

EGFR activation reverses gastrin suppression by MENIN by inducing its nuclear export

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INTRODUCTION: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are rapidly increasing malignancies in the US. They are strongly associated with loss-of-function mutations in the *Multiple Endocrine Neoplasia type 1 (MEN1)* gene which encodes the tumor suppressor MENIN. Gastrinomas secrete excess gastrin, representing the most malignant subtype of sporadic or hereditary GEP-NETs. Although MENIN epigenetically inhibits *gastrin* gene expression, ligand-receptor activation inducing *GASTRIN* removes MENIN suppression. However, the mechanism by which MENIN suppression is alleviated remains poorly defined. Exon 10 encodes all three nuclear localization signals (NLSs) of MENIN within its C-terminal domain. Moreover, NLS1 harbors a phosphorylation motif for protein kinase B, which mediates epidermal growth factor receptor (EGFR) signaling. Since MEN1-gastrinomas predominantly arise within the submucosal Brunner's glands rich in pro-proliferative EGFR ligands like epiregulin (EREG), we hypothesized that EGFR activation induces *GASTRIN* by reducing nuclear MENIN. **METHODS** We subcloned wild-type (WT) *MENIN* cDNA and the sequential C-terminal deletions into pcDNA carrying the FLAG epitope and validated with sequencing. Regulation of endogenous *GASTRIN* mRNA was determined after transfection of MENIN vectors into two human gastric cancer cell lines—AGS and KATOIII. The effect of EGFR signaling on MENIN's subcellular localization, stability, and repression of the gastrin gene was determined by immunoblotting, subcellular fractionation, immunofluorescent staining, and RT-qPCR. **RESULTS:** WT MENIN and constructs retaining 2 or 3 nuclear localization signals (NLSs) suppressed 50% of *GASTRIN* expression under basal conditions. Moreover, deleting all three NLSs abolished MENIN suppression. EREG treatment induced *GASTRIN* in the absence of the presence of WT or MENIN without 1-2 NLS. However, no induction of *GASTRIN* was observed with all 3 NLSs deleted. Constructs lacking all 3 NLSs exhibited reduced protein expression, which was reversed by the proteasome inhibitor MG132, suggesting decreased protein stability. NLS-deficient MENIN was highly localized to the cytoplasm whereas WT MENIN and deletions with >1 NLSs were distributed between the nucleus and cytoplasm under basal conditions. However, EREG treatment induced their cytoplasmic translocation, suggesting that EGFR signaling blocks MENIN's nuclear localization. **CONCLUSION:** EGFR activation induces *GASTRIN* gene expression by preventing nuclear import which requires the C-terminal NLSs.