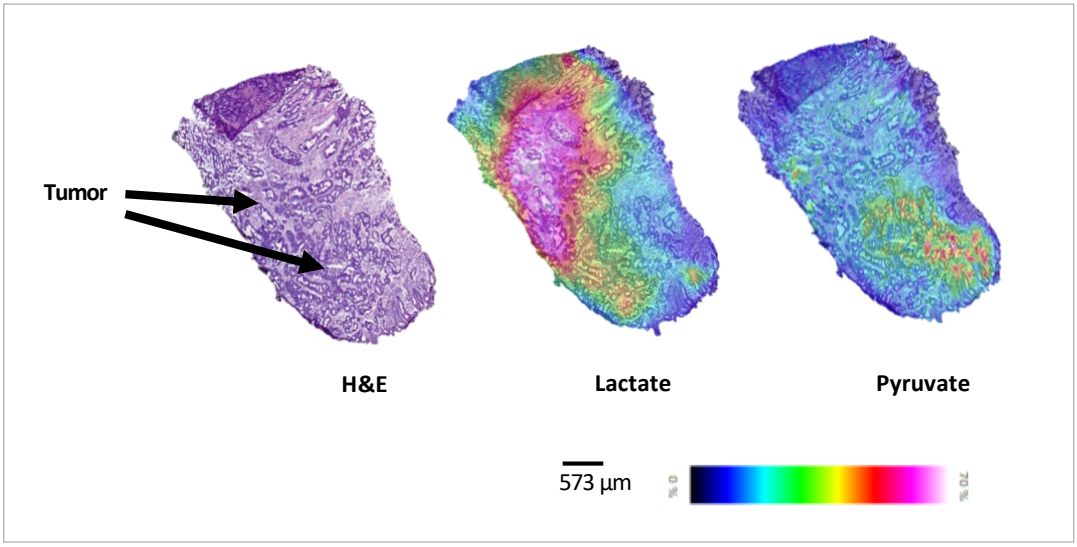
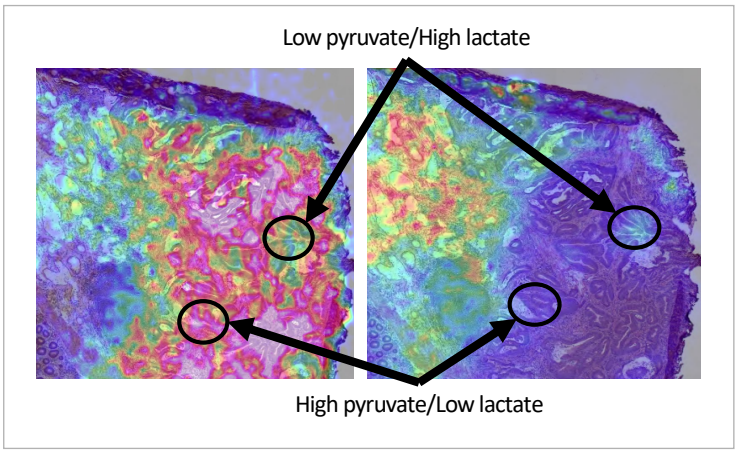


FIGURE 3. ROIs based on the differential metabolite distribution

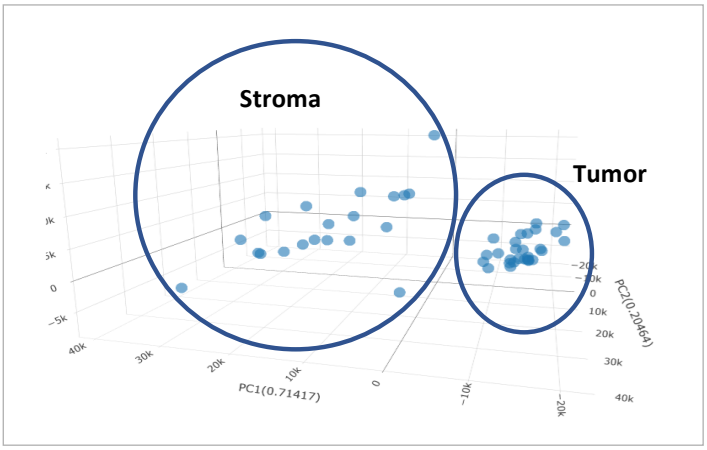


DATA DRIVEN SEPARATION OF HISTOLOGICAL REGION

Six (6) regions of interest (ROIs) were selected per sample according to metabolite accumulation and further segmented into stroma and tumor content in agreement with tumor panCK+ and stroma leucocyte CD45+ fluorescent staining.

Twenty-six (26) immune parameters from Immune Cell Profiling and the Immune Activation panels from Nanostring were simultaneously quantified in each compartment. The patient-normalized residuals were utilized to generate plots following dimensionality reduction. Principal component analysis (PCA) analysis on the dataset revealed that samples are separated in two groups (stroma and tumor) along PC1 and their difference is significant (Fig.4 & not shown). This information indicated that the quality of the automatic segmentation stroma/tumor was confirmed by deep-protein profiling.

FIGURE 4. PCA of all sample compartments



DIFFERENTIAL EXPRESSION OF ICOS AND CD127

Unsupervised hierarchical cluster was performed on both stroma components and the 26 targets. The stroma ROIs clustered predominantly by metabolic phenotypes (Fig.5).

Targets fell into 2 predominant clusters based on immune marker functions with immune checkpoint and inducible T cell costimulators being predominately represented in stromal ROIs Low pyruvate/ High Lactate. This indicated the role of lactate in modulating checkpoint expression in immune cell types and reflected a notable change in our understanding of the modulation of local immune responses to improve antitumor responses to immunotherapy.

To quantify differential protein expression in the two metabolic phenotypes, a linear mixed effects model was used to calculate fold-change (FC) and p-values for each protein (not shown). Two markers were statistically differentiated (Fig. 6). Of protein with significant higher expression in low pyruvate/high lactate phenotype, ICOS was associated with a successful humoral immune response and was upregulated following T-cell specific activation (HOT phenotype). In contrary, CD127 was upregulated in the high pyruvate/low lactate phenotype and was associated to a “memory” and exhausted T cell phenotype characterized by low proliferation in response to antigen stimulation (COLD phenotype).

The distinctive metabolic requirements of immune cell function provide a unique opportunity to differentiate effector from regulatory functions. Similarly, immune cells can be metabolically reprogrammed to modulate their effector functions and memory capability. These findings suggest that the metabolic crosstalk occurring in the TME may represent a unique opportunity for drugs that were originally developed for targeting cancer metabolism by providing the spatially resolved guidance to tailor treatments to the changing cancer cell trait distribution and coped with the dynamic heterogeneity (Fig.7).

FIGURE 5. Proteins clusters according to metabolic phenotypes

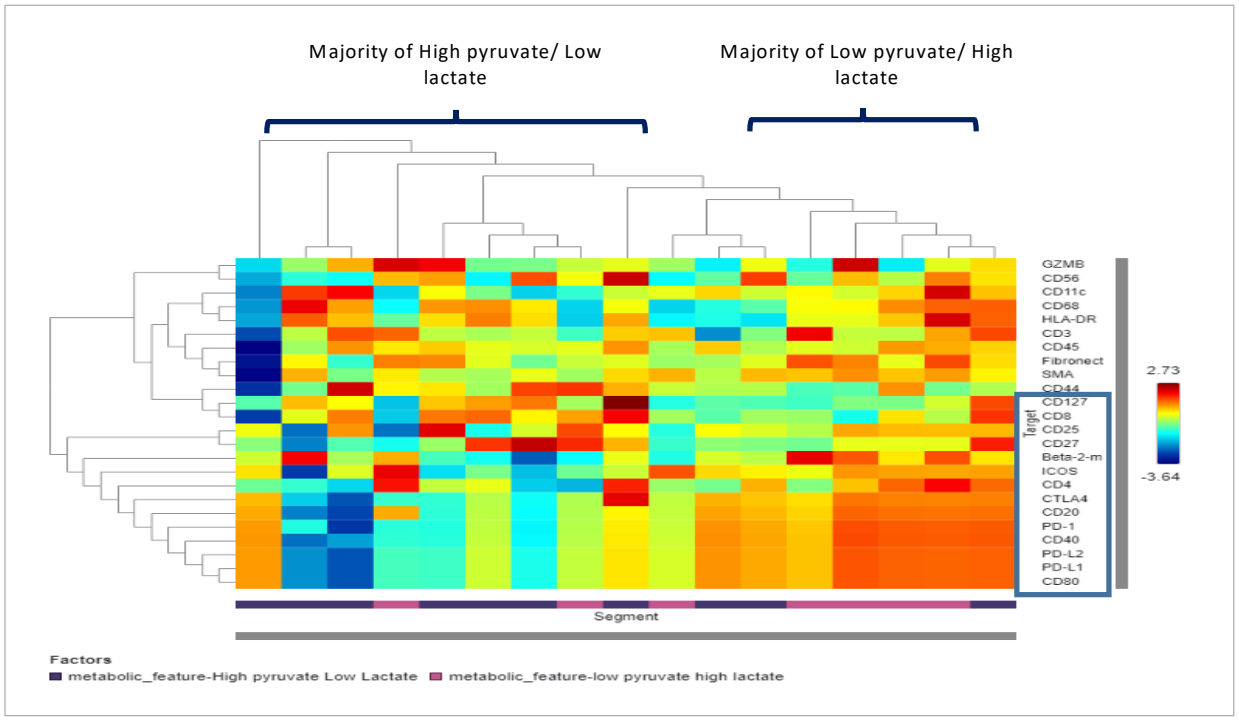


FIGURE 6. Differential expression of the two statistically significant proteins

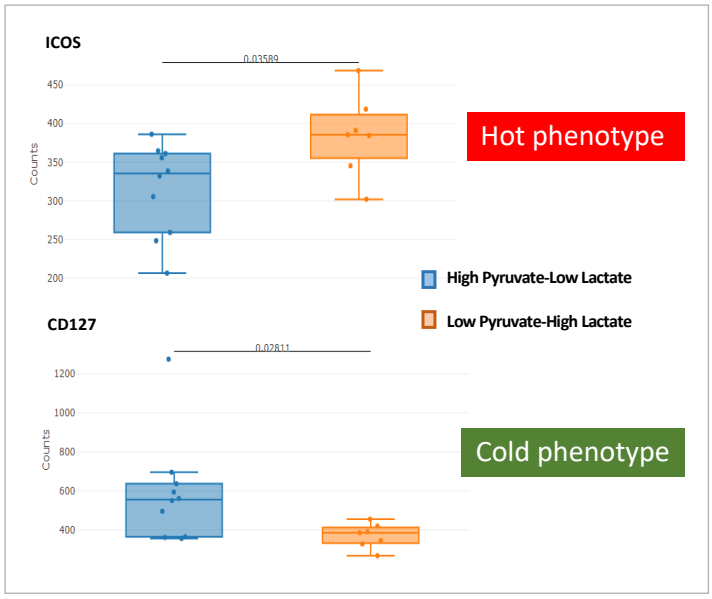
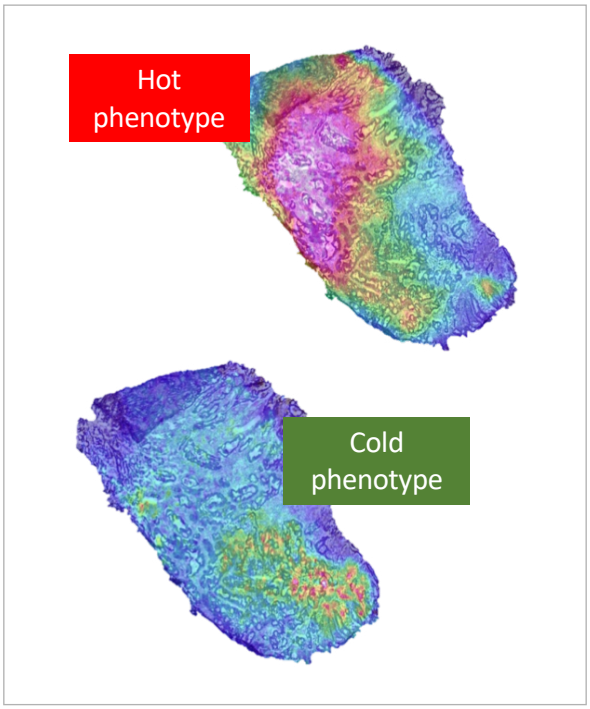


FIGURE 7. Intra-tumor metabolic heterogeneity and its involvement on immunotherapies strategies



CONCLUSION

This work has necessited the optimization of the alignment of the images from different modalities (molecular images and immunofluorescent images) as well as the development of a methodology to detect the lactate using MSI.

The combined workflow of QMSI and GeoMx DSP allows for a better understanding of both direct and indirect modulation of anti-tumor immunity though a better understanding of the tumor immune cell interface. One of the many unanswered questions pertains to the feasibility of targeting tumor cell metabolism in order to enhance immunotherapy response. In this paper, we demonstrated the role of lactate in the microenvironment of CRC tumors as a major driver for immune activation. On the contrary, the presence of pyruvate contributed to immune evasion and is intended at enabling combinatory therapeutic intervention aimed at resetting the bioavailability of this metabolite to correct the dysregulated immunological state. The mechanism elucidated in this work for the control of the immune response by metabolites, locally, in the diseased tissues of cancer have tremendous therapeutic implications. Metabolic interventions to trigger beneficial cytotoxic, inflammatory responses participates in the development of the next generation of immune-based therapeutics that can improve the intra-tumoral metabolic landscape.

[1] Unraveling disease complexity with integrated spatial systems biology approach

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