Chimeras- Greek Mythology or Scientific Reality?
Identification of Chimeric RNAs for Personalized Prevention of Breast Cancer

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Introduction

- ❖ Breast cancer is the most common cancer in the world.
- ❖ Breast cancer related deaths have decreased because of early breast cancer detection, reduced use of hormone replacement therapy and genomics based tailored treatments.
- ❖ *However*, the breast cancer prevention arena has not benefitted from genomic advances and for those at-risk for breast cancer, the only available prevention option is anti-estrogen based drugs.
- ❖ Personalized therapies based on individual molecular characteristics of patient tumors are desperately needed to improve breast cancer prevention.
- ❖ Fusions are created by joining the different parts of genes or chromosomes.
- ❖ Gene fusions, including the ones that are created by joining at the RNA level, can form during cancer development and thus present an attractive target for a prevention vaccine.

Objective

The objective of the current project is to identify novel actionable RNA fusions for the personalized prevention of breast cancer.

Hypothesis

We hypothesize that chimeric RNAs are relatively abundant in at risk breast tissue that can be targeted for personalized breast cancer prevention.

Methods

A list of RNA fusions was previously derived by RNA sequencing from at risk breast cancer tissue, breast tumors and normal breast tissue from healthy women.

- ❖ **Literature Search:** to learn interesting facts about the genes and any oncogenic role/ association with breast cancer or other cancers
- ❖ **Blat:** to locate the genes and fusion on chromosome, if the gene partners are located on same chromosome
- ❖ **cBioPortal/Survival Curves:** to predict association of genes with breast cancer patient survival and prognosis
- ❖ **Open Reading Frame (ORF) Finder and Fusion Junction:** to identify fusion junction and if the fusion is predicted to make a unique protein.
- ❖ **QPCR:** to detect the expression of chimeric RNAs in a panel of breast cancer cell lines.

20 Novel Chimeric RNA in Breast Cancer

| RNA FUSIONS (BREAST CANCER)	EXON Boundaries	TNBC Fusions		HER2+ Fusions		HR+ Fusions		TCGA (Breast Tumors)
		# of Fusion Positive Samples	Total # Junction Crossing Reads	# of Fusion Positive Samples	Total # Junction Crossing Reads	# of Fusion Positive Samples	Total # Junction Crossing Reads	# Samples
NSF-LRRC37A3	Exon 1-12| Exon 5-12	4	218	7	274	7	217	5
F8-CLIC2	Exon 1| Exon 2-6	1	3	4	25	3	11	1
KIAA0753-PITPNM3	Exon 1-16	Exon 2-20	0	0	3	10	4	14	1
PRKCH-FLJ22447	Exon 1-12	Exon 2-3	3	7	5	7	3	9	26
PACSIN2-ARFGAP3	Exon 1-12	Exon 2-6	2	2	2	3	3	4	1
UBE3C-DNAJB6	Exon 1	Exon 2-8	1	12	0	0	1	2	2
NCOR2-UBC	Exon 1-15	Exon 2	1	1	1	2	2	3	3
GALK2-FGF7	Exon 1-10	Exon 3-4	1	1	1	5	2	5	1
ARIH2-SLC25A20	Exon 1-5	Exon 5-9	1	1	0	0	1	1	2
B4GALT1-SMU1	Exon 1-2,3| Exon 2-12	2	4	1	2	1	2	1
WNK1-ERC1	Exon 1-24| Exon 6-19	1	1	0	0	0	0	1
SCCPDH-CNST	Exon 1-5| Exon 4-9	1	8	0	0	0	0	1
NOXRED1-TMED8	Exon 1-5	Exon 2-6	1	2	1	1	0	0	1
ACAP2-XXYLT1	Exon 1-22	Exon 3-4	1	1	1	1	0	0	1
MBD5-ORC4	Exon 1-2| Exon 2-15	1	1	1	1	0	0	2
UBE2G1-ANKFY1	Exon 1-3	Exon 3-25	1	3	6	6	1	1	1
AKT3-SDCCAG8	Exon 1| Exon 7-18	0	0	0	0	1	1	3
BACE2-FAM3B	Exon 1-7| Exon 2-7	0	0	0	0	1	1	3
ADCY9-SRL	Exon 1-2	Exon 2-6	1	1	1	2	0	0	6
TMCO3-TFDP1	Exon 1-7	Exon 3-12	1	7	1	1	0	0	6

Results

NSF-LRR37A2 Fusion Junction

N-Ethylmaleimide Sensitive Factor(NSF), Vesicle Fusing ATPase: Required for vesicle-mediated transport. Catalyzes the fusion of transport vesicles within the Golgi cisternae. Is also required for transport from the endoplasmic reticulum to the Golgi stack

Leucine Rich Repeat (LRR) 37A2: LRRs are frequently involved in the formation of protein–protein interactions.
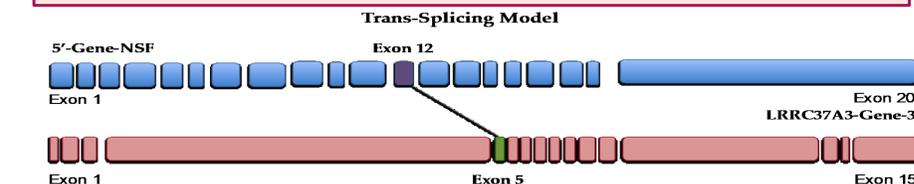
NSF: Chromosome 17q21
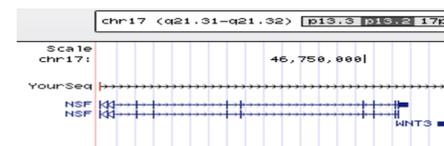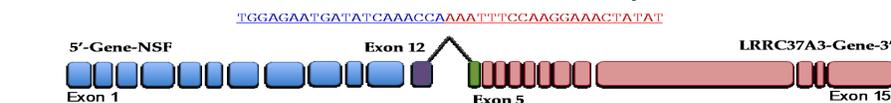

LRRC37A2: Chromosome 17q24
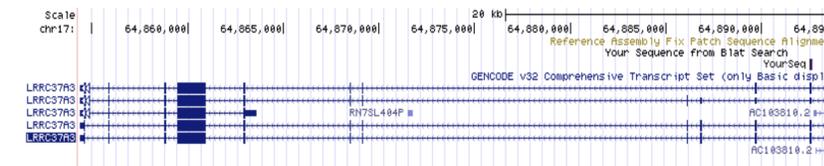
Fusion Sequence:

CTGCAAGTGATGAGAGGAGACTTCCTTGCTTCTTTGGAGAATGATATCAAACCA |FUSION BREAK|
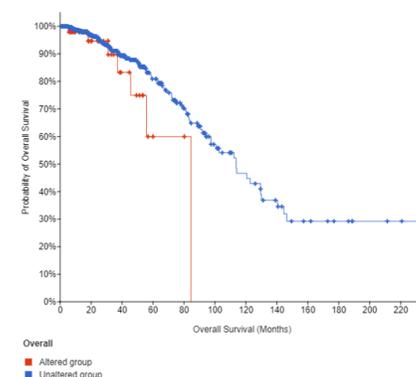AAATTTCCAAGGAAACTATATTGAAAATAACTTGACTGAATTACACAAGGATTCATTTGAAGGCCTGCTATCCCTCCAGTATTTAGATTTATCCTGCG

NSF [Exon 1-12]|LRRC37A2 / 3 [Exon 5-15] Fusion Transcript Model

Trans-Splicing Model

NSF [Exon 1-12] | LRRC37A3 [Exon 5-15] Fusion Junction

TGGAGAATGATATCAAACCAAAATTTCCAAGGAAACTATAT

NSF-LRR37A2 : Survival association (TCGA data set)

Logrank Test P-Value: 0.100

After about 80 months, breast cancer patients with mutations in NSF and LRRC37A2 typically did not survive.
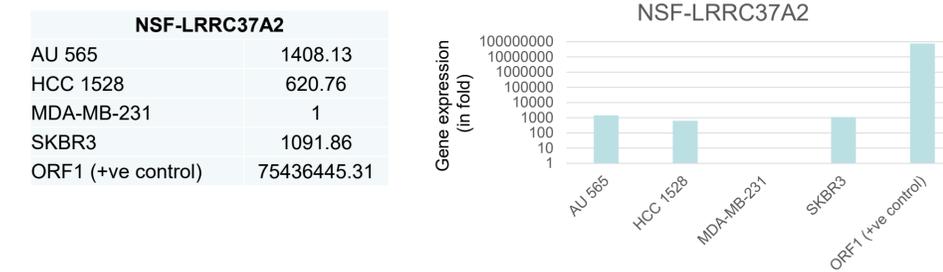
NSF-LRR37A2 : 1 novel ORF

NSF [Exon 1-12] | LRR37A3 [Exon 5-15] Fusion ORF

```
>lcl|ORF1
MTIEIDFLQKKSIDSNPYDTDKMAAEFIQQFNNQAFSVGQQLVFSFNEKL
FGLLVKDIEAMDPSILKGEPATGKRQKIEVGLVVGNSQVAFEKAENSSLN
LIGKAKTKENRQSIINPDWNFEKMGIGGLDKEFSDIFRRAFASRVFPPEI
VEQMGCKHVKGILLYGPPGCGKTLLARQIGKMLNAREPKVVNGPEIINKY
VGESEANIRKLFADAEEEQRRLGANSGLHIIIFDEIDAICKQRGSMAGST
GVHDTVVNQLLSKIDGVEQLNNILVIGMTNRPDLIDEALLRPGRLEVKME
IGLPDEKGRLQILHIHTARMRGHQLLSADVDIKELAVETKNFSGAELEGL
VRAAQSTAMNRHIKASTKVEVDMEKAESLQVTRGDFLASLENDIKPNFGG
NYISYIDGNVWKAYSWTEKLILRENNLTELHKDSFEGLLSLQYLDLSCNK
IQSIERHTFEPLPFLKFINLSCNVITELSFGTFQAWHGMQFLHKLILNHN
PLTTVEDPYLFKLPALKYLDMGTTLVPLTTLKNILMMTVELEKLIVPSHM
ACCLCQFKNSIEAVCKTVKLHCNSACLTNTTHCPEEASVGNPEGAFMKVL
QARKNYTSTELIIEPEEPSDSSGINLSGFGSEQLDTNDESDVTSTLSYIL
PYFSAVNLDVKSLLLPFIKLPTTGNSLAKIQTVGKNRQRLNRVLMGPRSI
QKRHFKEVGRQSIRREQGAQASVENTAEEKRLGSPAPRELKQPHTQQGPE
KLAGNAVYTKPSFTQEHKAAVSVLKPFSKGAPSTSSPAKALFQVRDRWKD
LTHAISILESAKARVTNMKTSKPIVHSRRKYRFHKTRSRMTHRTPKVKKS
PKVRKKSYLSRLMLSNRLPFSAAKSLINSPSQGAFSSLRDLSPQENPFLE
VSAPSEHFIENNNTKDTTARNAFEENVFMENTNMPEGTISENTNYNHPPE
ADSAGTAFNLGPTVKQTETKWEYNNVGTDLSPEPKSFNYPLLSSPGDQFE
IQLTQQLQSVIPNNNVRRLIAHVIRTLKMDCSGAHVQVTCAKLVSRTGHL
MKLLSGQQEVKASKIEWDTDQWKTENYINESTEAQSEQKEKSLEFTKELP
GYGYTKKLILALIVTGILTILIILLCLIEICCHRRSLQEDEEGFSRDSEA
PTEEESEALPX
```

NSF [Exon 1-12]|LRRC37A2 [Exon 5-15] Fusion Transcript: PCR Validation

QPCR: NSF-LRRC37A2 mRNA expression in breast cancer cell lines

NSF-LRRC37A2	
AU 565	1408.13
HCC 1528	620.76
MDA-MB-231	1
SKBR3	1091.86
ORF1 (+ve control)	75436445.31

NSF-LRRC37A2 chimeric mRNA is expressed in high levels in 3 breast cancer cell lines. (Data not shown for the rest of cell lines that do not express the gene product).

Summary

- ❖ RNA fusions PACSIN2-ARFGAP3, F8-CLIC2, and NSF-LRRC37A2 were investigated and fusion junctions were successfully determined.
- ❖ Positive correlations were noted between genes with in a fusion pair.
- ❖ Each fusion generates at least 1 unique ORFs, suggesting that these fusions are predicted to be translated, and these novel proteins can be selectively targeted with a vaccine, sparing the normal cells .
- ❖ The mRNA expression of NSF-LRRC37A2 fusion found to be present in three of out of the twelve samples, suggesting that NSF-LRRC37A2 is likely to be present in breast cancer patients.

Conclusions

Chimeric RNAs appear to be relatively abundant in breast cancer. Next steps will involve preclinical testing of a neo-antigen peptide vaccine generated from these fusions in animals and eventually at-risk breast cancer patients