Glioblastoma (GBM) is the deadliest brain tumour. Epithelial-Mesenchymal-like transition (MES) is a crucial mechanism that influences GBM motility, conferring GBM its highly infiltrative nature. Kisspeptin (KP-10), a neuropeptide predominately produced in the hypothalamus, can influence tumour cell motility, but its comprehensive mechanisms in GBM motility remain poorly understood. This study aims to elucidate the mechanism of kisspeptin in regulating GBM motility, through MES, in KISS1R-expressing GBM cells (KISS1R-GBM). The effects of KP-10 on MES were examined with rhodamine-phalloidin immunocytochemistry analysis. The transwell cell migration and invasion assays were performed to study the effects of KP-10 on cell motility. Shotgun proteomic analyses were conducted to understand the proteomic profiles of KP-10-treated GBM cells. PANTHER, Gene Ontology (GO) and Reactome pathway enrichment analyses were performed to determine protein functional roles and associated regulatory networks mediated by KP-10. Finally, the expressions of KP-10-regulated MES-related markers were validated with JESS Simple Western analysis. KP-10 (10nM) stimulated the formation of lamellipodia and filopodia from 3 to 48 hours, indicating KP-10 induced MES in KISS1R-GBM. KP-10 only promoted chemotactic cell migration and cell invasion at 24 hours, implying KP-10 promoted GBM cell motility through induction of MES. The proteomic analysis and enrichment analyses revealed KP-10 mediated upregulation of proteins involved in cytoskeleton reorganisation (actin-binding, cytoskeletal and pseudopod-enriched proteins), protein synthesis (chaperonin and ribosomal protein) and glycolysis, suggesting KP-10 induced active protein translation and glucose metabolism which concomitantly support dynamic actin remodelling for MES-mediated pseudopodia formation. KP-10 also mediated upregulation of MES-related markers (Snail, Slug, ZEB1 and N-cadherin), confirming KP-10 activated MES in KISS1R-GBM. In summary, the current data shows for the first time that the neuropeptide, KP-10 (at nanomolar concentration), induces MES-mediated dynamic cytoskeleton remodelling to promote cell invasion in KISS1R-GBM, suggesting the possibility of GBM infiltration could be targeted by KISS1R in the KISS1R-expressing GBM.

**Declaration of Interest Statement:** None