ImaBiotech
Revealing Biological Secrets

WHITE PAPER #2

SPATIALLY-RESOLVED TUMOR GENE EXPRESSION ANALYSIS IDENTIFIES SERPINE1 AS A MOLECULAR SWITCH OF CRC PROGRESSION

SUMMARY

During tumor progression, cancer cells come into contact with new cell types in the microenvironment, but it is unclear how tumor cells adapt to new environments. Here, we optimized and integrated spatial transcriptomics with laser microdissection (LMD) coupled to gene expression to discern genes that are differentially expressed in tumor cells during the course of cancer progression. The development of optimized protocols allows the possibility to identify region of interest from staining or immunostaining and micro-dissect enough material for gene expression and analysis. Using CRC tumors at different progression stages, we identified a key driver of tumor metastases. Our results demonstrated the power of spatial transcriptomic approaches in uncovering mechanisms that allow tumors to invade into the microenvironment and help discover biomarkers as potential therapeutic targets.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in the world. CRC primary tumors often metastasize to the liver, which accounts for most of CRC related death. The molecular mechanism of tumor metastasis remains poorly understood. Prevention of tumor metastasis is dependent upon the better understanding of the molecular mechanism governing this complicate process. However, the extensive interactions among tumor cells and tumor microenvironment (TME) have complicated the efforts in dissecting the metastatic process and there is no convincing evidence to date suggesting that the metastatic process links to specific genetic alterations in CRC tumors. The understanding of each ETM components that contribute to the tumor metastases remains elusive and it has been a significant challenge to deconvolute the transcriptional molecular networks to dissect the tumors at different progression stages.

In the present study, we genomically characterized CRC tumors using samples with different pathological stages. We used microdissection of the tumor cells in each clinical sample to allow the identification of tumor specific genes with biological relevance in disease progression (Fig 1). We then reveals key drivers of cancer progression including SERPINE1 and discussed how this gene may provide insight into the future design of anti-invasive therapies for CRC.

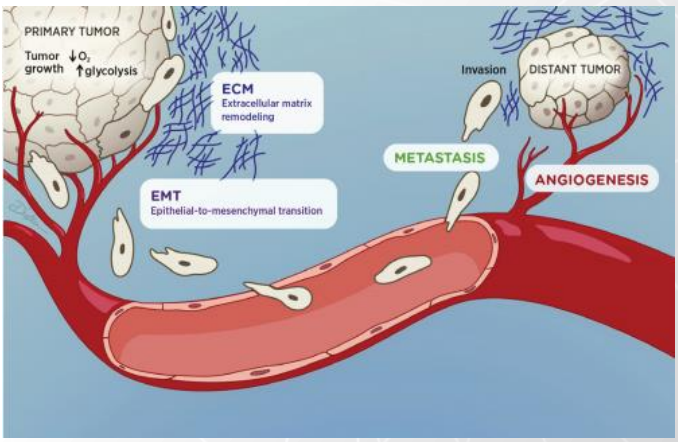

Fig 1. Features of cancer progression

TUMOR SPECIFIC GENE ANALYSIS

Our patient cohort consisted of twelve (12) resected frozen CRC tumors with different pathological stages (pTs) from pT1 to pT4, 3 patient samples per pT were used. We then analyzed these patients using laser capture microdissection that was coupled to nCounter for mRNA expression analysis using the NanoString PanCancer Progression Panel (Fig. 2).

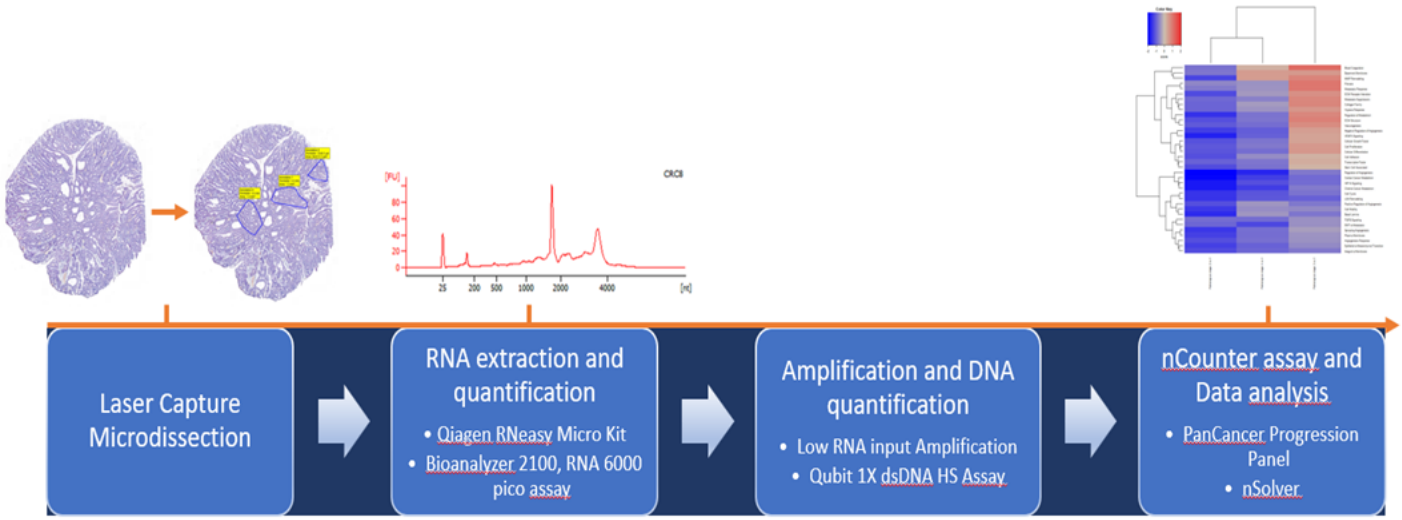

Fig 2. Workflow: tumor-specific transcriptomic analysis of CRC

TRANSCRIPTIONAL COMPARISON

For the analysis, Pt1 & pT2 samples were pulled together and described as **Early stage without metastases**. The same was performed with the Pt3 & Pt4 samples that were described as **Late stage with metastases** in the analysis. The distribution of the fold changes and p-values of the differential gene expression in Late stage versus Early stage CRC samples was shown in Fig. 3 as volcano plot. By comparing this differential transcriptomics profile between the Late stage versus the Early stage CRCs, we identified at least 2 up-regulated genes in the late stages CRCs with absolute fold change ≥2 and p<0.01.

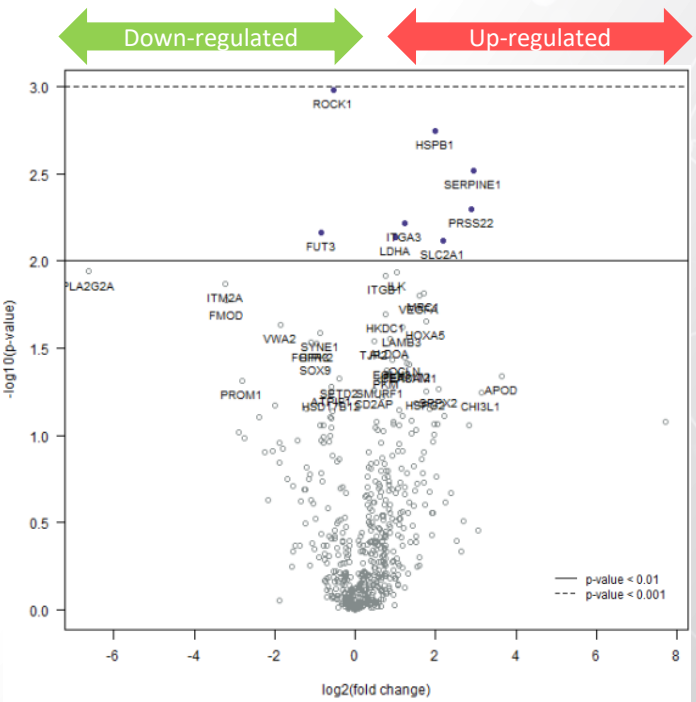

Fig 3. Volcano plots for differential gene expression in Late stage versus Early stage tumors

Analysis of the differential gene expression with the hallmark gene sets included in the panCancer progression panel showed that many functional pathways such as Cell proliferation, Angiogenesis and Epithelial to Mesenchymal Transition (EMT) are upregulated in CRCs with metastases (Fig.4). In contrast TGFB signaling was downregulated in late stage CRCs compared to early stage CRCs without metastases.

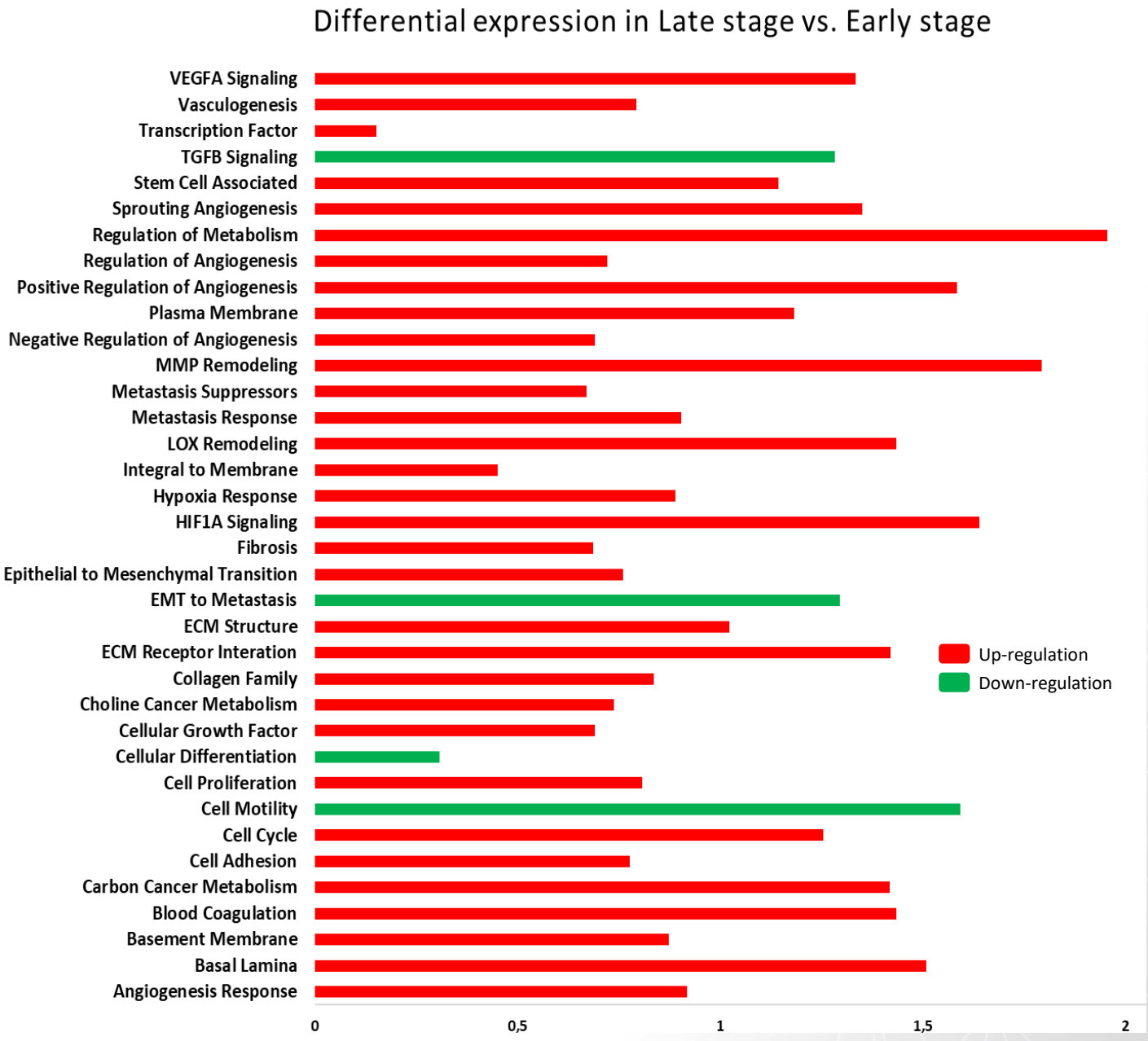

Fig 3. Summary plot for the representation of differential gene expression for Late vs. Early stage CRC tumors of functional pathways in the PanCancer Proliferation panel

EMT AS THE DRIVER OF METASTASES CASCADE

From each upregulated identified functional pathways, the gene set involving EMT was the most signifcantly upregulated pathway in Late stage with metastases. Specifically, SERPINE1 was statistically differentiated indicating a major role of this gene in CRC disease progression to metastases.

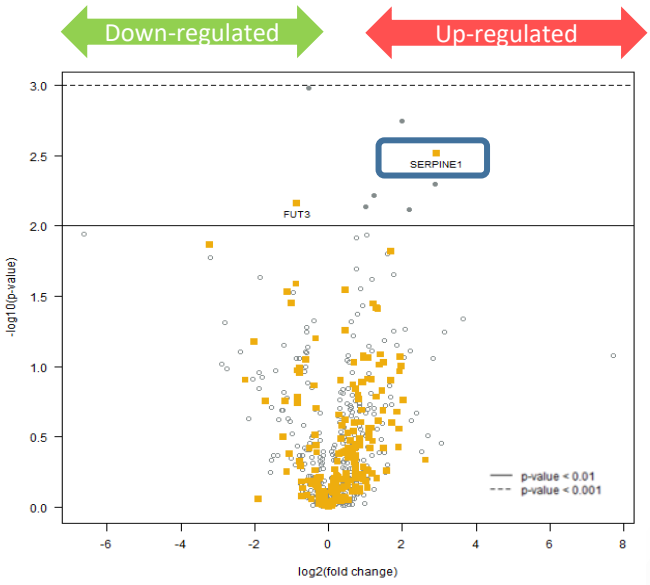

Fig 5. Volcano plot for differential gene expression in ETM pathway (orange dots) in Late stage versus Early stage tumors

CONCLUSION

This work required the optimization of the extraction of good quality RNA from few thousand cells collected with Laser Microdissection in order to get an accurate gene expression reading. A customized protocol was developed for RNA amplification and transcriptome analysis of each micro-dissected tumor pieces.

The understanding of the genetic trajectories primary to metastases of CRC in patients is crucial to identify the molecular events pushing the disease towards an increasing malignant phenotype. This information is important to plan innovative therapeutic strategies aimed to reverse the progression and drive the at-risk patients in clinical trials for new therapies development. In the present study using our targeted spatial gene expression workflow, we compared the expression levels of gene expression of primary tumors from CRC patients with and without metastases, to identify metastases-initiating mRNA target regulation. Statistical analysis revealed that SERPINE1 within the EMT functional pathway was strongly related to cancer progression and metastases with statistically significant difference. Therefore, the results of the present study suggest that the Plasminogen Activator Inhibitor 1 (PA1) or SERPINE1 may affect the prognosis of patients with CRC. Current findings support the hypothesis that targeting of PA1 function (by small molecule drugs) and/or gene expression (by histone deacetylase inhibitors) may constitute a clinically-relevant molecular approach to the therapy of CRC associated with increased PAI-1 levels.

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