Identifying mutation-independent signaling pathway activation in molecular subtypes of triple negative breast cancers

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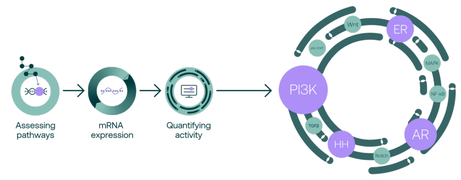
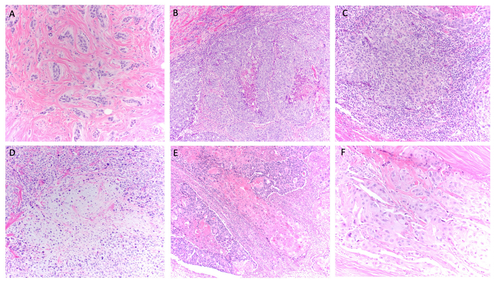
INTRODUCTION

Triple negative breast cancer (TNBC) is a rare and aggressive disease indicated by the absence of estrogen receptors (ER), progesterone receptors (PR), and minimal to no expression of human epidermal growth factor receptor 2 (HER-2). Using DNA methylation profiling, we previously identified three subgroups with distinct clinicopathologic and molecular signatures. Here, RNA expression-based pathway analysis is used to identify targetable pathways.

METHODS

We analyzed 44 TNBC cases diagnosed at NYU Langone Health between March 2011 and April 2018. Samples were compared based on DNA methylation and mutational analyses, histology, and Ki-67%. We assessed mRNA expression levels of pathway-specific target genes with RT-qPCR using the OncoSIGNal pathway profiling assay. Signal transduction pathway analyses used to measure activity of the estrogen receptor (ER), androgen receptor (AR), phosphoinositide-3-kinase (PI3K), mitogen-activated protein kinase (MAPK), Hedgehog (HH), Notch, and transforming growth factor beta (TGF- β) pathways.

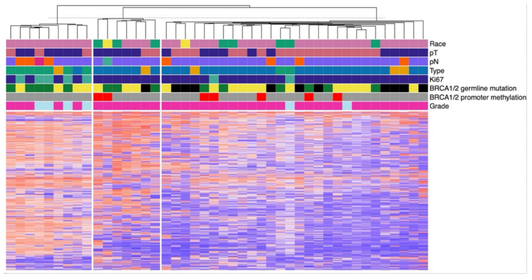




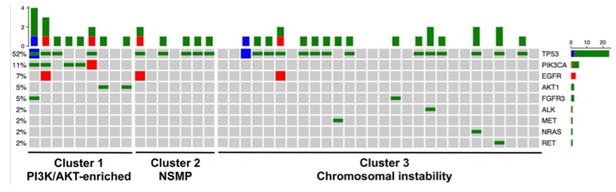
Histologic heterogeneity is visualized in various TNBC samples. Adapted from Lin, et al., 2023.

RESULTS

- Three distinct clusters identified with DNA methylation: **apocrine histology and PI3K/Akt mutations** cluster (Cluster 1), no **recurrent mutations** cluster (Cluster 2), and **DNA instability** cluster (Cluster 3).
- Cluster 1 showed no increased activity in PI3K pathway by RNA expression
- Cluster 1 showed increased AR and MAPK signaling, with a single case of increased ER signaling in the absence of ESR1 activating mutations.
- Cluster 2 showed upregulation in PI3K pathway in 4/7 (57%) of cases.
- Cluster 3 showed upregulation of PI3K in 11/25 (44%) of cases.
- PI3K pathway activation was associated with high Ki-67 >15%, however not all high Ki-67 cases showed high PI3K activity.
- Invasive ductal carcinoma (IDC) morphology was characterized by a nearly even split between MAPK (13/28, 46%) and PI3K (12/28, 44%), with single HH and TGF- β driven cases.
- In 5/28 (18%) of IDC we did not identify an upregulated pathway.



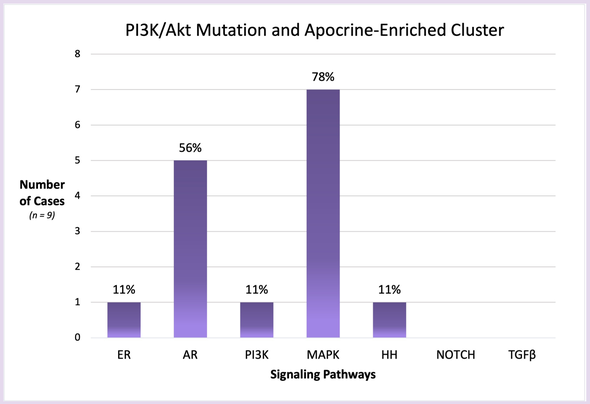
Molecular classification of TNBC: We have previously shown that TNBC form three distinct clinical epigenetic groups using genome-wide DNA methylation profiling. Adapted from Lin, et al., Mod Pathology 2023.

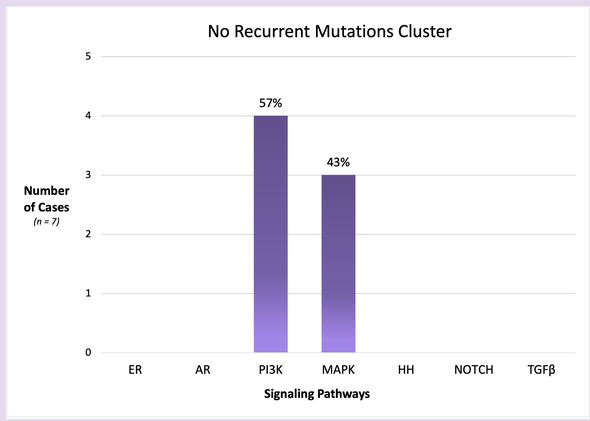


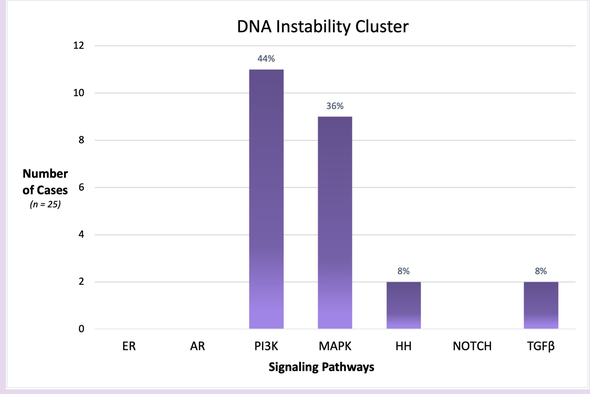
Mutational landscape of TNBC: Correspondingly, the three epigenetic clusters showed different mutational landscape. Cluster 1 was characterized by frequent mutations in PI3K and AKT1 genes, and Cluster 3, which was enriched in BRCA1/2 germline mutations and promoter hypermethylation shown in the heatmap above, was characterized by genome-wide chromosomal instability (not shown). Cluster 2 did not show recurrent mutations or chromosomal instability. Adapted from Lin, et al., Mod Pathology 2023.

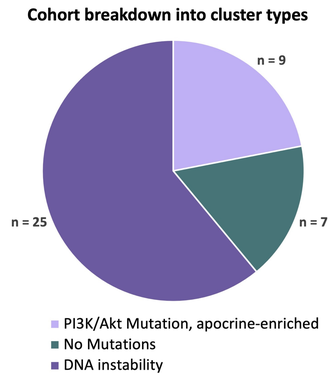
Therefore we sought to investigate pathways upregulated in each molecular group.

RNA EXPRESSION IDENTIFIES UPREGULATED PATHWAYS IN EPIGENETIC CLUSTERS:









DISCUSSION

RNA expression-based pathway activity and DNA mutational and methylation analyses provide complimentary information about the molecular landscape of TNBC.

PI3K mutations are early drivers and lead to distinct DNA methylation signatures, however they are not associated with increased PI3Kpathway activity by RNA expression.

In contrast, TNBC without PI3K mutations may show activation of the PI3K pathway in the absence of PI3K/Akt mutations.

Our observation calls into question: which biomarker is best suited to predict response to PI3K inhibitors in TNBC? This may include expanding the number of patients for which PI3K/Akt pathway inhibition might be a therapeutic option, as well as explaining the lack of response in PI3K mutated tumors.

REFERENCES & ACKNOWLEDGEMENTS

Lin, L. H., Tran, I., Yang, Y., Shen, G., Miah, P., Cotzia, P., Roses, D., Schnabel, F., Darvishian, F., & Snuderl, M. (2023). DNA Methylation Identifies Epigenetic Subtypes of Triple-Negative Breast Cancers With Distinct Clinicopathologic and Molecular Features. Modern pathology :36(11), 100306.

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