Application of innovative cell type deconvolution approach to study colorectal tumor metastasis

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ABSTRACT

- Colorectal Cancer (**CRC**) annual US incidence of ~150,000 new cases. Five year mortality rate over 35% percent → >50,000 deaths per year. Metastasis (METS) predictor of death/recurrence
- Thirty-six pT3 patients at DHMC and ~200 pT3 patients TCGA
- HiTIMED Cell Type Proportion demonstrates metastasis related cell types, cell type independent EWAS analyses
- Highlights angiogenic component in mismatch repair proficient tumors and T-cell suppression in deficient tumors

INTRODUCTION

- **DNA Methylation:**
  - Reversible somatic alteration, methyl group to cytosine-guanine dinucleotide (CpG); can silence tumor suppressors
- **Phenotype Variation:**
  - Differences in sex, age, tumor location, etc.
  - Lymph node metastasis, Distant metastasis, both, or neither
  - Mismatch repair deficiency (e.g., Lynch Syndrome)
- **Goal:** Develop statistical analysis pipeline to analyze CRC data to identify cell-types/CpGs related to prognosis/treatment
- **Experimental Design:**
  - Compare two cohorts and provide statistical testing to identify targetable molecular alterations
- **Tasks:**
  - Statistical comparison of cell type proportions
  - Epigenome-Wide Association Study (EWAS) on metastasis related differentially methylated CpGs, adjusting for cell type
  - Interpretation of pathways, cell-type specific methylation

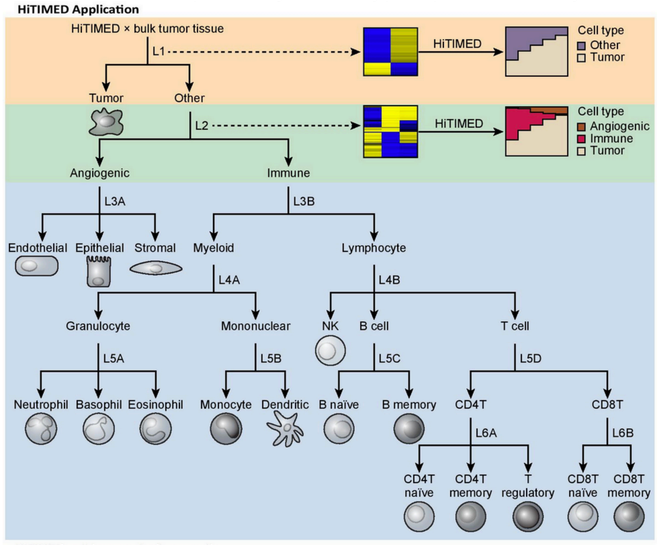


Figure 1: HiTIMED general workflow

METHODS

- **Preprocessing: Assisted by DAC and Salas labs at Dartmouth**
  - pOOBAH and Dye Bias correction, SeSAMe
  - Beta-values: proportion methylated alleles across cells
- **HiTIMED**: **Hierarchical Tumor Immune Microenvironment Epigenetic Deconvolution**
  - Estimate proportions of up to 17 cell types from DNAm
  - Validated against older models for improved accuracy
  - Characterizes phenotypical differences within tumor immune microenvironment (tumor, angiogenic, immune)
- **CellDMC:**
  - Identifies Differentially Methylated Cell Types (DMCTs)
  - Studies differential methylation conditional on cell type
- **EWAS:**
  - Compares epigenetic differences between observational groups such as differential methylation
  - Assesses CpGs individually for differential methylation but often confounded by differences in cell types
  - Stratified analysis by MMR deficiency and compared pT3 patients without METS to METS (LN and Distant separately compared)

RESULTS

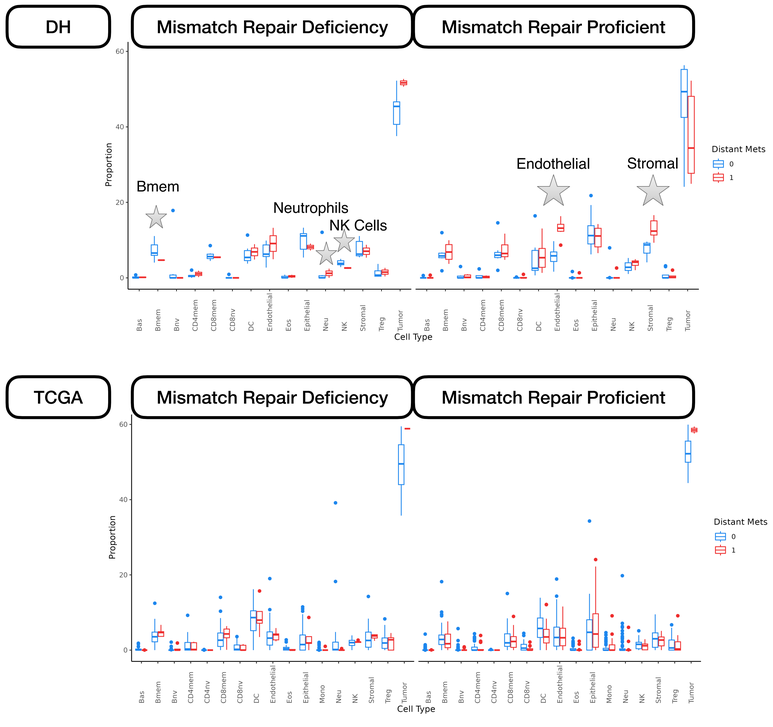
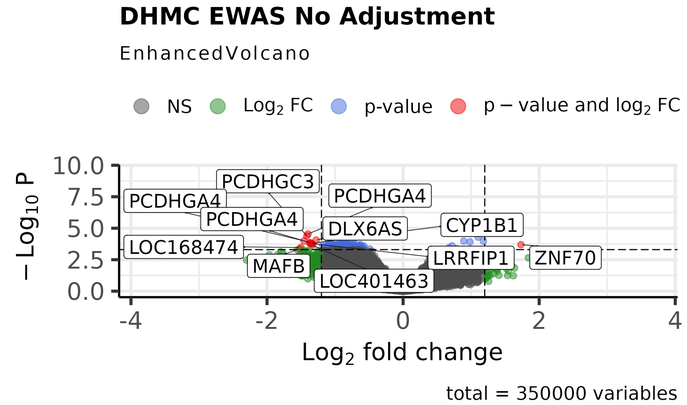


Figure 2: Cell Type proportions for each Cohort, segmented by MSI

RESULTS



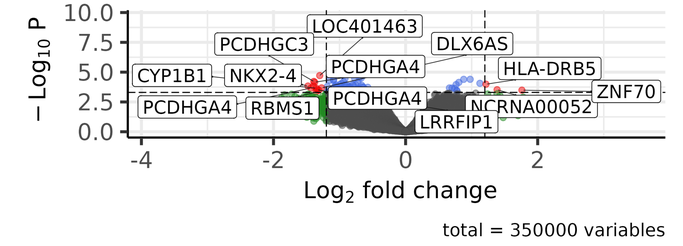


Figure 3: DHMC differentially methylated CpGs associated with METS

CONCLUSION

- **Future Directions:**
  - Filter out genes using GoRegion
  - Further testing of specific genomic regions: e.g., promoters, enhancers, etc.; repeat elements; copy number burden, etc.
  - Suggestions welcome!
- **Limitations:**
  - Cannot infer whether metastasis will develop as metastasis identified at time of diagnosis
  - Expanding cohort for DH with COBRE funding
- **Data and Code Availability:**
  - Link to deconvolution: https://github.com/SalasLab/HiTIMED
- **Funding:**
  - JL is supported by NIH P20GM104416 and P20GM130454

Acknowledgements:
Christensen and Salas Labs, Genomics Core and PSR

References: Available using QR code:

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