Inhibition of Tumor Cell Migration through Slit/Robo/TUBB3 Pathway

Shirley Yee; Tao Yang; Guofa Liu
Department of Biological Sciences
University of Toledo, Toledo, OH

ABSTRACT

Purpose: To investigate the involvement of Slit and Robo signaling in glioblastoma progression.

Studies have shown that the Slit-Robo signaling pathway is important in guiding neural and non-neural cell migration through attraction and repulsion, and more recently has been found that Slit and Robo also have important roles in tumorigenesis, cancer progression, and metastasis. Specifically, Robo1 binds to a tubulin protein known as TUBB3. Though the mechanisms have yet to be fully explored, a connection between the presence of the Slit protein and the rate of cancer cell invasion, specifically glioblastoma, may exist, which can lead to a better understanding of the Slit/Robo/TUBB3 pathway and the promising development of new drugs to target specific cancers. Here, the effects of Slit2 were investigated for human embryonic kidney (HEK) and glioblastoma multiforme (T98G) cells. HEK cells showed no difference with the presence of Slit2 medium in the wound healing assays, but interestingly, Slit2 instead promoted migration in T98G cells. On the other hand, Taxol, a drug that stabilizes microtubules, may play a role in inhibiting cell migration. From the Western blot and immunoprecipitation, it suggests that a connection exists between Robo1-TUBB3 and Slit2 regulation of this interaction.

BACKGROUND

Glioblastoma

- Glioblastomas (GBM) are tumors that arise from astrocytes, the cells that make up the supportive tissue of the brain
- Location: cerebral hemispheres of brain
- Represent about 15% of all primary brain tumors, and more than 50% of all gliomas
- Glioblastoma multiforme is the most malignant
  - Classified as a grade IV glioma, meaning that it is the most aggressive, invasive, and undifferentiated type of tumor
- Little is known about the etiology, treatment is difficult, and disease is usually incurable

Slit-Robo Pathway

- Slit protein is a large secreted factor where Slit2 acts as a ligand for Robo1, which is a highly conserved transmembrane receptor
  - Recent studies reveal that intracellular and extracellular domains of the binding site determine the signaling responses induced by the Slit binding
- Slit-Robo pathway is involved in development and regulating many physiological processes
  - Slit2 is known to regulate cell function which includes cell migration, proliferation, adhesion, and death
- Many studies have shown that the interactions of Slit/Robo inhibit cell invasion and motility in glioblastoma

METHODS AND RESULTS

Wound Healing Assay

Figure 1 | Effects of Slit2 medium on HEK cells.

a) Comparing the before (at 0 hr) and after (at 34 hr), the value of P ≤ 0.0001 (***) in all of the groups except the Slit2 medium group with DMSO, meaning that the data for those groups were statistically significant.

b) Representative images at 4X magnification of HEK scratch assays immediately after the scratches were made and then 24 hours later. Control medium was switched out for Slit2 medium at 0 hours for the Slit2 groups before the scratch. For every milliliter of medium, 0.4 μL of the drug (either DMSO or Taxol) was added.

Figure 2 | Effects of Slit2 medium on T98G cells.

a) Comparing the before (at 0 hr) and after (at 24 hr) in all four groups, the value of P ≤ 0.0001 (***) and (****), meaning that the data was statistically significant.

b) Representative images at 4X magnification of T98G scratch assays immediately after the scratches were made and then 24 hours later. Control medium was switched out for Slit2 medium at 0 hours for the Slit2 groups before the scratch. For every milliliter of medium, 1 μL of the drug (either DMSO or Taxol) was added.

Figure 3 | Slit2 induces binding of TUBB3 to Robo1 in HEK cells while Taxol reduces the interaction between Robo1 and TUBB3.

Human embryonic kidney (HEK) cells were transfected with Robo1 only, TUBB3 only, and Robo1/TUBB3, respectively. 2M Taxol was added 6 hours before immunoprecipitation and Slit2 conditioned medium was added to the treatment group and incubated for 20 minutes. Cell extracts were incubated with anti-HA antibody and protein A beads overnight for immunoprecipitation. Anti-HA (α-HA) antibody detected Robo1, while anti-FLAG (α-FLAG) recognized TUBB3. The cell lysates verified the expression of Robo1-HA and TUBB3-FLAG in HEK cells.

SUMMARY

1. From the HEK wound healing assays, cell migration occurred after 24 hours in all groups, but there were no effects on the HEK cells when treated with Slit2 conditioned medium.

2. From the T98G wound healing assays, cell migration occurred after 24 hours in all groups, with the finding that Slit2 promoted glioblastoma cell migration.

3. 2M Taxol reduced the binding of TUBB3 to Robo1 in HEK cells.

4. Slit2 conditioned medium induced the binding of TUBB3 to Robo1 in HEK cells.

FUTURE DIRECTIONS

- Study the interaction of Robo1 and TUBB3 in T98G cells
- Investigate the mechanism of how 2M Taxol reduce the interaction of ROBO1 and TUBB3 in T98G cells
- Investigate the mechanism of how 2M Taxol inhibits T98G cell migration

ACKNOWLEDGMENTS

Special thanks to Dr. Guofa Liu and Tao Yang, and funding by the Office of Undergraduate Research at the University of Toledo.