Multifaceted Therapeutic Approaches Targeting Oncogenic Pathways to Combat Triple-Negative Breast Cancer

Arisha Arora[1], Siddhartha Sankar Ghosh[1,2]*

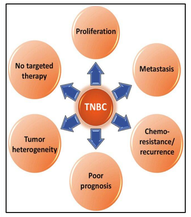
[1] *Department of Biosciences and Bioengineering,* [2] *Centre for Nanotechnology, Indian Institute of Technology Guwahati, IITG, Guwahati-781039, Assam, India*

Correspondence to: Prof. Siddhartha Sankar Ghosh ***(Email: sghosh@iitg.ac.in)***

Abstract No: 862 Presentation No: PS5-03-27

MOTIVATION

Need for novel targeted therapies for TNBC

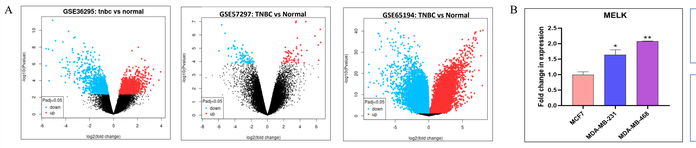


- Triple-negative breast cancer (TNBC) is the **most aggressive** subtype of breast cancer linked to high incidence of **metastatic cases**
- Therapeutic options are limited due to absence of conventional receptors (ER, PR and HER2)
- Identifying promising **biological targets** and **novel therapeutic approaches** is crucial
- Signalling pathways such as **Wnt and MAPK** represent emerging paradigms in TNBC treatment, converging to drive key cancer hallmarks
- We propose to employ drug repurposing and protein therapy as novel therapeutic strategies to combat TNBC

RESULTS

1. Drug repurposing against MELK as a promising therapeutic approach

Microarray analysis reveals significant DEGs in TNBC, highlighting MELK as an upregulated oncogene



Key Protein Interactors of MELK by String Network: AURKA, Cyclin B1, Cyclin B2, FOXM1, CDK1,

Pathway Analysis of MELK by EnrichR: Cell Cycle, p53 signaling, FOXM1 signaling, cellular senescence

Fig. 1: (A) Volcano Plot represents fold change in DEG's in selected databases, determined by GEO-based microarray analysis (B) MELK expression, determined by qRT-PCR

Molecular Docking and Simulation-based screen of 1293 FDA-approved drugs against MELK

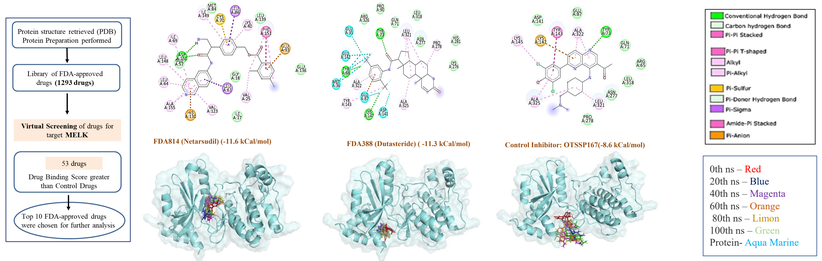
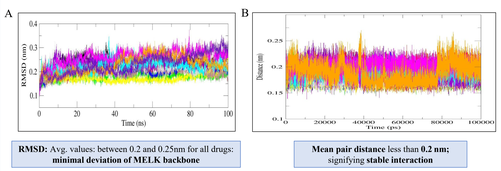


Fig. 2: 2D interaction profile and 3D interaction of top drugs and control with 20 ns time interval for 100 ns in MELK kinase domain



Drug	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
FDA814	-214.696	-32.753	172.168	-20.044	**-123.224**
FDA576	-209.339	-70.123	206.734	-21.601	-94.329
FDA388	-180.107	-19.679	113.485	-20.793	**-107.093**
FDA353	-162.346	-40.679	157.658	-19.756	-65.123
FDA680	-165.763	-57.481	175.547	-22.580	-70.277
FDA804	-212.580	-36.378	167.686	-23.180	-104.253
FDA911	-199.743	-38.586	163.951	-22.791	-97.088
FDA276	-176.879	-99.500	218.524	-21.403	-79.258
FDA880	-214.943	-42.219	193.903	-22.791	-86.050
FDA100	-184.039	-6.818	118.480	-20.462	-92.839
Control Inhibitor	-152.511	-35.574	135.002	-14.872	-67.756

RMSD: Avg. values: between 0.2 and 0.25nm for all drugs: **minimal deviation of MELK backbone**

Mean pair distance less than **0.2 nm;** signifying **stable interaction**

MMPBSA analysis shows **lowest binding energy for drugs: Netarsudil and Dutasteride**

Fig. 3: MDS parameters over 100 ns. (A) RMSD analysis (B) Pair distance between the MELK and 10 drugs (C) MMPBSA free energy analysis of top 10 drugs and control

Experimental validation of top repurposed drugs: Netarsudil and Dutasteride

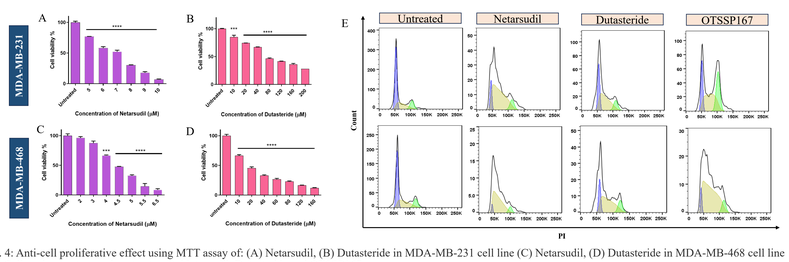


Fig. 4: Anti-cell proliferative effect using MTT assay of: (A) Netarsudil, (B) Dutasteride in MDA-MB-231 cell line (C) Netarsudil, (D) Dutasteride in MDA-MB-468 cell line (E) Cell cycle arrest upon drug treatment: Netarsudil, Dutasteride and OTSSP167

RESULTS

Experimental validation of the repurposed drugs

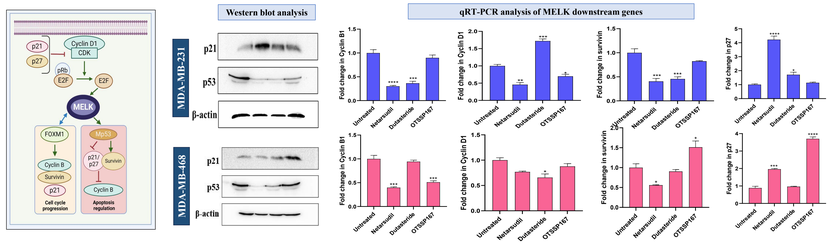
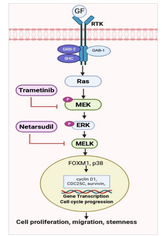


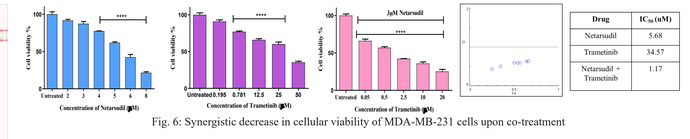
Fig. 5: Representation of functional network of MELK and its regulation of downstream target genes in TNBC. Immunoblots depicting changes in the expression of p21 and p53. qRT-PCR study shows expression level changes in cell cycle and apoptosis marker genes: cyclin B1, cyclin D1, p27 and survivin in TNBC cells following treatment

The findings depict potential of Netarsudil and Dutasteride as repurposed drugs against MELK to combat TNBC

2. Synergistic Combination Therapies inhibiting activated MAPK and Wnt signaling to combat TNBC

A. Vertical inhibition of MAPK pathway components MELK and MEK kinases





Drug	IC_{50} (nM)
Netarsudil	5.68
Trametinib	34.57
Netarsudil + Trametinib	1.17

Fig. 6: Synergistic decrease in cellular viability of MDA-MB-231 cells upon co-treatment

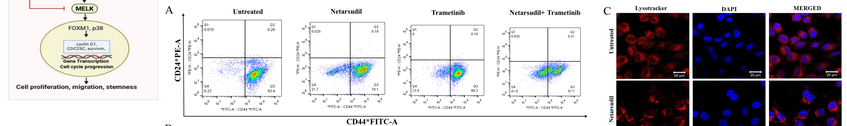
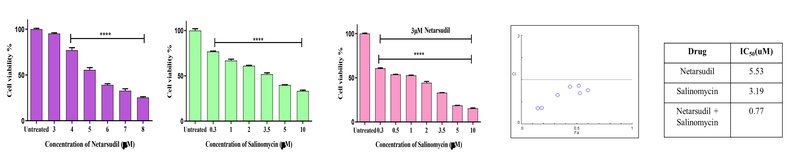


Fig. 7: Anti-cancer effects of the co-treatment:
(A) Synergistic decrease in cancer stemness of aggressive MDA-MB-231 cells
(B) Downregulation of MAPK signalling molecules along with induction of autophagy
(C) Induction of lysosomal dysfunction upon treatment

B. Targeting parallel pathways: MAPK and Wnt signaling to combat TNBC



Drug	IC_{50}(uM)
Netarsudil	5.53
Salinomycin	3.19
Netarsudil + Salinomycin	0.77

Fig. 8: Synergistic decrease in cellular viability of MDA-MB-231 cells upon co-treatment

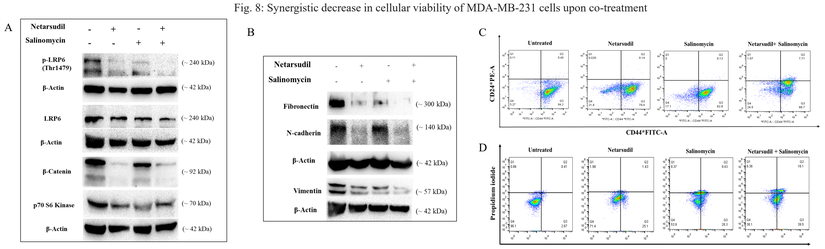


Fig. 9: Anti-cancer effects of the co-treatment: (A) Downregulation of Wnt and MAPK molecules (B) Marked decrease in expression of mesenchymal markers (C) Decrease in stemness of MDA-MB-231 cells (D) Induction of Apoptosis upon co-treatment

The findings depict drug combination strategies using Netarsudil against oncogenic pathways to combat TNBC

RESULTS

3. Protein Therapy to restore Wnt signaling pathway suppression in TNBC

Downregulated PEDF expression in TNBC

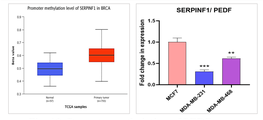


Fig. 10: Promoter methylation of PEDF gene leading to epigenetic downregulation in TNBC cells

Heterologous expression and purification of rPEDF in *E.coli* cells

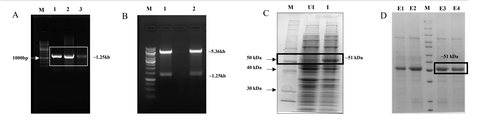


Fig. 11: (A) Clone confirmation by PEDF gene amplification by PCR (B) double digestion (C) IPTG-induced PEDF overexpression in *E. coli* cell lysates (D) rPEDF protein purified via Ni–NTA chromatography

Characterization of rPEDF

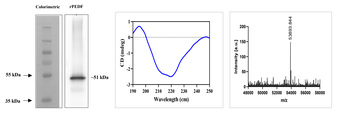
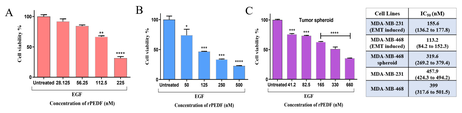


Fig. 12: Confirmation of rPEDF structure by Western Blot, CD spectra analysis and MALDI-TOF study

Anti-proliferative effect of rPEDF in TNBC cells and tumor spheroid



Cell Lines	IC_{50} (nM)
MDA MB 231 (EMT induced)	155.4 (136.2 to 177.8)
MDA MB 468 (EMT induced)	113.2 (84.2 to 152.3)
MDA MB 468 spheroid	319.4 (269.2 to 379.4)
MDA MB 231	457.2 (424.3 to 494.2)
MDA MB 468	382 (317.6 to 501.5)

Fig. 13: Dose-dependent decrease in viability of TNBC cells (A) MDA-MB-231 (B) MDA-MB-468 and (C) 3D tumor spheroid upon rPEDF treatment

rPEDF treatment inhibits Wnt signaling and EMT in TNBC

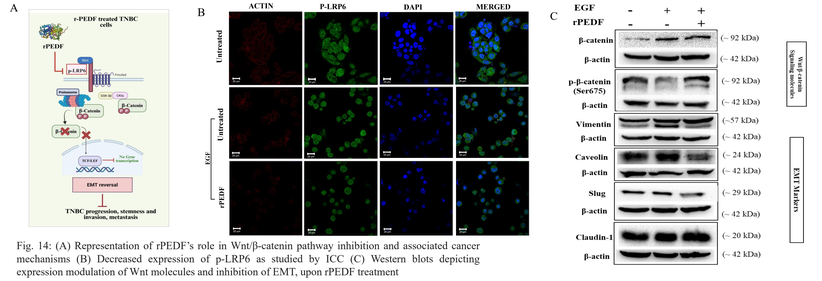


Fig. 14: (A) Representation of rPEDF's role in Wnt/β-catenin pathway inhibition and associated cancer mechanisms (B) Decreased expression of p-LRP6 as studied by ICC (C) Western blots depicting expression modulation of Wnt molecules and inhibition of EMT, upon rPEDF treatment

Broad anti-cancer effects achieved in TNBC upon rPEDF treatment

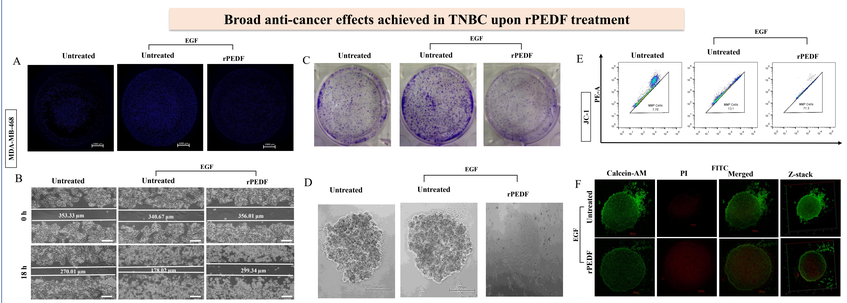


Fig. 15: Reduction in (A) invasion ability (B) migration potential (C) colony forming, and (D) sphere forming ability of TNBC cells after rPEDF treatment (E) Oxidative stress-induced depolarization of mitochondrial transmembrane potential in treated TNBC monolayers (F) cytotoxity in tumor spheroids upon rPEDF treatment

The study presents rPEDF as a promising protein-based therapeutic to suppress TNBC-associated Wnt pathway and EMT

DISCUSSION

- The study highlights the **therapeutic promise of repurposed drugs** (Netarsudil, Dutasteride and Salinomycin) and **recombinant PDEF** protein in targeting **MAPK and Wnt** pathways to suppress TNBC cell proliferation and metastasis.
- These approaches demonstrate strong translational potential, bridging the gap between the **identification of therapeutic targets and the development of tailored treatment options** for managing TNBC.

REFERENCES

1. Arora et al., (2025), *Advanced Therapeutics,*e00173
2. Pitner et al., (2017), *Expert Opin Ther Targets,* 21: 849-859
3. Boshuizen et al., (2022), *Cell Press,* 2020.05.031
4. Sen et al.,(2023), *J Biomol Struct Dyn,* 2192810

ACKNOWLEDGEMENT

We acknowledge the Department of Biotechnology, **Government of India** for the Translation Programme, IITG. We also acknowledge the **Param-Ishan facility** of IIT Guwahati. Additionally, we acknowledge the **Prime Minister's Research Fellow (PMRF) program for a research grant for supporting Arisha Arora** (PMRF ID-1901673)