Simultaneous Inhibition of EZH2 and Activation of Dopamine D1 in an Isotropic Triple-Negative Breast Cancer Microgel Model

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Introduction

Enhancer of zeste homolog 2 (EZH2) is the enzymatically active core subunit of the PRC2 complex, encompassing EED, SUZ12, RbAp46 and RbAp48[1].

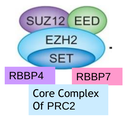


Figure 1b: *EZH2 regulates transcriptional activity: as part of PRC2, EZH2 methylates histone 3 at lysine 27 (H3K27), which contributes to transcriptional silencing, EZH2 also has a PRC2-independent role in transcriptional activation and is capable of methylating a number of non-histone protein substrates. OFF and ON refer to transcriptional silencing and activation, respectively. PMID: 26845405*

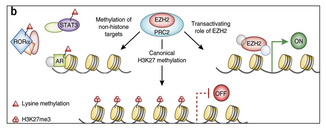


Figure 1A: *A.)* Polycomb group proteins 2 complex*; SET domain provides a catalytic site for EZH2 to trimethylate H3 at K27 and K9. This recruits the histone binding proteins RBBP4 and RBBP7.*

EZH2 is upregulated in Triple Negative Breast Cancers (TNBC) is a potential driver of metastasis[2].

Normal epithelial tissues form well-organized polarized single cell layers regulated by the surrounding microenvironment and extracellular matrix (ECM).

In cancer progression, proliferation and invasion into ECM disrupts the well-organized single cell layers. Conventional *in vitro* models utilize monolayers to investigate the effect of drugs on cancer cells but does not accurately recapitulate the *in vivo* microenvironment as the cells are subjected to homogeneous growth conditions.

Microgels are miniature and compartmentalized hydrogels that reproduce the viscoelastic properties of decellurized tissue in completely isotropic cell culture conditions.

Dopamine D1 receptor (D1R) activation in TNBC cell line induces apoptosis, autophagy, inhibit the invasion, and regress mammary tumors[3].

The oncogenic nature of EZH2 in driving aggressiveness in TNBC cells led us to simultaneously target D1R and EZH2 to completely ablate tumor growth and metastasis.

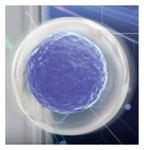
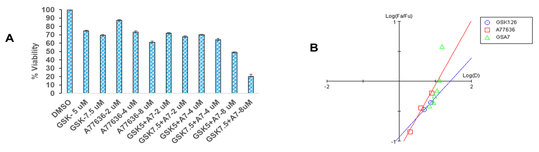


Figure 1C: *The microgel system yields a systematic method to investigate cells grown in truly isotropic conditions for applications across various disease models, yielding physiological relevance and tissue mimicry as opposed to traditional 2D monolayers. Figure adapted from Rima, et. al, Lab on a Chip.[5]*

Hypothesis

Activation of dopamine D1 receptor and simultaneous inhibition of EZH2 will be highly effective and efficacious in suppressing TNBC progression and subsequent metastasis leading to TNBC tumor ablation.

Combination of D1R agonist A77636 and EZH2 inhibitor GSK126 synergistically inhibit TNBC cell viability.



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Dose GSK126	Dose A77636	Effect	CI
5.0	2.0	0.28012	1.21661
7.5	2.0	0.36197	0.97484
5.0	4.0	0.30596	1.40619
7.5	4.0	0.41258	0.97615
5.0	8.0	0.50807	0.85746
7.5	8.0	0.79358	0.23617

Figure 2: *GSK126 and A77636 synergistically inhibit TNBC cell viability. MDA-MB-231 cells were treated individually and in combination with GSK126 and A77636 for 72h. MTT assay was performed to assess the cell viability. The Chou-Talalay method[4] was applied to determine the combination index to demonstrate synergy of these two agents.*

GSK126 and A77636 inhibit TNBC cell migration.

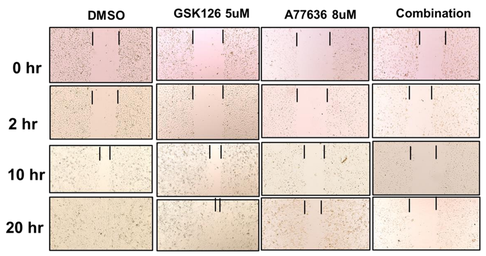

Figure 3*: Effect of EZH2 inhibitor GSK126 and A77636 alone or in combination on migration potential in MDA-MB-231 cells. Cells were treated with the indicated concentrations of GSK126 and A77636 alone and in combination and wound healing assay was performed. Representative photograph at each dose and time point is provided.*

SKF38393 and GSK126 disrupts PRC2 complex

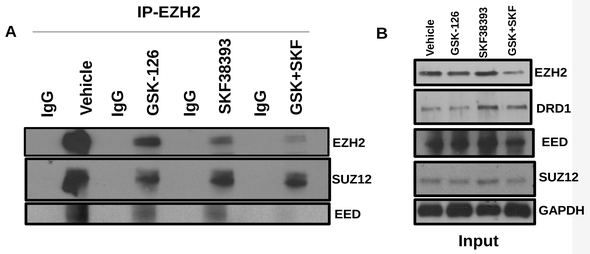

Figure 4: *Effect of GSK126 and SKF38393 alone or in combination on the protein expression of EZH2, SUZ12 and EED after immunoprecipitation with EZH2 in MDA-MB-231 cells. A) MDA-MB-231 cells treated with 10 μM GSK126 or 100 nM SKF38393 alone and in combination for 48 immunoprecipitated with EZH2. Immunoblot probed for the indicated antibodies. B) 25μg lysate was subjected to Western Blotting and probed for the indicated antibodies as input. GAPDH, loading control as input.*

Engineering a spheroid-inducing system

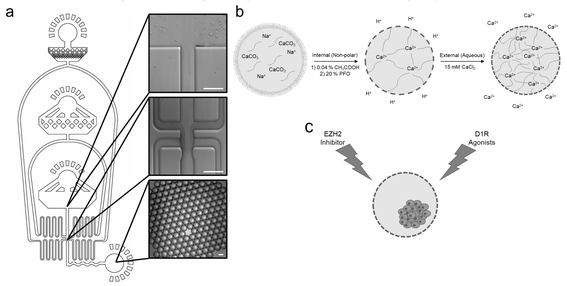

Figure 5: *Spheroid-inducing isotropic system. (a) Flow-focusing microfluidic droplet generation of monodisperse isotropic microgels. (b) Crosslinking schematic. (c) The proposed mechanism for drug screening via 3D culture. All scale bars are 100 μm. Figure adapted from* Rima, et. al, Lab on a Chip.[5]

GSK126 and D1R agonists suppress tumor growth

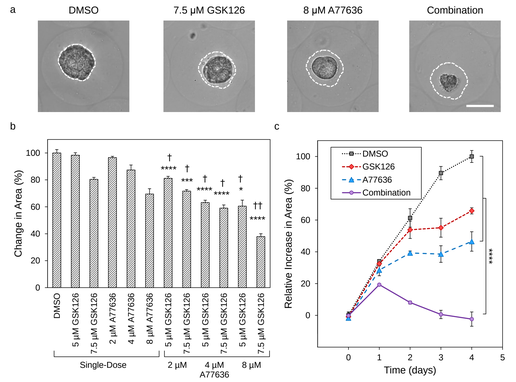

Figure 6: *Combination therapy in 3D microgel culture. (a) Representative images of microgels under GSK126 and A77636 treatment. (b) Changes in tumor area as a function of combinatorial treatment with GSK126 and A77636. (c) Growth kinetics under GSK126 and A77636 drug treatment demonstrates tumor reduction for dual therapy.*

Combination of A77636 and GSK126 induce cell death mediated by necrosis

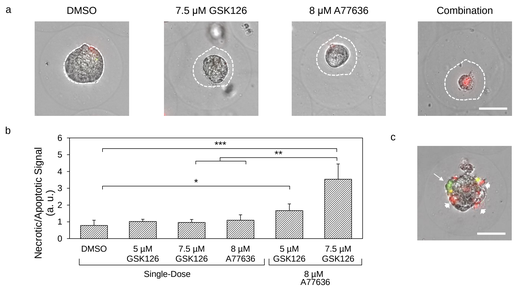

Figure 7: *Reduction in size of tumor is mediated by cell death via necrosis instead of apoptosis as defined by Yo-Pro and ethidium homodimer-1 staining. (a) Representative images. (b) Quantification of necrotic/apoptotic signal. (c) Identification of necrotic and apoptotic cells.*

Combination therapy degrades EZH2

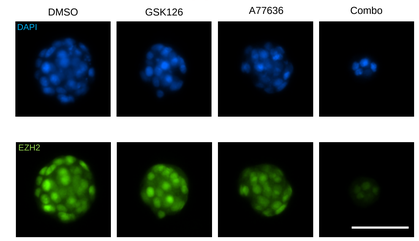

Figure 8: *Combination therapy leads to the degradation of EZH2. Representative images of treated spheroids and immunofluorescence; DAPI (blue) EZH2 (green).*

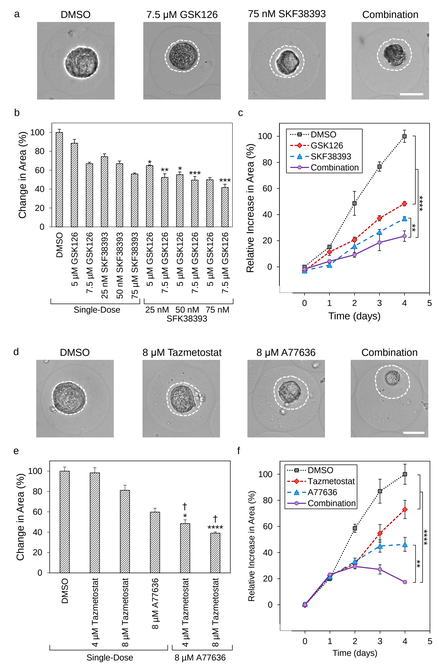

Figure 9: *Combination therapy with other D1R agonists (SKF38393) and an FDA-approved EZH2 inhibitor (Tazmetostat). (a) Representative images of microgels under GSK126 and SKF38393 treatment. (b) Changes in tumor area as a function of combinatorial treatment with GSK126 and SKF38393. (c) Growth kinetics under GSK126 and SKF38393 drug treatment demonstrates tumor reduction for dual therapy. (d) Representative images of microgels under Tazmetostat and A77636 treatment. (e) Changes in tumor area as a function of combinatorial treatment with Tazmetostat and A77636. (f) Growth kinetics under Tazmetostat and A77636 drug treatment demonstrates tumor reduction for dual therapy.*

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