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Cell migration dynamics after alteration of cell-cell contacts in fibrosarcoma and glioblastoma cell lines

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Cell Migration Dynamics After Alteration of Cell-Cell Contacts in Fibrosarcoma and Glioblastoma Cell Lines

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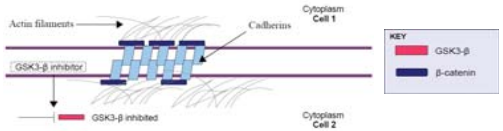
Abstract

Cell migration is a vital component of metastasis. In this study, our intent was to study cell migration by alteration of the Wnt/GSK-3 Pathway. Since $BeSO_4$ is a known GSK-3 kinase inhibitor, we hypothesized that this agent would cause cell migration to decrease as a result of β-catenin stabilization. Two human cell lines, HT-1080 (fibrosarcoma) and A172 (glioblastoma), were used to observe migration levels in the presence and absence of $BeSO_4$. Our results show that cell migration is diminished for cells that were pre-treated with $BeSO_4$, in comparison to the untreated (control) cells.

Background

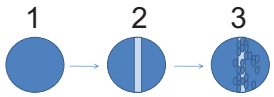
In order for a cell to migrate, during metastasis or under other conditions, cell-cell contacts must be broken, and cell-substrate contacts (i.e. focal adhesions) must be formed to give the moving cell traction. An enzyme known as GSK-3 kinase regulates the function of a bi-functional protein called β-catenin. In the cytoplasm, β-catenin serves as a key structural component of adherens junction, the points of contact between neighboring cells. These cell-cell contacts contain transmembrane cadherins that link to dynamic membrane and cytoskeletal substructures, of which β-catenin is a key constituent. Under normal conditions, GSK-3 kinase phosphorylates β-catenin, which then undergoes ubiquitin-dependent proteolysis. However, using a GSK-3 kinase inhibitor, such as $BeSO_4$, would allow for the stabilization of β-catenin, and thus potentially increase cell-cell adhesion and decrease cell migration.



Methods

- Coulter Counter
  - Used to count cells in order to assess effects on cell division.
  - $BeSO_4$ is known to affect cell division in many cell types, but its effect on HT-1080 cells has never been studied.
- In Vitro FluoroBlok Tumor Cell Migration Assay
  - $BeSO_4$ treated and untreated cells were allowed to migrate from the apical surface to the basal surface through the membrane via 3 µm pores. Cells that had migrated to the basal surface were stained with a fluorescent dye and mean fluorescence was measured to determine cell migration.
- Wound-Healing Assay
  - A pipette tip was used to make an initial wound (cell-free area), which was then monitored over time to observe the amount of cell migration.

1. Grow cells to preferred confluence in +/- $BeSO_4$ media.
2. Use a pipette tip to scrape away cells in a line across the dish.
3. Observe cell migration back into the cleared area.



Results

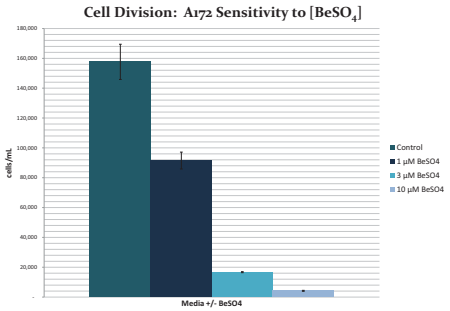


Figure 1a. *Low micromolar $BeSO_4$ inhibits cell proliferation in glioblastoma cells. A172 cells were dosed with $BeSO_4$ on Day 0 and counted after 9 days with 1:4 splits every 3 days.*

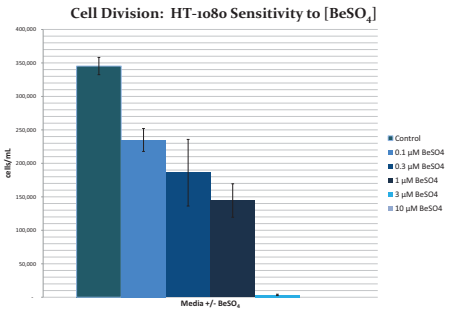


Figure 1b. *Low micromolar $BeSO_4$ inhibits cell proliferation in fibrosarcoma cells. HT-1080 cells were dosed with $BeSO_4$ on Day 0 and counted after 8 days with 1:8 splits every 2 days.*

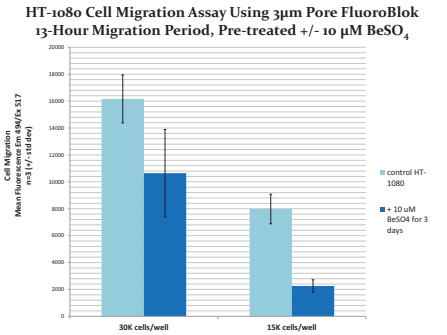


Figure 2a. *Untreated and 10µM $BeSO_4$ (3-days pre-treated) HT-1080 cells were allowed to pass through 3µm pores within the membrane via cell migration. Cells that had migrated to the basal side of the membrane were stained with a fluorescent dye, and the mean fluorescence was measured to quantify cell migration. Cells were seeded into the migration assay chambers at higher density (30,000 cells/well) and lower density (15,000 cells/well) to determine the optimal conditions for the experiment.*

Figure 3a. *HT-1080 cells were pretreated with +/- $BeSO_4$ for 36 hours. Plates were approximately 80-85% confluent. A wound was made at t=0 (using a pipette tip) and cell migration was observed at t=0, t=3, t=6, and t=9 hr.*

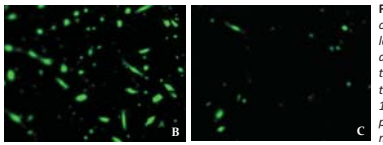


Figure 2 (b & c). *Microscopy of basal side of membrane, showing fluorescently-labeled cells that had migrated from the apical side. B) Untreated HT-1080 cells that had passed through the 3µm pores in the membrane. C) Pre-treated (3 days) 10µM $BeSO_4$ HT-1080 cells that had passed through the 3µm pores in the membrane.*

Figure 3b. *A172 cells were pretreated with +/- $BeSO_4$ for 74 hours. Plates were approximately 95-100% confluent. A wound was made at t=0 (using a pipette tip) and cell migration was observed at t=0, t=13, and t=23 hr.*

Conclusion

After confirming the sensitivity of A172 and HT-1080 to $BeSO_4$ in the Proliferation Assay, the FluoroBlok Tumor Cell Migration Assay and the Wound-Healing Assay demonstrated that both cell lines show a decrease in cell migration when treated with $BeSO_4$. We hypothesize that $BeSO_4$ treatment causes stabilization of β-catenin, which allows for stronger cell-cell contacts, and therefore decreased cell migration. Further studies are planned to test this hypothesis.

Acknowledgements

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References

Sineva, GS, & Pospelov, VA. (2010). Inhibition of gsk3beta enhances both adhesive and signaling activities of beta-catenin in mouse embryonic stem cells. *Biology of the Cell*, *102*(10), 549-560.

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