Targeting nuclear export for triple-negative breast cancer therapy

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Introduction

Triple-negative breast cancers (TNBC) are exceedingly heterogeneous in genetic mutations

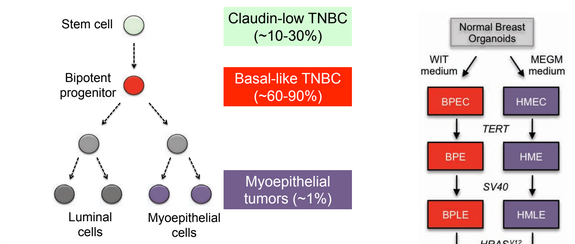
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TP53	**77.7%**	**0.000**	**0.000**	**0.000**
PIK3CA	12.6%		0.000	0.001
FRG1	6.8%	0.012		
PTEN	5.8%		0.000	0.000
FBXW7	4.9%		0.013	0.045
OTUD7A	3.9%			0.047
CARD11	3.9%			0.047
CDH1	2.9%		0.030	0.047
FRMD7	2.9%			0.047
HIST1H3B	1.9%			0.047
RHOA	1.9%			0.047
EZH2	1.9%			0.047
CBFB	1.0%			0.047

Within the TCGA database, 103 out of ~ 1,000 breast primary tumors are TNBC.

We employed MutSig to identify significant mutations in the TNBC subset.

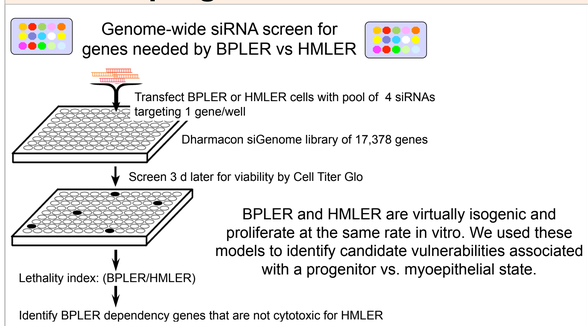
Beside p53 and a handful of other genes, most genetic lesions were exceedingly rare.

TNBC are commonly arrested in a progenitor-like epigenetic state



Despite genetic diversity, most TNBCs (classified as basal-like) resemble normal mammary progenitors that can be efficiently propagated in chemically-defined conditions in vitro. →

A genome-wide siRNA lethality screen for selective dependencies linked to a progenitor-like state



BPLER and HMLER are virtually isogenic and proliferate at the same rate in vitro. We used these models to identify candidate vulnerabilities associated with a progenitor vs. myoepithelial state.

Results

1. TNBC cell lines were recurrently selectively sensitive to perturbation of currently druggable cell fitness networks, including nuclear export, RNA splicing and the ubiquitin-proteasome system.

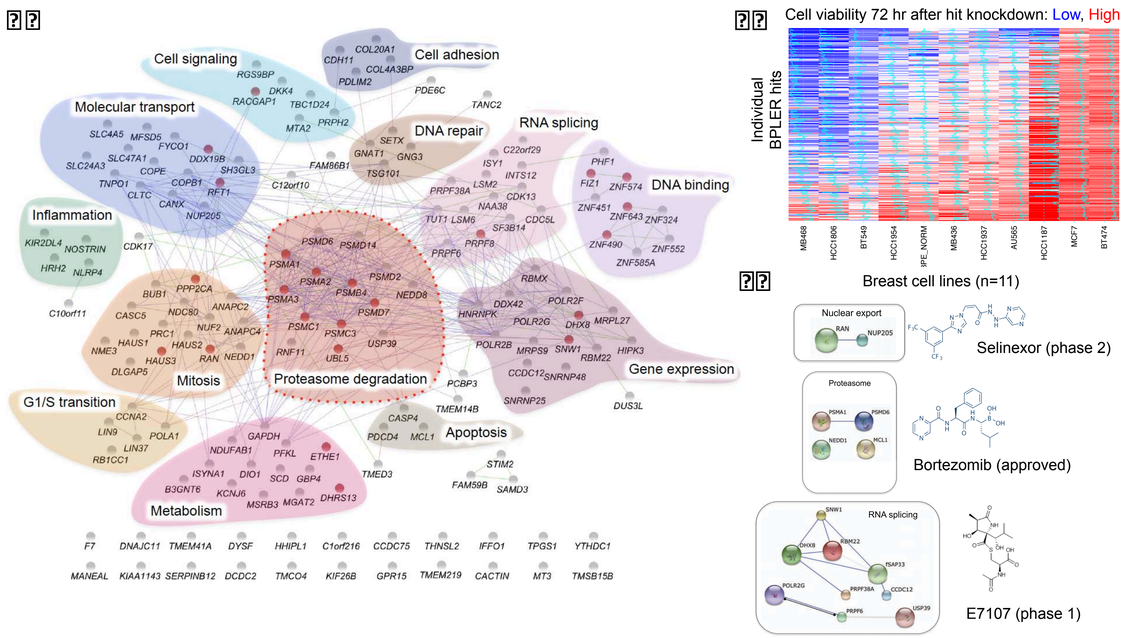


Figure 1: (A) Protein-protein interaction (PPI) functional network of BPLER dependency genes identified by unbiased genome-wide siRNA screening. **(B)** Heatmap of cell viability after knockdown of BPLER dependency genes in the indicated cell lines. **(C)** Druggable dependency genes shared by at least 30% of TNBC cell lines. For each network, representative clinical-stage inhibitor drugs are shown.

2. The nuclear export inhibitor selectively killed TNBC lines in vitro and in vivo. Up regulation of nuclear envelope genes was associated with exceptional response in these models.

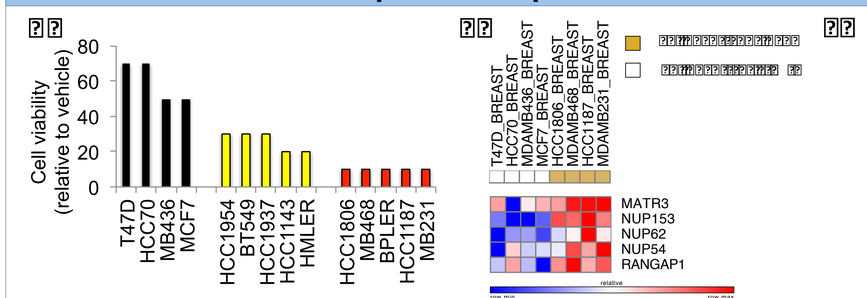
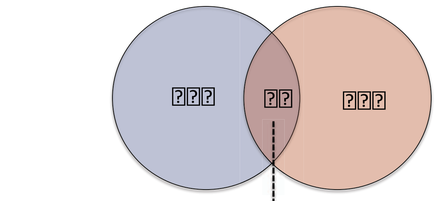


Figure 2: (A) Cell viability of TNBC cell lines after treatment with selinexor (0.5 uM) for 48 hr in vitro. Luminal T47D and MCF7 cells were used as control. **(B)** mRNA expression of nuclear envelope genes significantly correlated with exceptional TNBC sensitivity to selinexor in vitro, as determined by RNA-seq. **(C)** Volume of pre-established xenograft tumors from HCC1187 TNBC cell line upon treatment with selinexor (10 mg/kg) or vehicle twice a week by oral gavage. Arrows indicate treatment days.

Results (cont'd)

3. TP53-effector genes also stood out as candidate markers of TNBC sensitivity to selinexor.



Genes whose expression is associated with sensitivity to nuclear export inhibitors across 41 breast cell lines

Genes required for response to selinexor in TNBC MB231 cells as determined by CRISPR knock-out screening

3 out of 4 linked to TP53 function

Conclusions

- Differentiation arrest is a common step in the pathogenesis of TNBC.
- A genome-wide siRNA screen identified actionable TNBC vulnerabilities associated with poor differentiation, notably nuclear export, RNA splicing and proteasome function.
- The nuclear export inhibitor selinexor was selectively active against TNBC cell lines in vitro and in vivo.
- Response to selinexor in vitro was at least in part mediated by genes implicated in TP53 function.
- Thus, TNBCs appear to coopt nuclear export as one mechanism to dampen TP53-dependent signaling.