# Functional Study of a Voltage-Gated Potassium Channel during Medulloblastoma Cell Migration

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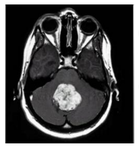
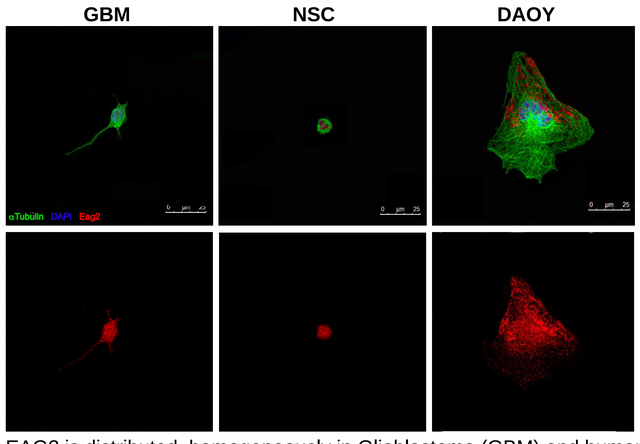
## Medulloblastoma (MB) and EAG2



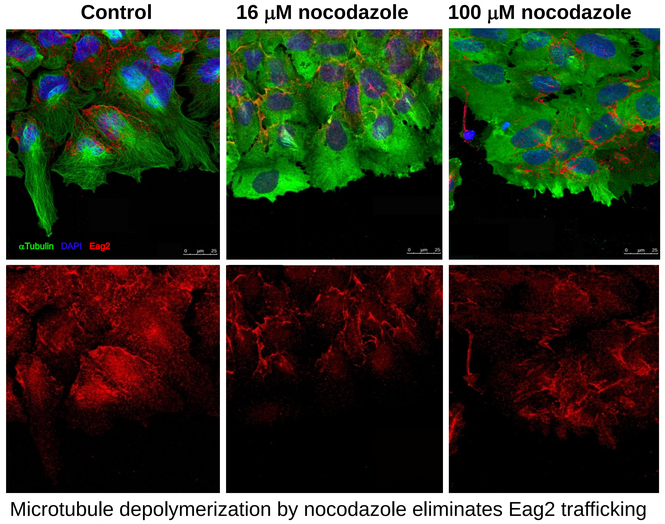
Medulloblastomas (MB), the most common pediatric central nervous system (CNS) tumor, is a neuroepithelial tumor [1] characterized by its rapid progression, aggressive nature, and tendency to metastasize along the brain-spinal cord axis. Little is known about the functional effectors driving deregulated MB cell migration and metastasis. Studies with mouse MB tumor models have revealed that the expression of EAG2, a voltage-gated potassium channel, is markedly upregulated during MB tumorigenesis. Abundant expression of the EAG2 gene has also been detected in MB human specimens [2]. In the present work, we have studied the functional role of EAG2 using two established human MB cell lines, DAOY and VMB11.

### EAG2 is localized at the trailing edge in migrating MB cells



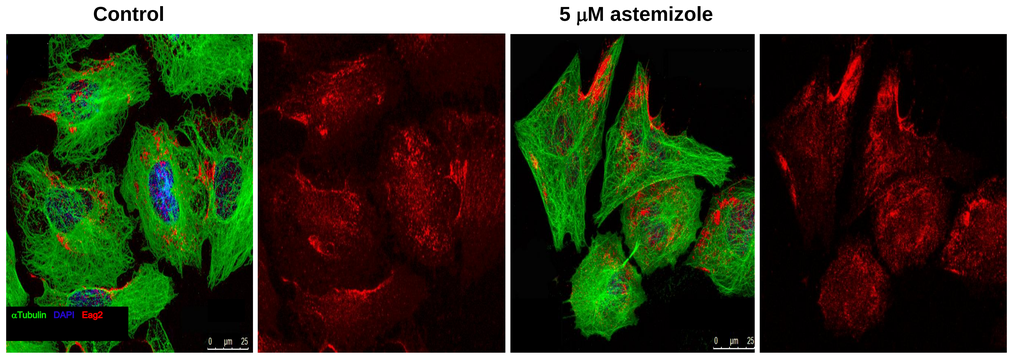

EAG2 is distributed homogeneously in Glioblastoma (GBM) and human neural stem cells (NSC), while localized at the trailing edge in migrating MB cells (DAOY).

### EAG2 enrichment to the trailing edge is microtubule dependent



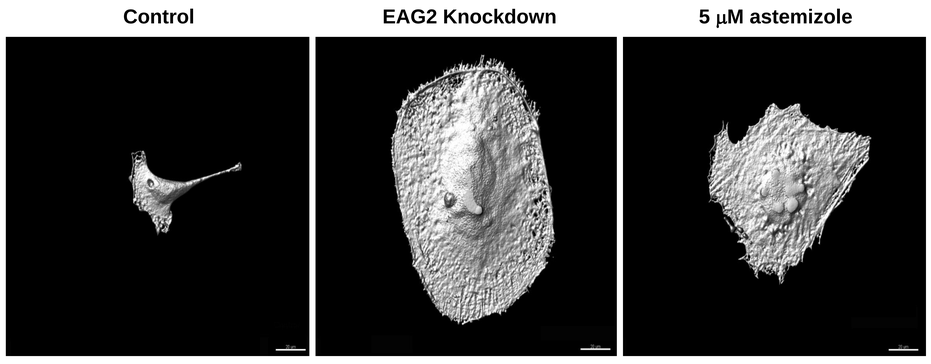

Microtubule depolymerization by nocodazole eliminates Eag2 trafficking to the trailing edge of MB cells .

### EAG2 localization at the trailing edge does not depend upon $K^+$ channel activity



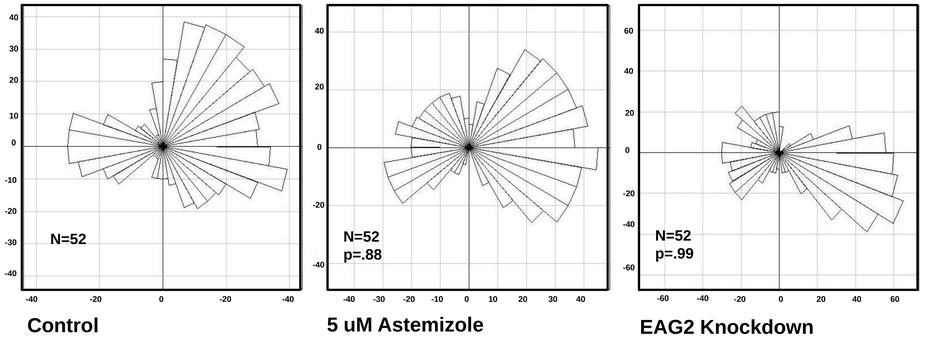

Astemizole, an Eag2 channel inhibitor, did not affect EAG2 enrichment to the trailing edge of VMB11 cells, suggesting that Eag2 trafficking is independent of Eag2 channel activity.

### EAG2 regulates cell volume and polarity during MB migration



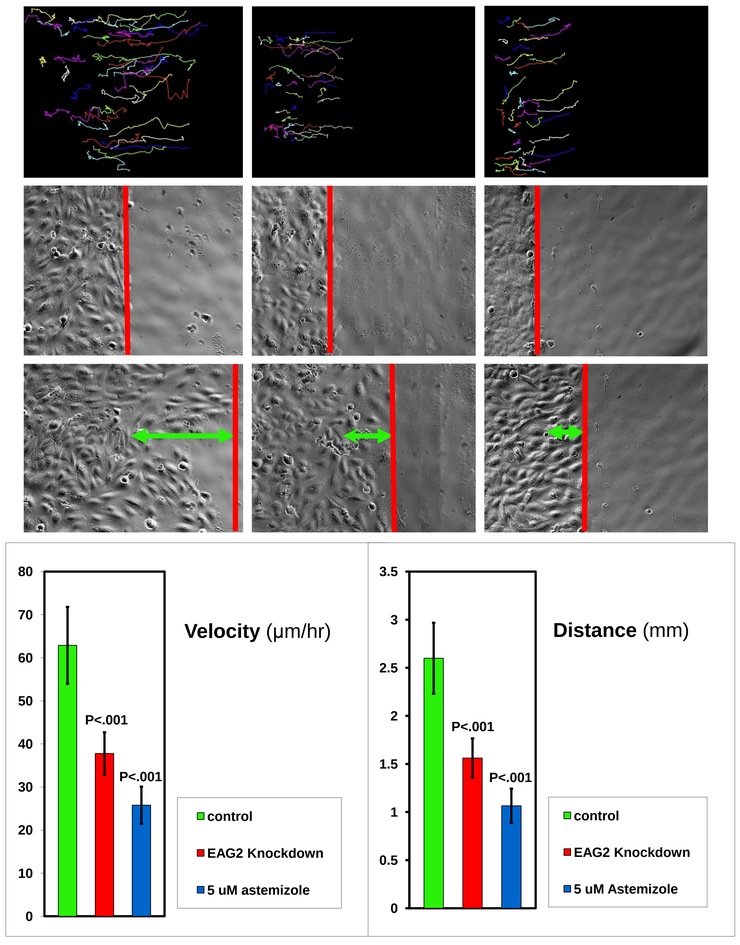

3D reconstruction of fixed VMB11 cells showing increase in cell volume [3] and loss of polarity due to EAG2 knockdown and Eag2 channel inhibition.

### EAG2 does not affect the directionality of migrating MB cells




Directionality, calculated as the ratio of displacement to accumulated distance, did not differ in control VMB11 cells as compared to EAG2 knockdown cells or astemizole treated cells during scratch wound induced migration.

### EAG2 knockdown or channel inhibition impairs the migration of MB cells




We utilized live cell imaging of scratch wound induced migration of VMB11 cells overnight to measure the distance and velocity of migration, which were significantly reduced by EAG2 knockdown and $K^+$ channel inhibition.

## Conclusions

- EAG2 is localized at the trailing edge of MB cells. Its trafficking is microtubule dependent
- $K^+$ channel inhibitor does not affect Eag2 trafficking in MB cells
- EAG2 knockdown and $K^+$ channel inhibitor eliminate the polarity of MB cells and significantly retard their migration
- These findings suggest a possible role for EAG2 in MB cells migration and metastasis.

References

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2. Huang, X., Ketova, T., Litingtung, Y. & Chiang, C. Isolation, enrichment, and maintenance of medulloblastoma stem cells. *J Vis Exp*. 43, 2086 (2010).
3. Huang, X. et al. Voltage-gated potassium channel EAG2 controls mitotic entry and tumor growth in medulloblastoma via regulating cell volume dynamics. *Genes Dev.* 26, 1780-96 (2012).

Acknowledgements

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