S4-05
Interrogating the landscape of long noncoding RNAs in breast cancer to identify predictors of tamoxifen resistance

Dr. Feng has disclosed that he is a co-inventor on a patent entitled Noncoding RNAs Uses Thereof. Dr. Feng has also disclosed that he is a founder of PFS Genomics, a molecular diagnostics company focused on breast cancer.
Disclosures:

I have received research funding from:
- Celgene Corporation
- Medivation
- Astellas
- Varian

I have participated in Advisory Boards for:
- GenomeDX Biosciences
- Medivation/Astellas

I am the President and Founder of PFS Genomics, a molecular diagnostics company focused on breast cancer.

I am a co-inventor on a patent entitled “Noncoding RNAs and Uses Thereof,” filed by the University of Michigan, which covers the discovery of some of the long noncoding RNAs described in this presentation.

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Looking beyond protein-coding genes

What is the purpose of this pervasive non-coding RNA?

- There are many different species of non-coding RNAs
- Of these different species, long noncoding RNAs (lncRNAs) most closely resemble protein-coding genes in structure, and potentially in function

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San Antonio Breast Cancer Symposium, December 8-12, 2015

Questions at hand:

What are the lncRNAs that populate breast cancer?

Could lncRNAs be important clinically?

Are they functional in disease?

We recently performed a large-scale IncRNA discovery effort, using 7,256 RNA sequencing (RNA Seq) libraries from tumors, normal tissues, and cell lines from 25 independent studies.

Iyer et al, Nature Genetics, 2015
Findings from our IncRNA compendia

- We discovered 48,952 unannotated long noncoding RNAs, which significantly increases the number of known IncRNAs.
- The genomic diversity of IncRNAs eclipses that of coding transcripts (~60,000 IncRNAs vs ~30,000 protein coding genes).
- 7,942 of these IncRNAs are lineage- or cancer-specific.
- Similar to Oncomine, this data can be interrogated at [http://mitranscriptome.org](http://mitranscriptome.org) (Iyer et al, Nature Genetics, 2015)

What are the IncRNAs that populate breast cancer?

- 1076 human samples
- Identified the most differentially expressed IncRNAs in breast cancer (versus normal tissue)
- Used unsupervised hierarchical clustering to identify breast cancer subtypes
Top ER+ vs. ER- IncRNAs

Nomination of BRCAT431 as a top outlier IncRNA in ER+ breast cancers

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Questions at hand:

What are the IncRNAs that populate breast cancer?

Is BRCAT431 important clinically in breast cancer?

Is BRCAT431 functional in disease?

BRCAT431 expression is associated with signatures of aggressive disease

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BRCAT431 expression is increased in the luminal B subtype of breast cancer

Questions at hand:

What are the IncRNAs that populate breast cancer?

Is BRCAT431 important clinically in breast cancer?

Is BRCAT431 functional in disease?
Knockdown of BRCAT431 inhibits oncogenic phenotypes in cell lines

**Extent of knockdown**

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<th>MCF7</th>
<th>T47D</th>
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**Proliferation assays**

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**Invasion assays**

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**Colony formation assays**

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BRCAT431 promotes tamoxifen resistance

**Relative Expression**

- Parental MCF7
- TamR MCF7
- shNT + 1uM Tam
- TamR MCF7 + shBRCAT431-1 + 1uM Tam
- TamR MCF7 + shBRCAT431-2 + 1uM Tam
- Parental MCF7 + 1uM Tam

**Percent Confidence**

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How does BRCAT431 function?

RNA pulldown followed by mass spectrometry nominates the RNA binding protein HnRNPL as a top BRCAT431 interactor

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<tr>
<th>Protein Name</th>
<th>#Unique Peptides</th>
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<th>AS Counts</th>
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Knockdown of HnRNPL reverses BRCAT431-mediated invasion

- Vector
- BRCAT431

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Conclusions

- We have developed a compendia of novel long noncoding RNAs enriched in breast cancer and specifically in ER+ disease
- BRCA431 is a top outlier lncRNA in ER+ breast cancer and is associated with aggressive clinical and preclinical phenotypes
- Mechanistically, BRCA431 interacts with HnRNPL to promote disease aggressiveness
- LncRNAs should be explored as biomarkers and therapeutic targets in cancer

Acknowledgments

Feng Lab
Sumin Han
Teng Ma
Chao Zhang
Karl Wilder-Romans
Joe Evans
John Prensner
Vishal Kothari
Bhavna Malik
George Zhao
Laura Chang

Chinnaiyan Lab
Arul Chinnaiyan
Yashar Niknafs
Rohit Malik
Matthew Iyer
Xuhong Cao

Funding support
Bayer Family Fund for Breast Cancer Research
Department of Defense
NIH
American Society of Clinical Oncology
Fund for Cancer Research

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