Exploring the Metastasis Process in Prostate Cancer using Interaction Networks

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Introduction

Prostate cancer (PC) remains to be one of the leading causes of cancer death in North American men[1]. Metastatic events indicate the advanced stage of cancer and poor patient survival. Deeper insights in the pathways that cause a primary tumor to metastasize into distant sites are critical for development of new biomarkers and therapies and are clinically significant towards improving patient outcomes.

Conventional strategies use a single-gene approach where aberrant genes are considered independently from other genes. However, understanding the function of genes in a complex disease like PC requires investigation of genes within the context of their interaction networks (collectively called interactome)[2]. This idea is based on the observation that disease condition is characterized by aberrant networks of genes (subnetworks), which interact at the protein level or at the protein-DNA level.

OptDis algorithm[3] has been reported to efficiently identify subnetworks with the best possible discrimination between tumor classes and provide better insights into the biological mechanisms. Our project aims to investigate differences in the transcriptome between primary tumors and metastatic tumors and biological pathways/function that could shed more light in the biology behind the metastasis process using OptDis.

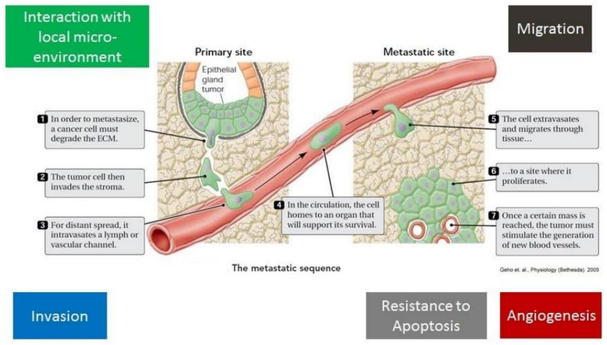


Figure 1.: The Metastatic Process

Dataset

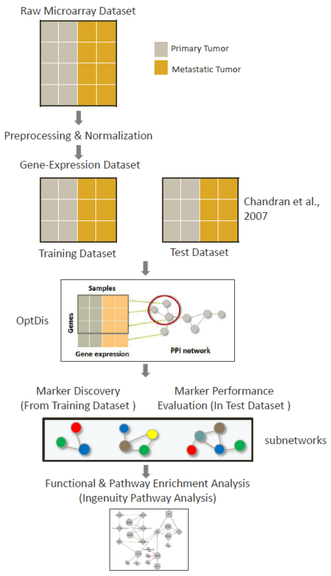
We used a publically available gene-expression prostate cancer dataset representing primary and metastasis tumors from various metastatic sites – Grasso et *al*, 2012[4] (GSE35988). All the metastatic tumor samples are from patients with castrate resistant prostate cancer (CRPC), an aggressive type of PC which is hormone therapy or chemotherapy resistant. Changes in expression identified using this study design could be dependent upon differences between prostate adenocarcinoma and CRPC or between primary PC and metastatic PC.

Table 1. : Description of the dataset

Platform	Disease State	Number of Samples	Remarks
Agilent Whole Human Genome Microarray	Benign Normal	28	
	Primary Prostate Tumors	59	
	Metastatic Tumors	35	All Metastatic tumors are CRPCs and are derived from various metastatic sites

Methods

Figure 2.: Workflow of the Project.



Results

A. Exploratory Analysis

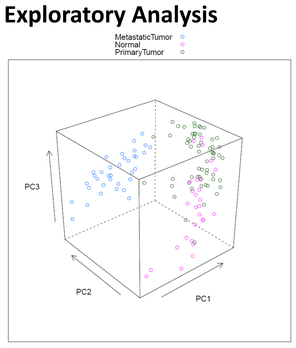


Figure 3.: Plot of sample types with respect to the first three principal components.

B. Classification Performance

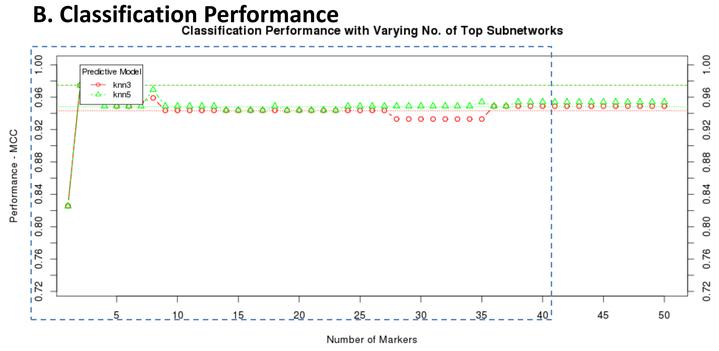


Figure 4.: Classification performance of markers derived from Grasso cohort in Chandran cohort.

C. Method Comparison

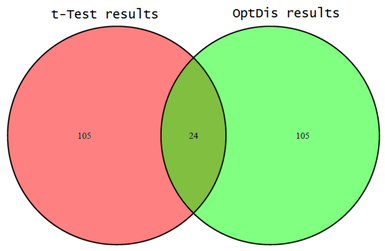


Figure 5.: Venn diagram of genes discovered from t-test and OptDis.

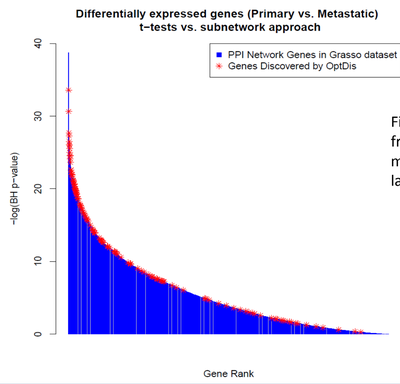


Figure 6.: BH p-values of genes from t-test (primary vs. metastasis) with OptDis genes labelled.

Results

D. Sub-Network Analysis

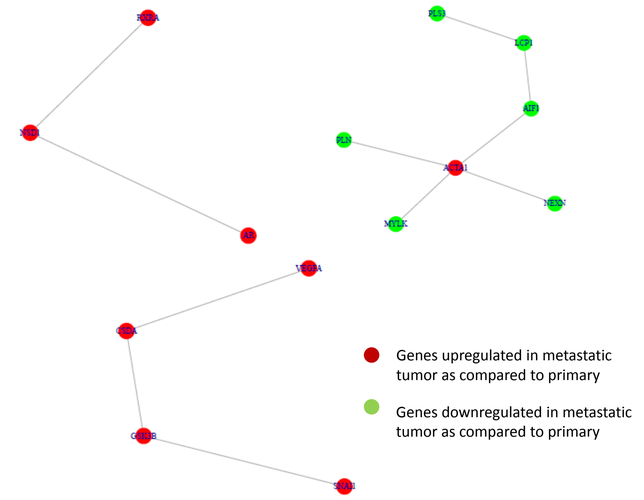


Figure 7.: Three examples of sub-networks discovered from OptDis (Out of Top-40 sub-networks).

CRPC Grasso: Primary –> Metastasis, SubNetworks 1 to 40

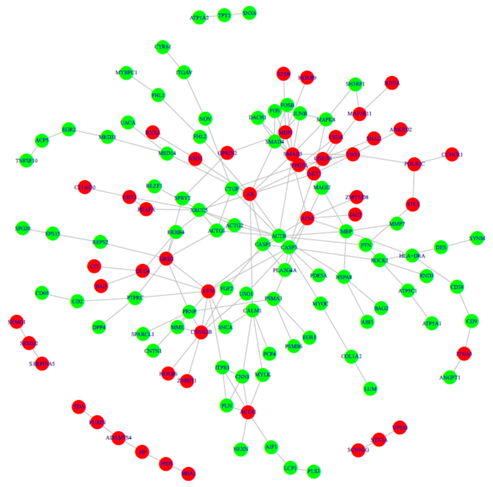


Figure 8.: Genes from the top 40 sub-networks discovered using OptDis.

E. Pathway Enrichment

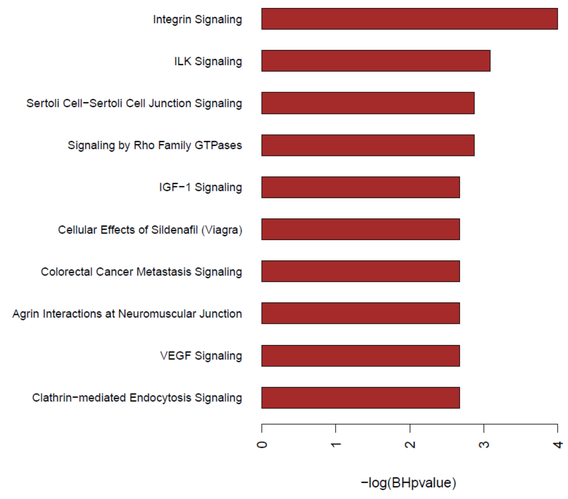


Figure 9.: Negative log BH p-values of top 10 pathways (out of 108 significant pathways with BH p-value <0.1) from IPA.

Integrin Signaling

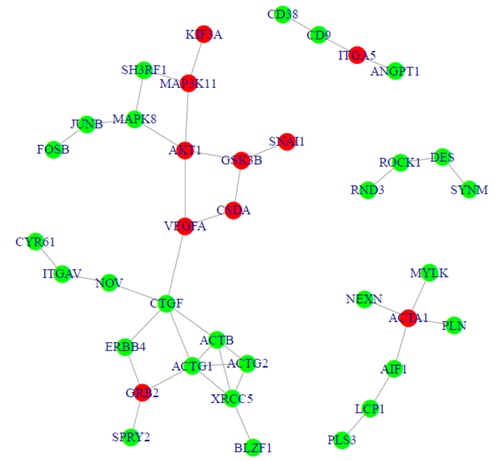


Figure 10.: Network showing the genes enriched in the Integrin Signaling pathway.

Discussion

Among the subnetwork markers discovered by OptDis that discriminate between the primary and castration-resistant metastatic tumors, we chose the top 40 subnetworks to examine pathways underlying the metastasis process. Genes within these subnetworks include genes with previous links to prostate cancer metastasis, such as the growth factor VEGFA, CTGF, and genes without previous associations, such as the hemoglobin chain HBA2.

The 128 genes from OptDis showed significant enrichment in 108 pathways using a cutoff of BH p-value < 0.1 in IPA. In comparison, using the top 128 differentially expressed genes found using Student's t-test yielded no significantly enriched pathways.

The most enriched pathways in analysis of OptDis results using the IPA tool have known links to metastasis and CRPC. Integrin and integrin-linked kinase (ILK) signalling pathways were most highly enriched. Integrins are transmembrane glycoproteins that mediate binding to components of the extracellular matrix (ECM), and influence signalling into and out of cells by activating kinases of the Ras/Rho GTPase pathways, which were also highly enriched in this analysis. The ability of primary tumor cells to invade the stroma before entering the bloodstream, and to establish and grow in secondary sites in metastasis, is facilitated by integrins and ILK signalling[6].

Conclusions

- 108 pathways were enriched in OptDis results, indicating that the sub-network approach could provide more insight on the biological mechanisms than traditional statistical methods.
- Genes and pathways identified with OptDis frequently had previous links to metastasis and CRPC in the literature, supporting the validity of this approach.
- The genes and pathways identified without previous links to metastatic CRPC present candidates for future study.

References

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