A core cell cycle-regulated network that coordinately upregulates centrosome amplification and centrosome clustering proteins is in overdrive in Androgen Receptor-low Triple-negative breast cancer

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Background

- There are no approved targeted treatments for Triple-Negative Breast Cancer (TNBC: ER-/PR-/HER2-)
- Racial disparity in breast cancer outcomes
  - Black women are twice as likely to be diagnosed with TNBC than White women (38 vs 19 per 100,000), and thus have fewer targeted treatment options
  - Black women with breast cancer have a 40% higher mortality rate than White women with breast cancer
- Quadruple Negative BC
  - Recent studies have proposed the use of androgen receptor (AR) expression to further stratify TNBC patients
  - Approx. 65-88% of TNBC patients are AR-negative, and are classified as having Quadruple Negative BC (QNBC)
  - Black women are more likely than White women to have QNBC, which is generally more proliferative, has a higher likelihood of belonging to more aggressive basal-like subtypes, younger age at diagnosis, chemoresistance, and shorter disease-free survival
- Centrosome Amplification and Centrosome Clustering
  - Malignant cells often contain an excessive number of centrosomes gained in a process known as centrosome amplification
    - Result of cell–cell fusion, centrosome fragmentation, de novo centriole formation, and cytokinesis failure
  - Possessing only two centrosomes is critical to the proper division of the cell
  - Cells that bear additional centrosomes should be at risk for multipolar divisions that can lead to aneuploidy and cell death; however, cancer cells cluster excess centrosomes into two polar groups by a process called centrosome clustering, that allows them to assemble pseudo-bipolar mitotic spindles, promotes chromosomal instability, intratumoral heterogeneity, and disease progression
- KIFC1, a well established kinesin motor protein that mediates clustering, mainly acts by sliding microtubules and bundling them closer to the poles. It cannot single handedly accomplish its centrosome clustering function. Thus, we focused on the Chromosomal Passenger Complex (CPC), which is localized at the kinetochore and collaborate with KIFC1.
- The CPC—composed of Aurora B kinase (AURKB), INCENP, survivin (BIRC5) and borealin (CDCA8)—and some of its targets are required for clustering in many human cancer cell lines
- The CPC functions in resisting the pulling forces of the spindle, maintaining sister-chromatid cohesion, and allowing for the correction of faulty connections between chromosomes and the spindle
- In cancer cells, the CPC functions in overdrive, and these kinetochore genes are coordinately overexpressed.

Central Question: What mechanisms co-upregulate the centrosome amplification genes, with the centrosome clustering genes, specifically the CPC genes in AR-low TNBCs?

Goal: Answering this question may allow us to identify potential actionable treatment targets, and expand our understanding of what drives the tumor biology of AR-low TNBCs.

Methodology

Gene Expression Profiling Interactive Analysis (GEPIA): (Used in Fig. 1 and Fig. 2.)

- We analyzed RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects, for the expression profiles of centrosome clustering proteins KIFC1, AURKB, BIRC5, and CDCA8.

University of Alabama and Birmingham Cancer Data Analysis (UALCAN): (Used in Fig. 1, Fig. 2, Fig. 3, and Fig. 5.)

- To better understand connections of our focus genes ATAD2, E2F1, and FOXM1, and the expression of our centrosome clustering genes of interest, we performed additional *in silico* analyses of publicly-available gene expression datasets from the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN). We examined comparisons based on expression in normal vs primary tumor, TP53 mutation status, race, and breast cancer subtypes.

Kaplan-Meier Plotter (KM Plotter) Data: (Used in Fig. 2 and Fig. 3.)

- We used the KM Plotter tool to analyze publicly-available breast cancer microarray data to evaluate the prognostic significance of our genes of interest
- For the probe, we used the Jetset optimal microarray probe set, and the optimal cutoff was used for survival analysis, and no restriction of subtypes) in relation to recurrence-free survival over the course of 120 months.

bc-GenExMiner Data: (Used in Fig. 4.)

- We used bc-GenExMiner "targeted" gene correlation analysis of 10 genes in TNBCs (all RNA sequencing data, TNBC status determined by immunohistochemistry).
- We evaluated the Pairwise Pearson's correlation coefficient among the 10 genes of interest, to better understand their patterns of expression in AR-low TNBCs.

Summary of Findings

Centrosome amplification (CA) is a well-established hallmark of cancer, and leads to spindle multipolarity and cell death, unless centrosome clustering pathways are co-upregulated alongside CA pathways. Our data demonstrate a pan-cancer pattern of upregulation of crucial centrosome clustering proteins, especially CPC components. In breast cancer datasets, we found significant upregulation of both centrosome clustering proteins KIFC1, AURKB, BIRC5, and CDCA8, and the oncogenes FOXM1, ATAD2, and E2F1, in tumor samples compared to normal samples. Both these genes sets were highly overexpressed in TNBCs (which show the highest levels of CA among breast tumors), in breast tumors of African-Americans, and in TP53-mutant breast cancers. Overexpression of each of these genes resulted in a poorer recurrence-free survival among breast cancer patients. Our analyses also showed that in AR-low TNBCs, these two gene sets are in overdrive, and result in the profound dysregulation of cell cycle controls and proliferation, which underlie poor outcomes. Thus, AR-low TNBCs and/or TP53-mutant TNBCs not only show severe CA and robust centrosome clustering, but also exhibit dysregulation of a core cell cycle network, that ultimately lead to an aggressive disease course, poor outcomes, and racial disparities in outcomes.

Our work illuminates a core cell-cycle network that links centrosome amplification and clustering pathways. Profound dysregulation of this core network drives an aggressive clinical course in AR-low and/or TP53-mutant TNBCs.

p53-p21-DREAM Pathway

It is known that the Dimerization partner, RB-like, E2F and Multi-vulval class B (DREAM) complex and RB coordinately halt entry into G1 of the cell cycle.

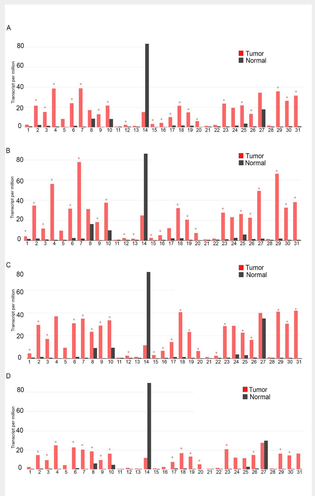
Cell-cycle regulated genes are broadly categorized into two groups: G1/S regulator genes and G2/M regulator genes.

- While both DREAM and RB operate in G1/S, entry into the cell cycle is primarily regulated by RB at this early stage.
- G2/M regulator genes function in mitosis, spindle assembly, chromosome segregation, and cytokinesis. These genes contain a cell cycle genes homology region (CHR) in their promoters and are primarily regulated by DREAM.
- In G0, wild type p53 induces the expression of CDK inhibitor p21, inhibiting CDK1's kinase activity. When CDK1 activity is inhibited, Rb-related proteins p130 and p107 are hypophosphorylated. Together, these hypophosphorylated proteins recruit the other members of the DREAM complex, which in turn represses the expression of G2/M genes. In the event that p53 has a loss-of-function mutation or is absent, CDK1 activity would be high, which would lift the repression of G2/M gene expression. Thus, the cell would exit G0 and express G1/S genes (including E2F1, a potent driver of CA, and ATAD2) prematurely, and overexpress G2/M genes.

Nexus of dysregulation surrounding FOXM1 in TP53-mutant and AR-low TNBC (Fig. 6)

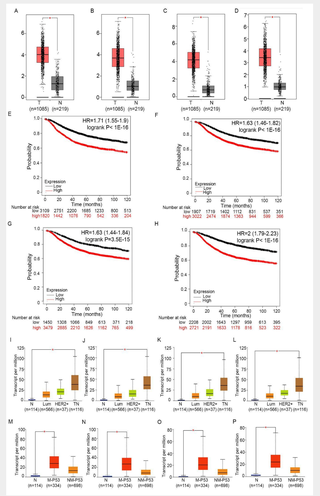
- In TNBCs, AR normally induces the expression of the transcription factor SPDEF, which constrains FOXM1 expression by disrupting a positive feedback loop that amplifies FOXM1's expression. This constraint is normally reinforced by the p53-p21-DREAM pathway.
- However, when AR is underexpressed (e.g., in AR-low TNBCs) or not expressed, or when p53 is mutated (as in ~50% of TNBCs), these regulatory pathways are defunct and FOXM1 expression is prematurely activated and strongly dysregulated. FOXM1 is a transcription factor for KIFC1, the CPC genes, and MKI67, as well hundreds of other target genes. Overexpression of FOXM1 target genes promotes CA, centrosome clustering, cell proliferation, chemoresistance, and resistance to apoptosis. All of these are markers of poor prognosis.

Fig. 1. Centrosome clustering proteins KIFC1, AURKB, BIRC5, and CDCA8 are overexpressed in a variety of tumor tissues



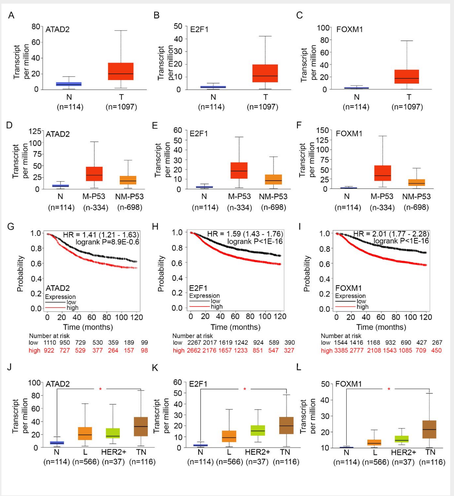
(A-D). Expression of KIFC1, AURKB, BIRC5, and CDCA8, respectively, in normal/tumor tissues from 31 different cancer types. Red asterisk denotes a significantly higher expression in tumor tissues compared to corresponding normal tissues. 1: Adenoid Cystic Carcinoma; 2: Bladder Urothelial Carcinoma; 3: Breast Invasive Carcinoma; 4: Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; 5: Cholangiocarcinoma; 6: Colon adenocarcinoma; 7: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; 8: Esophageal carcinoma; 9: Glioblastoma Multiforme; 10: Head and Neck Squamous Cell Carcinoma; 11: Kidney Chromophobe; 12: Kidney renal clear cell carcinoma; 13: Kidney Renal Clear Cell Carcinoma; 14: Acute Myeloid Leukemia; 15: Brain Lower Grade Glioma; 16: Liver Hepatocellular Carcinoma; 17: Lung adenocarcinoma; 18: Lung Squamous Cell Carcinoma; 19: Ovarian serous cystadenocarcinoma; 10: Pancreatic adenocarcinoma; 21: Pheochromocytoma and Paraganglioma; 22: Prostate adenocarcinoma; 23: Rectum adenocarcinoma; 24: Sarcoma; 25: Skin Cutaneous Melanoma; 26: Stomach Adenocarcinoma; 27: Testicular Germ Cell Tumors; 28: Thyroid Cutaneous Carcinoma; 29: Thymoma; 30: Uterine Corpus Endometrial Carcinoma; 31: Uterine Carcinosarcoma.

Fig. 2. Overexpression of centrosome clustering proteins KIFC1, AURKB, BIRC5, and CDCA8, is associated with poor prognosis, triple-negative status, and TP53-mutant status of breast cancers



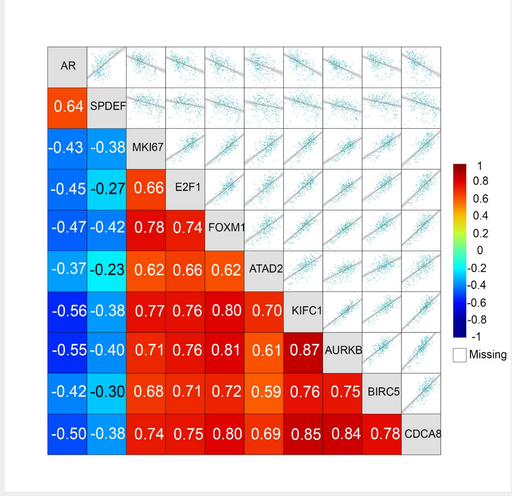
(A-D) Expression of KIFC1 (A), AURKB (B), BIRC5 (C), and CDCA8 (D), respectively, in breast invasive carcinoma (red), versus normal breast tissues (black). **(E-H)** Kaplan-Meier Survival analysis to evaluate the prognostic significance of centrosome clustering genes. The red line represents survival of patients with above-cutoff levels of KIFC1 (E), AURKB (F), BIRC5 (G), and CDCA8 (H) expression, respectively, in breast tumors, while the black line represents survival of patients with below-cutoff levels of the same clustering genes in relation to recurrence-free survival. **(I-L)** Breast tumor subtype analysis of the expression of centrosome clustering genes—KIFC1(I), AURKB (J), BIRC5 (K), and CDCA8 (L), respectively, in breast invasive carcinoma subclasses of the TCGA dataset, are shown using box whisker plots. The red asterisk indicates a statistically significant difference in expression (p<0.05); n=sample size; N=Normal, Lum=Luminal, HER2+=Her2-positive tumors, TN=triple-negative. **(M-P)** Expression of centrosome clustering genes—KIFC1(M), AURKB (N), BIRC5 (O), and CDCA8 (P), respectively, based upon the TP53 mutation status of breast tumors (M-P53: Breast tumors with mutant TP53, NM-P53: breast tumors with non-mutant TP53). The red asterisk indicates a statistically significant difference in expression (p<0.05); n=sample size per subtype.

Fig. 3. Analysis of the expression levels and prognostic significance of ATAD2, E2F1, and FOXM1 oncogenes among breast tumors.



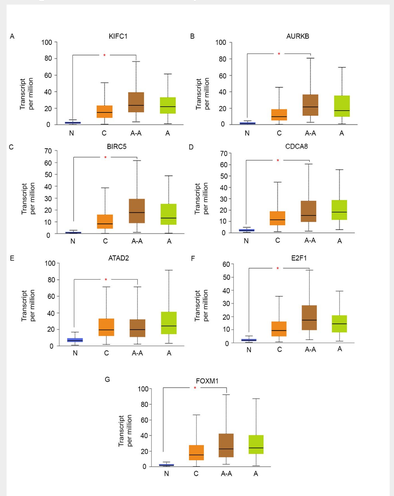
(A-C) Comparison of expression of the oncogenes ATAD2 (A), E2F1 (B), and FOXM1 (C), in breast tumor tissues versus normal tissues (GEPIA platform). The red box plot represents gene expression level in breast invasive carcinoma, while the black plot represents gene expression in matched normal breast tissues. **(D-F)** Analysis of TCGA dataset (UALCAN platform) for the expression of specific oncogenes of interest based upon the TP53 mutation status of breast tumors. Box whisker plots showing the expression of ATAD2 (D), E2F1 (E), and FOXM1 (F), respectively, in breast tumors categorized as having mutations in TP53 (M-P53), or having a non-mutant TP53 status (NM-P53). The red asterisk indicates a statistically significant difference in expression (p<0.05); n=sample size per subtype. **(G-I)** Kaplan-Meier Survival analysis to evaluate the prognostic significance of our oncogenes of interest in breast cancer. The red line represents survival of patients with above-cutoff levels of ATAD2 (G), E2F1 (H), and FOXM1 (I) expression, respectively, in breast tumors, while the black line represents survival of patients with below-cutoff levels of the same oncogenes in reission of specific oncogenes of interest. The expression of ATAD2 (J), E2F1 (K), and FOXM1 (L), respectively, in breast invasive carcinoma subclasses of the TCGA dataset, are shown using box whisker plots. The red asterisk indicates a statistically significant difference in expression (p<0.05); n=sample size per subtype; N=Normal, Lum=Luminal, HER2+=Her2-positive tumors, TN=triple-negative.

Fig. 4. Analysis of correlations between expression of key centrosome clustering genes, and expression of their potential upstream regulators in TNBC.



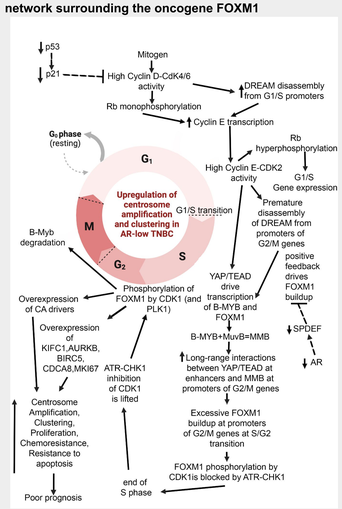
Bc-GenExMiner "targeted" gene correlation analysis of 10 genes (all RNA sequencing data, TNBC status determined by immunohistochemistry). Scatter plots depict Pearson's pairwise correlations, and the numbers inside the squares indicate the strength of the observed Pearson's pairwise correlations. Total n=4421 for each pairwise-comparison. Strong negative correlations are depicted in blue, and strong positive correlations are depicted in warm colors. P-Values for all pairwise correlations were statistically significant (p<0.0001).

Fig. 5. Expression levels of ATAD2, E2F1, and FOXM1 oncogenes in breast tumors of patients of different races.



(A-G) Box-whisker plots showing the expression levels of KIFC1 (A), AURKB (B), BIRC5 (C), CDCA8 (D), ATAD2 (E), E2F1 (F), and FOXM1 (G) in breast tumors from patients of different races (self-identified). "N" represents normal breast tissues with a sample size, n=114 for all. "C" represents Caucasians with a sample size, n=748. "A-A" represents African-Americans with a sample size, n=179, and "A" represents Asians with a sample size, n=61. Analysis of TCGA RNA sequencing data was performed on the UALCAN platform. The red asterisk indicates a statistically significant difference in expression levels between the indicated groups (p<0.05).

Fig. 6. Model depicting how loss of TP53 function and/or AR expression in TNBC dysregulates a core transcriptional network surrounding the oncogene FOXM1



The schematic above depicts how and why the centrosome clustering genes KIFC1, AURKB, BIRC5, and CDCA8, which are all primarily G2/M genes, and are extra sensitive to DREAM regulation, are overexpressed in TP53-mutant and/or AR-low TNBCs. Normally, the TP53-p21-DREAM and the AR-SPDEF pathways ensure the timely expression of G1/S and G2/M genes (especially FOXM1). Loss of TP53 function leads to premature and enhanced expression of G1/S genes that include E2F1, ATAD2 (a cofactor for E2F1) and numerous genes implicated in Centrosome Amplification. It also drives premature and enhanced expression of G2/M genes, especially FOXM1. Low activity of the AR-SPDEF pathway (in AR-low TNBCs) further deregulates FOXM1 expression, causing an abnormal buildup of this oncoprotein. Activation of abnormally high levels of FOXM1 at the G2/M boundary leads to overexpression of the hundreds of FOXM1 target genes, including genes that promote centrosome amplification and clustering, proliferation, chemoresistance, and resistance to apoptosis, precipitating a poor prognosis. Our model illuminates novel connections between centrosome amplification and clustering, and shows how these pathways are coordinately upregulated with proliferation and survival pathways in TP53-mutant and AR-low TNBCs. It highlights how a nexus of deregulation surrounding FOXM1 drives the tumor biology of AR-low TNBC.