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Selective Cytotoxicity of the NAMPT Inhibitor FK866 Toward Gastric Cancer Cells With Markers of the Epithelial-Mesenchymal Transition, Due to Loss of NAPRT

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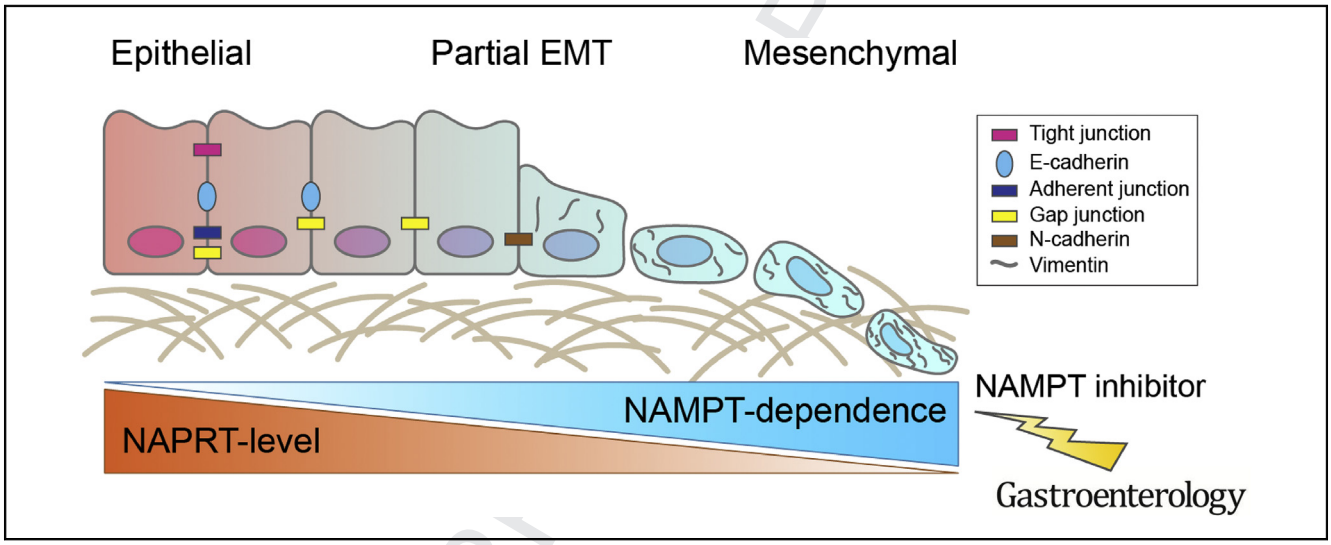

BACKGROUND & AIMS: Markers of the epithelial-to-mesenchymal transition (EMT) in gastric tumor tissues are associated with poor patient outcomes. We performed a screen to identify pharmacologic compounds that kill gastric cancer cells with EMT-associated gene expression patterns and investigate their mechanisms. **METHODS:** We identified 29 gastric cancer cell lines with a gene expression signature previously associated with an EMT subtype, based on data from RNA sequence analyses, and confirmed the mesenchymal phenotypes of 7 lines (Hs746T, SNU1750, MKN1, SK4, SNU484, SNU668, and YCC11), based on invasive activity and protein markers. We screened 1,345 compounds for their ability to kill cells with the EMT signature compared with cell lines without this pattern. We tested the effects of identified compounds in BALB/c nude mice bearing GA077 tumors; mice were given intraperitoneal injections of the compound or vehicle (control) twice daily for 24 days and tumor growth was monitored. Proteins associated with the toxicity of the compounds were overexpressed in MKN1 and SNU484 cells or knocked down in MKN45 and SNU719 using small interfering RNAs. We performed immunohistochemical analyses of 942 gastric cancer tissues and investigated associations between EMT markers and protein expression patterns. **RESULTS:** The nicotinamide phosphoribosyltransferase inhibitor FK866 killed 6 of 7 gastric cancer cell lines with EMT-associated gene expression signatures but not gastric cancer cells without this signature. The 6 EMT-subtype gastric cell lines expressed significantly low levels of nicotinic acid phosphoribosyltransferase (NAPRT), which makes the cells hypersensitive to nicotinamide phosphoribosyltransferase inhibition. Gastric cell lines that expressed higher levels of NAPRT, regardless of EMT markers, were sensitized to FK866 after knockdown of NAPRT, whereas overexpression of NAPRT in deficient EMT cell lines protected them from FK866-mediated toxicity. Administration of FK866 to nude mice with tumors grown from GA077 cells (human gastric cancer tumors of the EMT subtype) led to tumor regression in 2 weeks; FK866 did not affect tumors grown from MKN45 cells without the EMT expression signature. Loss of NAPRT might promote the EMT, because it stabilizes β-catenin. We correlated the EMT gene expression signature with lower levels of NAPRT in 942 gastric tumors from patients; we also found lower levels of *NAPRT* mRNA in colorectal, pancreatic, and lung adenocarcinoma tissues with the EMT gene expression signature. **CONCLUSIONS:** FK866 selectively kills gastric

cancer cells with an EMT gene expression signature by inhibiting nicotinamide phosphoribosyltransferase in cells with NAPRT deficiency. Loss of NAPRT expression, frequently through promoter hypermethylation, is observed in many gastric tumors of the EMT subtype. FK866 might be used to treat patients with tumors of this subtype.

Keywords: Stomach Cancer; Drug Screen; Partial Epithelial-to-Mesenchymal Transition; Synthetic Lethal.

Gastric cancer is a leading cause of cancer-related death worldwide. Few targeted therapies for gastric cancer are available because pharmacologically tractable, recurrent mutations are rare in gastric cancer. In gastric cancer, molecular heterogeneity affects prognosis and treatment outcomes. Multiple classification methods have been developed to define gastric cancer molecular subtypes based on a set of heterogeneous molecular features.[1,2] The microsatellite stable and epithelial-to-mesenchymal transition (EMT) subtype of gastric cancer was reported as a molecular subtype in the Asian Cancer Research Group (ACRG) cohort.[2] The other subtypes include the microsatellite instability subtype,[1,2] the microsatellite stable subtype with or without *TP53* mutation,[2] and the Epstein-Barr virus subtype.[1] Approximately, 15%–30% of gastric tumors are classified as the EMT subtype.[2]

Because EMT has been found to underlie malignant tumor progression and therapeutic resistance, pharmacologic interventions against EMT pathways have garnered growing interest. However, directly targeting EMT-induced signaling pathways, for example, by inhibiting transforming growth factor-β, Wnt, and NOTCH,[3–5] has shown limited success, reflecting a narrow therapeutic window or lack of direct tumoricidal effect with these approaches.[6] Alternatively, synthetic lethal approaches, which attack indirect dependences associated with specific cancer biomarkers, could provide novel therapeutic options: a good example is a poly(adenosine diphosphatase–ribosyl)transferase-1 inhibitor, olaparib, approved by the US Food and Drug Administration, which has a synthetic lethal effect on ovarian tumors containing BRCA1 and 2 mutations.

In the present study, we sought to characterize the EMT status of 29 gastric cancer cell lines and screen for small molecule pharmacologic compounds that have selective toxicity against EMT-subtype gastric cancer cell lines. We further investigated the mechanisms of EMT selectivity and the in vivo efficacy of the nicotinamide phosphoribosyltransferase (NAMPT) inhibitor FK866 and the associations in protein expression between EMT markers and nicotinic acid phosphoribosyltransferase (NAPRT), a response biomarker to FK866, using gastric cancer tissues.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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Methods

EMT Gene Signature Analysis

The EMT gene signature used in the present study was composed of 149 up- and 161 downregulated genes in gastric adenocarcinoma cohorts of the EMT subtype.[2] EMT-subtype cell lines were detected by using unsupervised hierarchical clustering with average linkage based on the Euclidean distance of the gene expression values. EMT signature scores were calculated by subtracting the average $\log_2$-scale expression value of the 161 downregulated genes from that of the 149 upregulated genes. Tumor samples displaying significantly high EMT signature scores were detected from a *q-q* plot for each of the 31 datasets.

Gastric Tumor Subjects and Tissue Microarray Analysis

The present study was approved by the institutional review board of Severance Hospital (Seoul, South Korea; 4-2015-0616, 4-2017-0978). Demographic and clinical information and tumor tissue samples were obtained from 942 patients with gastric cancer who had undergone curative-intent gastrectomy from 2000 through 2003 at Severance Hospital. Patient age, sex, tumor histology, Lauren classification, and pathologic TNM stages were evaluated as clinical parameters. The median follow-up time was 112 months (range, 1–163 months). Immunohistochemical analysis of sections of tissue microarray (TMA) blocks containing 942 gastric tumor tissue samples was performed using a Ventana XT Automated Stainer (Ventana

***Authors share co-first authorship.**

Abbreviations used in this paper: **ACRG, Asian Cancer Research Group; EMT, epithelial-to-mesenchymal transition; NAD^+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; TCGA, The Cancer Genome Atlas; TMA, tissue microarray.**

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