TP53-dependent and -independent transcriptomic responses to pan-14-3-3 inhibition under DNA damage in breast cancer cells

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Introduction

- ✓ The 14-3-3 family regulates p53-mediated DNA-damage responses, apoptosis, and cell-cycle control.
- ✓ Pan-14-3-3 inhibition (Difopein) enhances apoptosis mainly in *TP53*-wild-type (WT) tumors.
- ✓ Its impact in *TP53*-mutant (Mut) breast cancer remains unclear.
- ✓ This study compared *TP53*-dependent transcriptomic responses to pan-14-3-3 inhibition under genotoxic stress.

Methods and Materials

Figure 1. Study design



Difopein
1uM,24h vs Contorol

Doxorubicin
3uM, 2h

Downstream analyses
- ◆ RNA-seq → DEGs → Pathway enrichment
- ◆ Flow cytometry (Annexin V / PI) after 24h
- ◆ Western blot (PARP cleavage) after 24h

Results 1

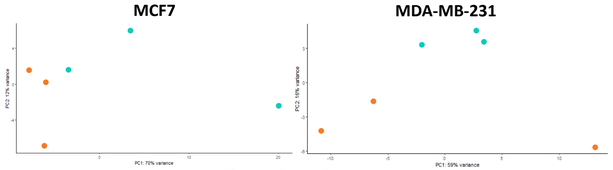
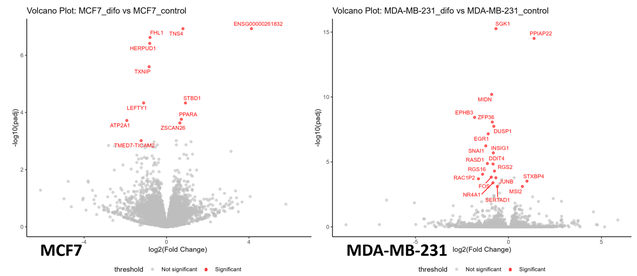


Figure 2A: PCA Plot

PCA plots show distinct clustering of samples in MCF7 (*TP53*-WT) and MDA-MB-231 (*TP53*-Mut) cells.

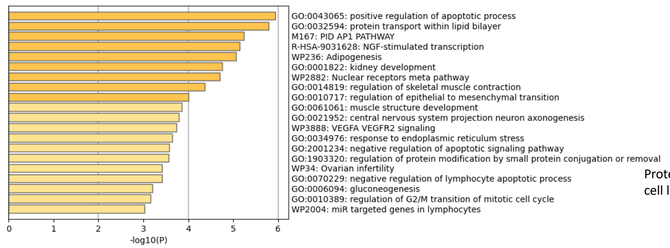
Results 2



Volcano plots showing differential gene expression in MCF7 and MDA-MB-231 cells after pan-14-3-3 inhibition.

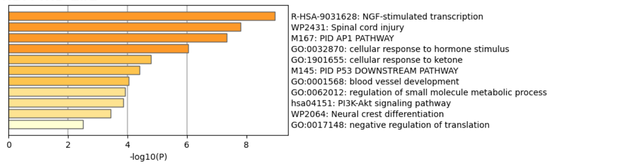
Figure 2B: Volcano Plot

MCF7



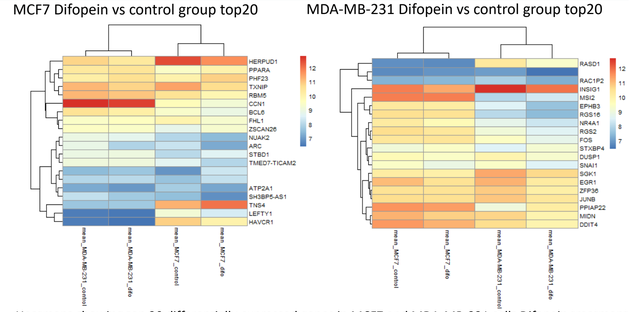
In MCF7 cells, enriched pathways include positive regulation of apoptotic process, protein transport within lipid bilayer, AP1 pathway, NGF-stimulated transcription, and adipogenesis.

MDA-MB-231



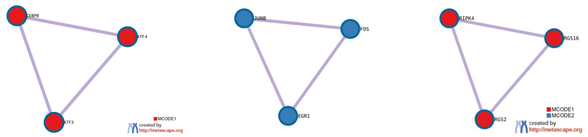
In MDA-MB-231 cells, enriched pathways include NGF-stimulated transcription, spinal cord injury response, AP1 pathway, cellular response to hormone stimulus, and PI3K-Akt signaling pathway.

Figure 2D:Pathway enrichment



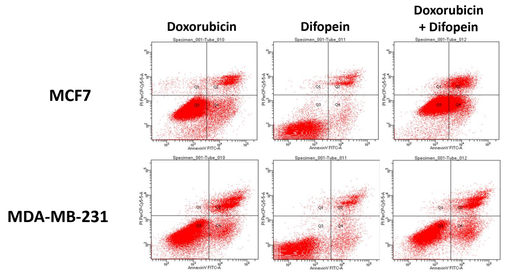
Heatmaps showing top 20 differentially expressed genes in MCF7 and MDA-MB-231 cells Difopein treatment.

Figure 2C: Heatmap



Protein-protein interaction (PPI) networks and MCODE components in MCF7 and MDA-MB-231 breast cancer cell lines. Network diagrams illustrate key protein interactions and clusters identified by MCODE analysis.

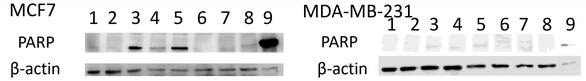
Figure 2E:PPI MCODE Components



In flow cytometry analysis, MCF7 cells showed a greater increase in Q2 and Q4 populations with the combination treatment of Doxorubicin and Difopein compared to either treatment alone.

Figure 3: Flow cytometry (Annexin V / PI)

Results 3



1:control, 2:DMSO, 3: Doxorubicin, 4:Difopein, 5: Doxorubicin + Difopein, 6: Doxorubicin + z-VAD-FMK, 7:Difopein + z-VAD-FMK 8: Doxorubicin + Difopein + z-VAD-FMK 9:Staurosporine

Western blot analysis of PARP expression and cleavage in MCF7 and MDA-MB-231 breast cancer cells under various treatment conditions.

Figure 4: Western Blotting

Discussion

- ✓ Pan-14-3-3 inhibition induced strong *TP53*-dependent apoptotic transcriptional programs in MCF7 cells, including activation of *ATF3*, *ATF4*, and *CEBPB*.
- ✓ TP53-Mut MDA-MB-231 cells exhibited limited apoptotic signaling and instead engaged alternative survival-associated regulators such as *RBMX* and *GTF2A2*.
- ✓ Divergent pathway enrichment patterns highlight distinct stress-response mechanisms under genotoxic stress.
- ✓ Functional assays confirmed transcriptomic differences between *TP53*-WT and -Mut cells.
- ✓ Apoptosis was more pronounced in *TP53*-WT cells, as shown by flow cytometry.
- ✓ PARP cleavage was detected exclusively in *TP53*-WT cells, indicating caspase-dependent cell death.
- ✓ These findings suggest that pan-14-3-3 inhibition may provide therapeutic benefit specifically in *TP53*-proficient breast cancers.

Conclusions

- ✓ Pan-14-3-3 inhibition triggers *TP53*-dependent transcriptional reprogramming under genotoxic stress.
- ✓ These findings support *TP53*-guided therapeutic strategies targeting the 14-3-3 family in breast cancer.

Contact

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References

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