Genomics Core – what we can do for your success

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Abstract

The genomics core facility at the Vancouver Prostate Centre offers genomics expertise to academic and clinical researchers to study genome structure and dynamics using high-throughput sequencing technology and microarrays. In addition to laboratory services that include DNA/RNA sample preparation, quality control, sequencing and microarray assays, the facility offers expertise in bioinformatics, biostatistics and computer science to process, analyze, integrate, and visualize complex data. Our facility manages research projects from experimental design to interpretation of data, as well as supporting grant applications and publications.

Expertise – Selected Contributions

Noteworthy publications

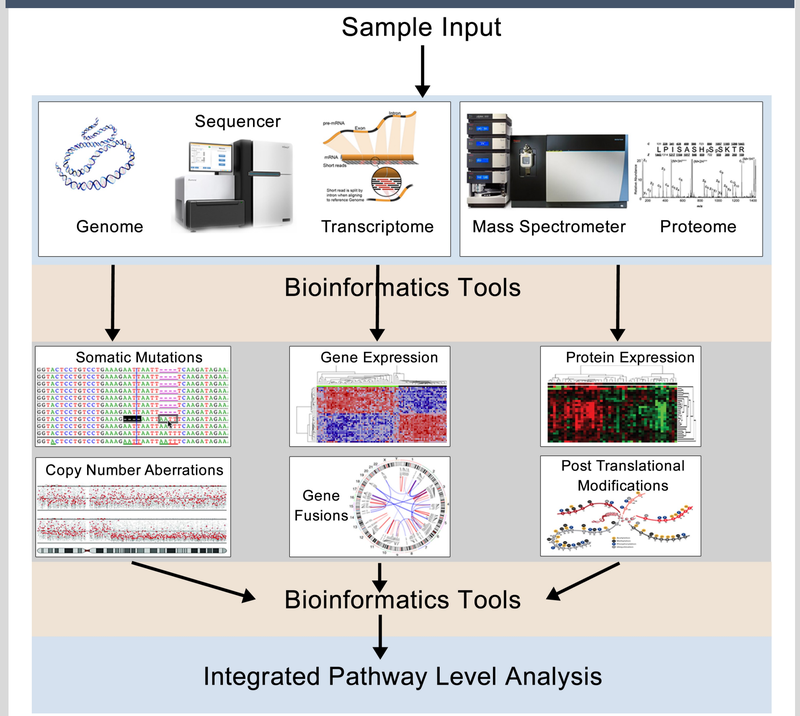
- Characterization of the neuroendocrine phenotype and transdifferentiation in prostate cancer: The Placental Gene PEG10 Promotes Progression of Neuroendocrine Prostate. Akamatsu S et al. (2015) Cancer. Cell Report (see **panel A**)
- HIT'nDRIVE: Patient-specific Multi-driver Gene Prioritization for Precision Oncology. Shrestha R et al. (2017) Genome Res (**panel B**)
- Stromal Gene Expression is Predictive for Metastatic Primary Prostate Cancer. Mo F. et al. (2017) Eur Urol. (**panel C**)
- Clonality Inference from Single Tumor Samples Using Low-Coverage Sequence Data. Donmez Net al. (2017) J Comput Biol. (**panel D**)
- Integrated Multi-omics Molecular Subtyping Predicts Therapeutic Vulnerability in Malignant Peritoneal Mesothelioma. Shrestha R et al (2018) In submission (**see poster #4 by Raunak Shrestha**)

Expertise – Recently Added

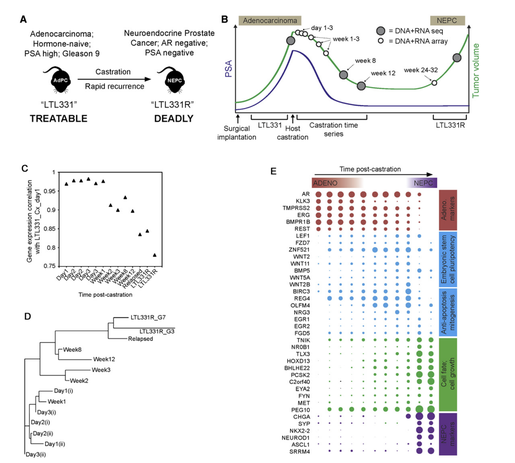
Examples of applications

- The Proteome of Primary Prostate Cancer. Iglesias-Gato et al. (2015) Eur Urol. (**panel E**)
- Profiling of human tumour cells using single cell RNA sequencing (**panel F**)

Expertise – Integration of multi-omic data

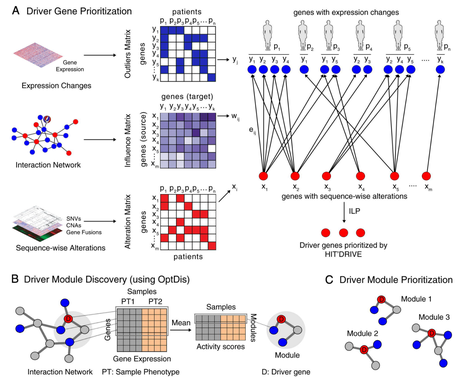


A) Characterization of the neuroendocrine phenotype and transdifferentiation in prostate cancer



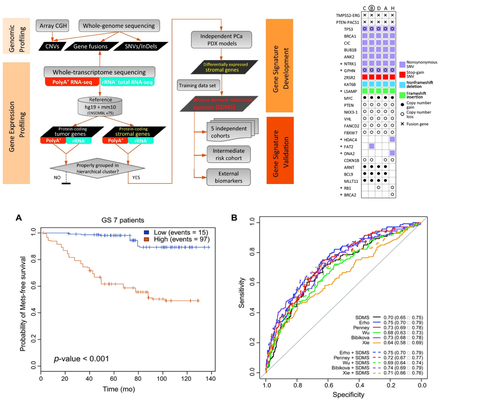
Modeling NE Transdifferentiation in Patient-Derived Xenograft LTL331. (A) Overview of the model (B) Schematic depicting time points at which tumors were collected along progression to NEPC (C) Plot showing divergence of gene expression profiles during transdifferentiation (D) Gene expression clustering demonstrating that weeks 2–12 post-castration are markedly different to earlier in the series (E) Selected genes exhibiting a significant trend of increasing mRNA expression during transdifferentiation. Circle size represents log2 fold changes in gene expression normalized to pre-castration levels.

B) 'Omics' Integration and precision oncology



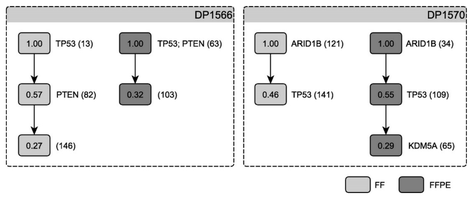
Overview of HIT'nDRIVE algorithmic framework. (A) HIT'nDRIVE integrates sequence-wise changes in genome with expression changes in transcriptome obtained from patients' tumor samples. The influence values derived from the protein interaction network indicate how likely a driver gene influences its downstream target genes in the network. (B) The predicted driver genes are used as seeds to discover modules of genes that discriminate between the sample phenotypes using OptDis. (C) Based on this the driver modules are ranked and thus prioritized.

C) Stromal gene expression is predictive for metastatic primary prostate cancer



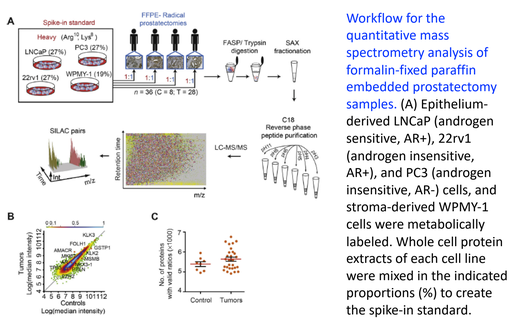
(A) Genomic and transcriptomic profiling workflows led to the discovery of a stromal gene signature. This signature was further validated in multiple large independent clinical cohorts, including those with intermediate-risk tumors (B) Matrix showing the genomic alterations identified in each PDX model. Genes with an alteration event not supported by the transcriptome sequencing data are marked with an asterisk. (C) Kaplan-Meier survival analysis for the stroma-derived metastasis signature (SDMS) for patients with intermediate-risk Gleason 7 tumors. (A) Kaplan-Meier curves show that intermediate-risk patients with high SDMS scores, based on a cohort median split (low/high), have worse outcome. (D) Addition of the SDMS to previously validated signatures (Wu, Bibikova, and Xie) improves the predictive power. Combined logistic regression models were trained in the Mayo Clinic I cohort and evaluated in the pooled validation cohort.

D) Analysis of clonality



Subclonal composition of two prostate tumors using matched fresh frozen and FFPE samples as predicted by CTPsingle. Known cancer-related genes containing nonsynonymous coding mutations are shown next to nodes based on their subclonal assignments. The numbers in parentheses give the total number of mutations assigned to each node, including synonymous mutations. The PTEN mutations in the FF and FFPE samples of DP1566 represent distinct mutations. The rest of the mutations are identical between FF and FFPE in both patients. FFPE, formalin-fixed paraffin embedded.

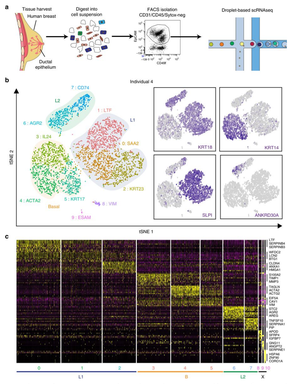
E) The proteome of primary prostate cancer



Workflow for the quantitative mass spectrometry analysis of formalin-fixed paraffin embedded prostatectomy samples. (A) Epithelium-derived LNCaP (androgen sensitive, AR+), 22rv1 (androgen insensitive, AR+), and PC3 (androgen insensitive, AR-) cells, and stroma-derived WPMY-1 cells were metabolically labeled. Whole cell protein extracts of each cell line were mixed in the indicated proportions (%) to create the spike-in standard.

Trypsin-digested extracts were further fractionated by anionic exchange. Each fraction was analyzed by LC-MS/MS using an Exactive-Q mass spectrometer. Peptides were identified and quantified using MaxQuant software suit. (B) Scatter plot of the relative abundance of all proteins shown as the log10 of the median intensity of the tumors (n = 28) and the benign neighboring prostate tissue (controls; n = 8). Relative density is color-coded, and the names of several prostate cancer relevant proteins are indicated. (C) Number of proteins with valid ratios: light (from prostatectomy origin)/heavy (from cell line origin) for each tumor and control sample.

F) Single-cell RNA sequencing



Application of high throughput droplet-mediated single-cell RNA sequencing (scRNAseq). Nguyen QH, et al. Nat Commun. 2018. PMID: 29795293.

(A) Overview of droplet-enabled scRNAseq approach where basal and luminal epithelial cells were sorted together and subjected to scRNAseq analysis. (B) Data from a single individual was analyzed using Seurat and the distinct clusters (0–10) are displayed in tSNE projection with selected marker gene for each cluster, and main epithelial cell types (Basal, L1, L2) are outlined. Feature plots of characteristic markers for the three main cell types are shown on the right showing expression levels as gradient of purple. (C) Heatmap showing the top ten marker genes for each cluster as determined by Seurat analysis with three selected genes per cluster highlighted on the right.

Publications

Characterization of the neuroendocrine phenotype and transdifferentiation in prostate cancer (1-3)

1. From sequence to molecular pathology, and a mechanism driving the neuroendocrine phenotype in prostate cancer. Lapuk AV et al. (2012). J Pathol. 227(3):286-97. PMID: 22553170.
2. Identification of DEK as a potential therapeutic target for neuroendocrine prostate cancer. Lin D et al. (2015) Oncotarget. Jan 30;6(3):1806-20. PMID: 25544761.
3. The Placental Gene PEG10 Promotes Progression of Neuroendocrine Prostate. Akamatsu S et al. (2015) Cancer. Cell Report. 2015 Aug 11;12(6):922-36. PMID: 26235627.

Cancer heterogeneity

4. The inter-tumor transcriptome heterogeneity of high-risk primary prostate cancer Wyatt A et al (2014). Genome Biology 2014 Aug 26;15(8):426. PMID: 25155515.

Characterization patient-derived xenografts (5-7)

5. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Choi SY et al. (2014) Adv Drug Deliv Rev. Dec 15;79-80:222-37. PMID: 2530533
6. Next generation patient-derived prostate cancer xenograft models. Lin D et al. (2014) Asian J Androl. May-Jun;16(3):407-12. PMID: 24589467
7. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Lin D et al. (2014) Cancer Res. Feb 15;74(4):1272-83. PMID: 24356420

Integrative analysis of genomic and transcriptomic data (8)

8. Integrated genome and transcriptome sequencing identifies a novel form of hybrid and aggressive prostate cancer. Wu C, Wyatt AW et al. (2012). J Pathol. 2012 May;227(1):53-61. PMID: 22294438

Development of methods for the detection of structural variants (9)

9. Nfuse: Discovery of complex genomic rearrangements in cancer using high-throughput sequencing. McPherson A et al. (2012). Genome Research. 22: 2250-2261. PMID: 22745232

Ultra-sensitive detection of single nucleotide variants and indels in circulating tumour DNA (10)

10. SiNVICT: Ultra-Sensitive Detection of Single Nucleotide Variants and Indels in Circulating Tumour DNA. Can Kockan et al. (2017) Bioinformatics Jan 1;33(1):26-34. PMID: 27531099

RNA editing (11)

11. Systematic identification and characterization of RNA editing in prostate tumors. Mo F. et al. (2014). PLoS One. Jul 18;9(7):e101431. PMID: 25036877

detection of fusions (12) - 12. Poly-gene fusion transcripts and chromothripsis in prostate cancer. Wu C et al. (2012) Genes Chromosomes Cancer. Dec;51(12):1144-53. PMID: 22927308.

Alternative splicing (13,14)

13. ORMAN: optimal resolution of ambiguous RNA-Seq multimappings in the presence of novel isoforms. Dao P et al. (2014) Bioinformatics. Mar 1;30(5):644-51. PMID: 24130305.
14. The role of mRNA splicing in prostate cancer. Lapuk AV et al., (2014) Asian Journal of Andrology. 2014 July-Aug; 16(4):515-21. PMID: 24830689.

Analysis of clonality (15,16)

15. Clonality inference in multiple tumor samples using phylogeny. Malikic S et al. (2015) Bioinformatics. May 1;31(9):1349-56. PMID: 25568283.
16. Clonality Inference from Single Tumor Samples Using Low-Coverage Sequence Data. Donmez Net al. (2017) J Comput Biol. Jun;24(6):515-523. PMID: 28056180

Pathway and subnetwork (17)

17. HIT'nDRIVE: patient-specific multi-driver gene prioritization for precision oncology. Shrestha R et al. (2017) Genome Res. Sep;27(9):1573-1588. PMID: 28768687

Identification of clinical variants (18-20)

18. Next generation sequencing of prostate cancer from a patient identifies a deficiency of methylthioadenosine phosphorylase, an exploitable tumor target. Collins CC et al. (2012): Mol Cancer Ther 11(3): 775-83. PMID: 22252602
19. Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance and response in castration-resistant prostate cancer. Azad AA et al., (2015). Clinical Cancer Research. 21:2315-24. PMID: 25712683
20. Genomic alterations in cell-free DNA and enzalutamide resistance in castration-resistant prostate cancer. Wyatt AW et al., (2016) JAMA Oncol. Dec 1;2(12):1598-1606. PMID: 27148695

Identification of a gene expression signature (21)

21. Stromal Gene Expression is Predictive for Metastatic Primary Prostate Cancer. Mo F. Eur Urol. 2017 Mar 19. PMID: 28330676

Potential therapeutic targeting (17,22)

17. HIT'nDRIVE: patient-specific multi-driver gene prioritization for precision oncology. Shrestha R et al. (2017) Genome Res. Sep;27(9):1573-1588. PMID: 28768687
22. A meta-analysis approach for characterizing pan-cancer mechanisms of drug sensitivity in cell lines. Wang K et al (2014). PLosOne 2014 July 18;9(7). PMID: 25036042.

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