mRNA translation is a therapeutic vulnerability necessary for bladder epithelial transformation

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Abstract

Using genetically engineered mouse models, this work demonstrates that protein synthesis is essential for efficient urothelial cancer formation and growth but dispensable for bladder homeostasis. Through a candidate gene analysis for translation regulators implicated in this dependency, we discovered that phosphorylation of the translation initiation factor eIF4E at serine 209 is increased in both murine and human bladder cancer, and this phosphorylation corresponds with an increase in de novo protein synthesis. Employing an eIF4E serine 209 to alanine knock-in mutant mouse model, we show that this single posttranslational modification is critical for bladder cancer initiation and progression, despite having no impact on normal bladder tissue maintenance. Using murine and human models of advanced bladder cancer, we demonstrate that only tumors with high levels of eIF4E phosphorylation are therapeutically vulnerable to eFT508, the first clinical-grade inhibitor of MNK1 and MNK2, the upstream kinases of eIF4E. Our results show that phospho-eIF4E plays an important role in bladder cancer pathogenesis and targeting its upstream kinases could be an effective therapeutic option for bladder cancer patients with high levels of eIF4E phosphorylation.

eIF4E is a translation initiation factor

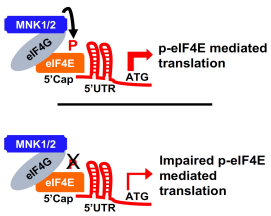

Figure 1. Simplified schema of eIF4E mediated translation. Mnk 1/2, a kinase phosphorylates the eIF4G bound eIF4E thus activating it and initiating translation. Hindrance in eIF4E phosphorylation leads to reduction in mRNA translation. Adapted from Gingras *et al.*, Annual Review of Biochemistry, 1999.

Optimal protein synthesis is required for efficient urothelial transformation

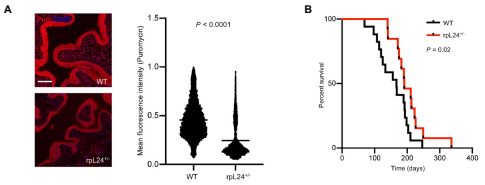

Figure 2. (A) Puromycin incorporation in WT and rpL24+/– urothelium. Representative IF images show less protein synthesis in rpL24+/– mice compared with WT counterparts. Quantification of > 5000 cells/genotype (WT [n = 3], rpL24+/– [n = 2], P < 0.0001, t test). (B) Kaplan-Meier survival analysis of WT (n = 17) and rpL24+/– (n = 13) mice treated with 0.075% BBN ad libitum (P = 0.02, log-rank test).

Urothelial carcinoma is associated with increased protein synthesis and phosphorylation of the translation initiation factor eIF4E

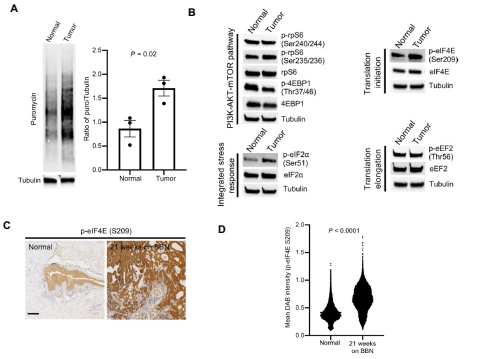

Figure 3. (A) Puromycin incorporation in normal and tumor organoids developed from WT and WT + BBN–treated mice. Representative puromycin Western blot. Quantification of n =3 biological replicates (P = 0.02, t test). (B) Candidate gene analysis of translation regulators by Western blot using normal and BBN tumor organoids (n= 3 biological replicates). The same tubulin blot is used in the PI3K-AKT-mTOR pathway and integrated stress response figures. The same tubulin blot is used in the translation initiation and translation elongation figures. (C) eIF4E S209 phosphorylation in WT and BBN-treated C57BL/6 mice. Representative eIF4E S209 IHC. (D) Quantification of > 5000 cells/genotype (Normal [n = 2], 21 weeks on BBN [n = 2], P < 0.0001, t test). Scale bars: 100 μm. Data are presented as mean ± SEM.

Phosphorylation of eIF4E at S209 is necessary for carcinogen-induced bladder tumor initiation and progression

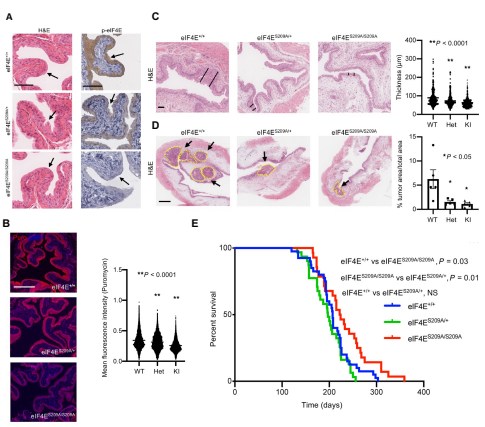

Figure 4. (A) Representative H&E and phospho-eIF4E S209 staining of the urothelium in eIF4E+/+ (WT), eIF–4ES209A/+ (Het), and eIF4ES209A/S209A (KI) mice. (B) Representative puromycin IF of eIF4E+/+ (WT), eIF4ES209A/+ (Het), and eIF4ES209A/S209A (KI) urothelium with quantification of > 5000 cells/genotype (n = 4 mice/genotype.**P < 0.0001, Dunnett's multiple-comparison test). (C) Representative H&E of 9-week BBN–treated eIF4E+/+ (WT), eIF4ES209A/+ (Het), and eIF4ES209A/S209A (KI) mouse urothelium used for thickness measurements (black lines demarcate urothelial thickness) with quantification (n = 6 mice/genotype, **P < 0.0001, Dunnett's multiple-comparison test). (D) Representative H&E of 15-week BBN–treated eIF4E+/+ (WT), eIF4ES209A/+ (Het), and eIF4ES209A/S209A (KI) mouse urothelium (dotted yellow lines demarcate tumors) with quantification (n = 6 mice/genotype, *P < 0.05 [eIF4E+/+ (WT) versus eIF4ES209A/+ (Het), P = 0.02; eIF4E+/+ (WT) versus eIF4ES209A/S209A (KI), P = 0.01], Dunnett's multiple-comparison test. Scale bar: 500 μm). (E) Kaplan-Meier survival analysis of BBN-treated eIF4E+/+ (n = 40), eIF4ES209A/+ (n = 31), and eIF4ES209A/S209A (n = 28) mice (log-rank test). Scale bars: 100 μm unless otherwise noted. Data are presented as mean ± SEM.

High eIF4E phosphorylation correlates with responsiveness to pharmacologic MNK1/2 inhibition

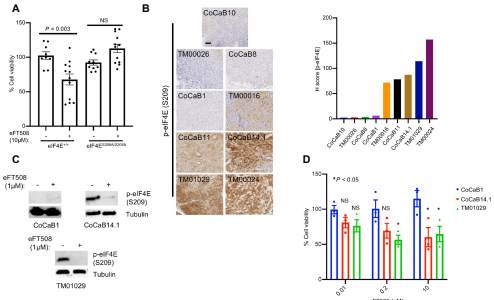

Figure 5. (A) Cell viability assay in eIF4E+/+ and eIF4ES209A/S209A bladder cancer organoids derived from BBN-treated mice (n = 3 biological replicates, P = 0.003, t test). (B) Phospho-eIF4E S209 staining across 9 bladder cancer PDX models with quantification. Scale bars: 500 μm. (C) Representative phospho-eIF4E Western blots across 3 bladder cancer organoid models treated with eFT508 (72 hours). (D) Cell viability assay of the CoCaB1, CoCaB14.1, and TM01029 PDX derived organoids treated with 0.01, 0.2, or 10 μM eFT508 for 72 hours. *P < 0.05 (CoCaB1 versus TM01029 at 0.2 μM, P = 0.03; CoCaB1 versus CoCaB14.1 at 10 μM, P = 0.03; CoCaB1 versus TM01029 at 10 μM, P = 0.04). Dunnett's multiple-comparison test. Scale bar: 100 μm. Data are presented as mean ± SEM.

eIF4E S209 phosphorylation is a requisite for a therapeutic response to eFT508 in bladder cancer

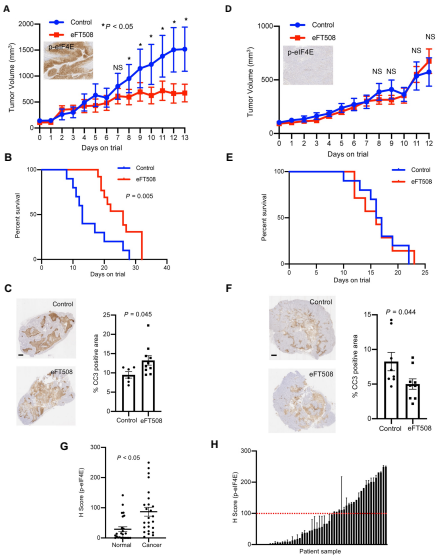

Figure 6. eFT508 treatment causes reduced tumor size and improved survival in phospho-eIF4E high bladder cancer PDX model. (A–C) Growth curve (Inset: representative phospho-eIF4E staining in the TM01029 PDX) (A), Kaplan-Meier curve (B), and representative IHC and bar graph of cleaved caspase 3 (CC3) (C) of the TM01029 (high phospho-eIF4E) PDX model treated daily with eFT508 10 mg/kg orally (n = 10 [control]; n = 13 [eFT50]). (D–F) Growth curve (Inset: representative phospho-eIF4E staining in the CoCaB1 PDX) (D Kaplan-Meier curve (E), and representative IHC and bar graph of cleaved caspase 3 (CC3) (F) of the CoCaB1 (low phospho-eIF4E) PDX model treated daily with eFT508 10 mg/kg orally (n = 9 [control]; n = 8 [eFT508]). (G) Phospho-eIF4E S209 levels in primary bladder cancer specimens compared with matched normal tissues (n = 25 patients, P < 0.05, t test). (H) Phospho-eIF4E S209 levels across 53 primary bladder cancer specimens demonstrating that 37% of patients express high levels of phosphorylated eIF4E. Scale bars: 1 mm. Data are presented as mean ± SEM.

Conclusions

- Bladder urothelium requires robust protein synthesis to promote the process of cellular transformation and tumor growth. This is mediated, in part, through hyperphosphorylation of the oncogene eIF4E.
- 37% of patients with invasive bladder cancer express high levels of p-eIF4E.
- eIF4E phosphorylation levels dictate the ability of bladder tumors to respond to the clinical-grade MNK1 and MNK2 inhibitor eFT508.
- This study and the prevalence of eIF4E hyperphosphorylation within muscle-invasive bladder cancer provide the preclinical rationale for conducting phase 2 studies in urothelial carcinoma patients.

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