The Epigenetic Regulation of Mesenchymal and Stem Cell Like Properties

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Background

Epithelial-Mesenchymal Transition (EMT) is a biological program that induces epithelial cells to morph into a mesenchymal stem cell-like phenotype.[2] Recently our lab identified that cell division is critical for the gain of stem cell properties during EMT but not for mesenchymal properties. Epigenetic programming is critical for both cell division and EMT regulation. Epigenetic regulators include various histone or chromatin remodeling proteins such as EZH2, HDACs, and DNMTs.

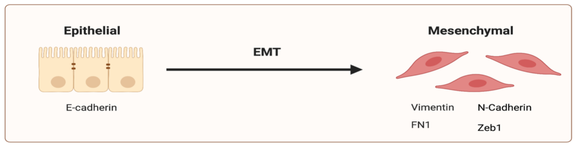


Figure 1: Basics of EMT (Epithelial to Mesenchymal Transition. [6]

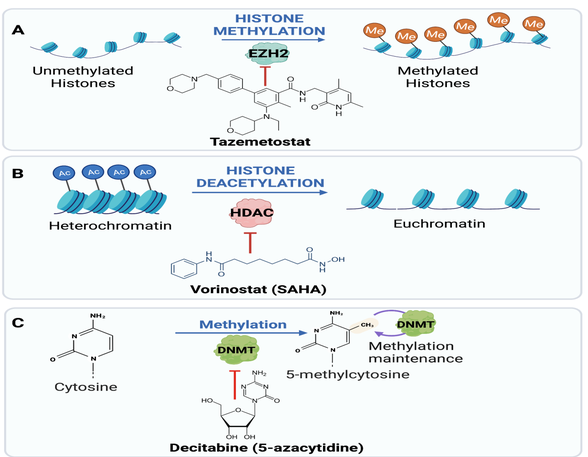


Figure 2: A) EZH2 (Enhancer of Zeste Homolog 2) is an enzyme that participates in histone methylation. Inhibition of EZH2 by Tazemetostat results in a prevention of histone methylation.[5] **B)** HDACs (Histone Deacetylases) are a class of enzymes that catalyze the removal of acetyl groups from histone proteins. Inhibition of HDACs by the drug Vorinostat results in a persistence of acetylation of histone proteins.[3] **C)** DNMTs (DNA methyltransferases) are a class of enzymes that catalyze the transfer of methyl groups to DNA. Decitabine is a DNMT inhibitor which ultimately prevents the methylation of DNA. [4]

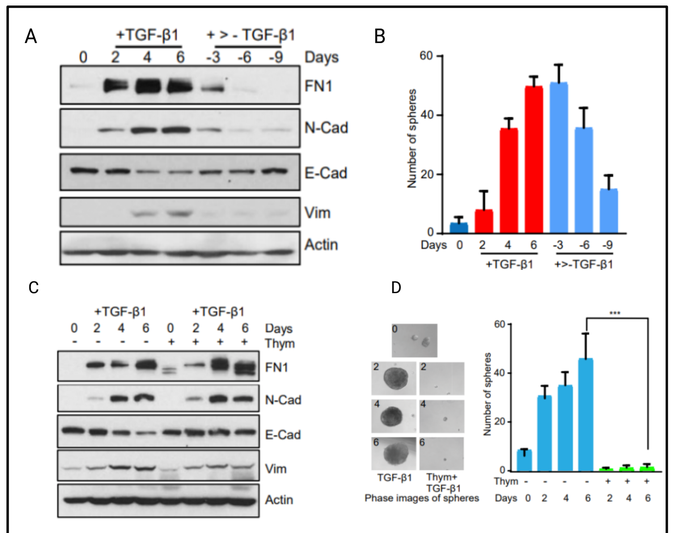


Figure 3: A) Western blot showing time course of TGF-β1 induction of EMT in MCF10A cells, leading to acquisition of mesenchymal properties by the normally epithelial cell line. **B)** The mammosphere data demonstrated acquisition of stemness properties upon TGF-β1 induction of EMT in MCF10A cells. **C)** MCF10A cell lines were exposed to TGF-β1 for 6 days in which they exhibited mesenchymal markers. This was not changed upon cell division inhibition. **D)** Upon cell division inhibition in MCF10A treated with 6 days of TGF-β1, stemness properties were inhibited. **Cell division inhibition leads to no change in mesenchymal properties, but is capable of inhibiting stemness acquisition in MCF10A cells[1].**

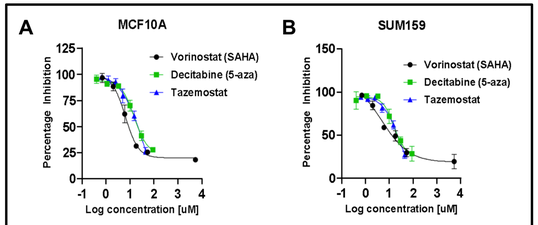
Hypothesis

Due to the link of epigenetic regulation in both EMT and cell division, we hypothesize that a particular class of epigenetic inhibitors will be able to unlink the gain of mesenchymal and stemness properties.

Methods

- EMT-enriched cell line: SUM159 cell line
- Epithelial cell line: MCF10A. EMT was induced using TGF-β1 for 6 days.
- Inhibitors Tazemetostat, Vorinostat, and Decitabine were utilized on cells.
- IC_{50} was determined using IncuCyte
- Protein analysis by Western Blot
- Stemness analysis by Mammosphere assay

Results



uM	SAHA (vorinostat)	Decitabine (5-aza)	Tazemetostat
IC50	5.4	15	30
IC25	1.4	8	10

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IC25	1	8	10

Figure 4: A) Dose response curve of cell viability upon drug treatment in MCF10A + 6 days TGF-β1, with calculated IC_{50} and IC_{25}. **B)** Dose response curve of cell viability upon drug treatment in SUM159, with calculated IC_{50} and IC_{25}. [6]

Mesenchymal Properties

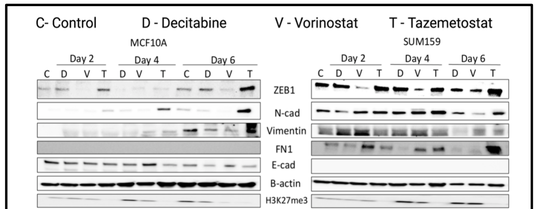

Figure 5: Western blot analysis of drug treated MCF10A and SUM159. Decitabine provided conflicting results, with more studies required. Vorinostat was capable of inhibiting mesenchymal properties and Tazemetostat has no effect on mesenchymal properties acquisition. [6]

Stemness Properties

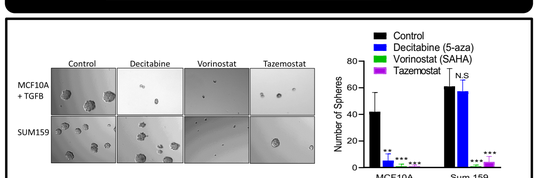

Figure 6: Mammosphere assay was conducted on drug treatment cell lines for period of 7 days. Results indicate that Decitabine prevents MCF10A cells from the gain of stemness properties, but is not able to revert the gain of stemness properties already found in SUM159. Vorinostat and Tazemetostat are capable of inhibiting stemness properties in both cell lines. [6]

Conclusions

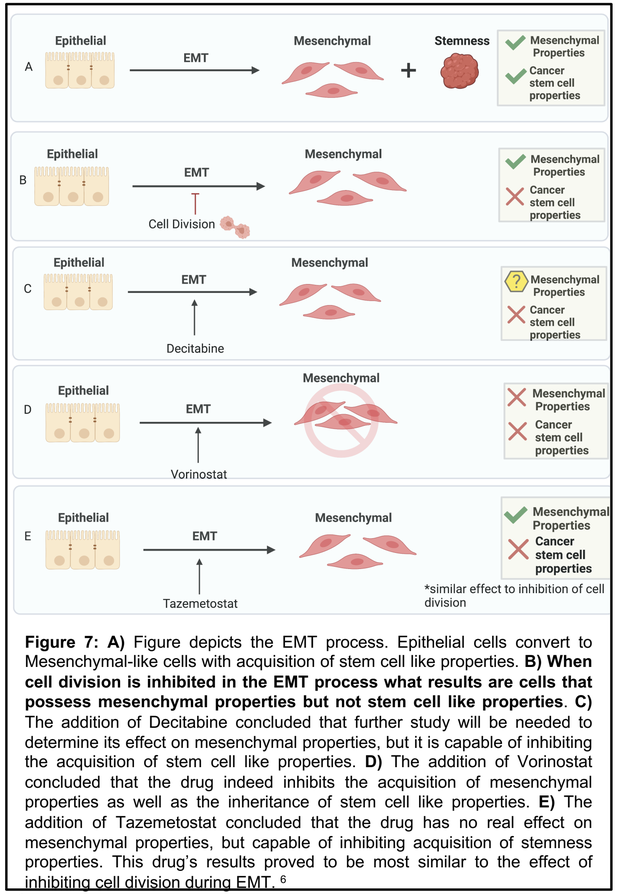

Figure 7: A) Figure depicts the EMT process. Epithelial cells convert to Mesenchymal-like cells with acquisition of stem cell like properties. **B) When cell division is inhibited in the EMT process what results are cells that possess mesenchymal properties but not stem cell like properties**. **C)** The addition of Decitabine concluded that further study will be needed to determine its effect on mesenchymal properties, but it is capable of inhibiting the acquisition of stem cell like properties. **D)** The addition of Vorinostat concluded that the drug indeed inhibits the acquisition of mesenchymal properties as well as the inheritance of stem cell like properties. **E)** The addition of Tazemetostat concluded that the drug has no real effect on mesenchymal properties, but capable of inhibiting acquisition of stemness properties. This drug's results proved to be most similar to the effect of inhibiting cell division during EMT. [6]

References

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6. Images Created with BioRender.com

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