Background

• The epithelial-mesenchymal transition (EMT) is a process activated during cancer that allows cells to acquire migratory capacities, stem cell properties, and increased metastatic potential.
  • Epithelial cells possess more E-cadherin, an epithelial protein, and cytokeratin (keratin protein in tissues).
  • Mesenchymal cells have more N-cadherin, a mesenchymal extracellular protein, and vimentin (intermediate filament protein).
• Along the EMT spectrum, there exists a hybrid E/M cell that exhibits advantageous plasticity, the ability to transit between states.
• Transforming growth factor-beta 1 (TGFβ1) induces EMT through ECM remodeling, and SB43152 inhibits the TGFβ1 signaling pathway, pushing cells to undergo mesenchymal-epithelial transition (MET).
• The cellular response to EMT-MET induction is unknown within cancer cells without plasticity.
• Another facet of hybrid E/M cells is the expression of epithelial and mesenchymal structural cellular markers, such as cytokeratin and vimentin filament proteins, respectively.
• The putative interaction of these markers is not understood in hybrid E/M cells.

Hypothesis

I hypothesize that hybrid E/M cells with plasticity will be more resilient to apoptotic stress altering the EMT state relative to non-plastic cells, and they will possess co-expression of vimentin and cytokeratin filaments.

Materials & Methods

Reagents
  • TGFβ1
  • SB431542 (TGFβ1 inhibitor)
  • Caspase-3/7 green dye (apoptotic marker)
  • Keratin 14 antibody
  • Vimentin antibody

Flow Cytometry was used to collect single cells of D2.A1 and D2.OR for scanning of E-Cadherin and N-Cadherin markers.

D2.A1 and D2.OR cells were imaged for confluence and Caspase-3/7 activity by Incucyte® for six days under the conditions of no treatment, treatment with TGFβ1, and treatment with SB431542.

The D2.A1 and D2.OR cells were then stained to be imaged for Keratin 14 (red) and Vimentin (green) using microscopy.

Figure 1. The epithelial-mesenchymal transition includes extracellular matrix remodeling of filament proteins, cytokeratin and vimentin. The hybrid E/M cell state likely exhibits a co-localization of the epithelial marker (cytokeratin) and the mesenchymal marker (vimentin).

Figure 2. D2.A1 and D2.OR cells were assessed for epithelial and mesenchymal markers using flow cytometry to establish their EMT status. D2.A1 predominantly co-expresses the epithelial and mesenchymal marker (EN+), thereby making it a hybrid E/M model. However, the majority of the D2.OR cells only express the mesenchymal marker (N+).

Figure 3. (A) D2.A1 and D2.OR cells were treated with TGFβ1 and SB431542 to determine their capabilities to transit between states on the E/M spectrum. Caspase-3/7 green dye was used to showcase cell viability through Incucyte® data. D2.OR cells faced higher apoptosis levels, whereas D2.A1 cells had a higher viability. (B) Normalized caspase activity, calculated as Caspase Levels/Confluency, was determined by the Incucyte® over time for D2.A1 and D2.OR cells per condition of treatment.

Figure 4. The D2.OR and D2.A1 cells were imaged through immunofluorescent microscopy for structural markers, Keratin 14 (red, epithelial) and vimentin (green, mesenchymal). D2.OR cells predominantly expressed vimentin independent of treatment with TGFβ1 or SB431542. D2.A1 cells expressed a co-localization of vimentin and keratin when untreated. Once treated with TGFβ1, D2.A1 cells attained mesenchymal-like properties and expressed vimentin, and they gained epithelial-like properties expressing keratin under the treatment of SB431542.

Conclusion

• Apoptotic resistance within D2.A1 hybrid E/M cells in comparison to D2.OR mesenchymal cells exemplifies the advantageous flexibility held by cancer cells with plasticity on the E/M spectrum.
• The co-localization of Keratin 14 and vimentin expressed by D2.A1 cells supports an understanding of the nature of structural plasticity.
• The ability for D2.A1 cells to express mesenchymal-like features with TGFβ1 and become epithelial-like under the treatment of SB431542 demonstrates the hybrid E/M cellular adaptivity within a tumor microenvironment.

References


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