Colocalization of HGFR/c-MET and Phosphorylated Forms of This Tyrosine-Kinase Receptor in Glioblastoma

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Abstract

Hepatocyte Growth Factor Receptor/Tyrosine-Protein Kinase Met (HGFR/c-MET) is a growth factor receptor that plays a critical role in mediating the effects of its ligand Hepatocyte Growth Factor (HGF). HGFR/c-MET exists in its dimeric form, with each subunit linked by a disulfide bridge, in the extracellular ligand-binding domain and a tyrosine kinase domain in the intracellular region. This receptor is overexpressed in a variety of human cancers, including glioblastoma, where it is thought to promote tumor cell migration and proliferation.

Anchoring of this receptor is modulated by phosphorylation of key tyrosine residues, which may be inactivated by mechanisms that allow for signaling through this receptor to be inhibited. A recent study investigated the role of HGFR/c-MET phosphorylation in glioblastoma using a novel combination of antibodies that allow for visualization of both non-phosphorylated and phosphorylated HGFR/c-MET in a single experiment.

Materials

- **Cells**: A-172 human glioblastoma cell line (ATCC HTB-18®)
- U-87 MG human glioblastoma cell line (ATCC HTB-13®)
- U-251 MG human glioblastoma cell line (ATCC HTB-19®)

**Primary Antibodies**

- Mouse Anti-Human HGFR/c-MET Monoclonal Antibody (R&D Systems, Catalog # NL007)
- Rabbit Anti-Human HGFR/c-MET Monoclonal Antibody (R&D Systems, Catalog # AF3950)
- Rabbit Anti-Human HGFR/c-MET Monoclonal Antibody (R&D Systems, Catalog # AF2480)

**Secondary Antibodies**

- Polyclonal Antibody (R&D Systems, Catalog # NL007)
- Polyclonal Antibody (R&D Systems, Catalog # AF3950)
- Polyclonal Antibody (R&D Systems, Catalog # AF2480)

**IHC Substrate/Chromogen**

- NorthernLights™ 557-Conjugated Donkey Anti-Mouse IgG Secondary Antibody (R&D Systems, Catalog # NL207)
- Alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) Superclonal™ Secondary Antibody (Invitrogen)

Methods

Cell Culture

Cells were cultured with growth media in T25 flasks at 37 °C and 5% CO2. They were exposed to Recombinant Human HGF protein (25 ng/mL; R&D Systems, Catalog # 294-HG) for 30 minutes at 37 °C and 5% CO2. Following stimulation, cells were fixed with paraformaldehyde and permeabilized with Triton X-100, and then stained with combinations of primary antibodies and secondary antibodies. Immunofluorescence images were acquired using a Nikon Ti microscope and deconvolved using NIS-Elements software.

Results

Upon stimulation with Recombinant Human HGF, the localization and colocalization patterns of phospho-HGFR/c-MET (Y1234/Y1235) were altered in a single-cell level, with both non-phosphorylated and phosphorylated forms colocalizing in a subset of cells. The localization of non-phosphorylated HGFR/c-MET (total protein) was primarily in the cytoplasm, while phosphorylated HGFR/c-MET (phospho-HGFR/c-MET; brown) was detected in paraffin-embedded sections of human glioblastoma tissue.

Conclusion

The study demonstrates that phosphorylation of HGFR/c-MET plays a critical role in the localization and function of this receptor, providing insights into potential therapeutic targets for glioblastoma treatment.

Acknowledgements

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References


Image 1

**A.** A-172 Cells
**B.** U-87 MG Cells
**C.** U-251 MG Cells

**Figure 1** Comparison of non-phosphorylated and phosphorylated HGFR/c-MET in human glioblastoma tissue. Non-phosphorylated HGFR/c-MET (total protein) is predominantly localized to the cytoplasm, while phosphorylated HGFR/c-MET (phospho-HGFR/c-MET; brown) is detected in paraffin-embedded sections of human glioblastoma tissue.