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Research Paper

Nicotinamide nucleotide transhydrogenase-mediated redox homeostasis promotes tumor growth and metastasis in gastric cancer

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ABSTRACT

Overcoming oxidative stress is a critical step for tumor growth and metastasis, however the underlying mechanisms in gastric cancer remain unclear. In this study, we found that overexpression of nicotinamide nucleotide transhydrogenase (NNT) was associated with shorter overall and disease free survival in gastric cancer. The NNT is considered a key antioxidative enzyme based on its ability to regenerate NADPH from NADH. Knockdown of NNT caused significantly NADPH reduction, induced high levels of ROS and significant cell apoptosis under oxidative stress conditions such as glucose deprival and anoikis. *In vivo* experiments showed that NNT promoted tumor growth, lung metastasis and peritoneal dissemination of gastric cancer. Moreover, intratumoral injection of NNT siRNA significantly suppressed gastric tumor growth in patient-derived xenograft (PDX) models. Overall, our study highlights the crucial functional roles of NNT in redox regulation and tumor progression and thus raises an important therapeutic hypothesis in gastric cancer.

1. Introduction

Gastric cancer (GC) is the most common gastrointestinal neoplasm and is a leading cause of cancer-related deaths worldwide [1–3]. The high growth ability and mortality rate of GC is evident as it spreads to distant organs; for example, peritoneal dissemination is a common development in patients with advanced GC even at initial diagnosis and carries a very poor prognosis [4]. However, its molecular mechanism has not been clear elucidated. Growing evidence points to the fundamental role of redox homeostasis in tumorigenesis and metastatic progression [5–7]. Yet, the regulation of NADPH metabolism in GC remains unclear.

In cancer cells, overcoming oxidative stress is a critical step for tumor progression. Redox homeostasis is dependent on a balance between the levels of antioxidants such as NADPH, used to maintain reduced glutathione (GSH), and oxidants such as reactive oxygen species (ROS). During tumor progression, cancer cells usually suffer from oxidative stress caused by ischemia, hypoxia and anchorage-independent growth when tumor growth exceeds the ability of available vasculature to supply tumor cells with oxygen [8–10]. Though ROS are essential for adequate signal transduction and are known to regulate crucial cellular processes. However, once the balance is broken, rapid increases in intracellular ROS may lead to cell apoptosis, and cells frequently demand for more NADPH to eliminate excess ROS.

NNT is considered a key antioxidative enzyme based on its ability to regenerate NADPH from NADH and is the major mitochondrial enzymatic source of NADPH, contributing 45% of the total NADPH supply [11]. NNT is at the critical interface between the NADH and NADPH pools in mitochondria; the NNT catalyzes the reaction $NADH + NADP^+ \leftrightarrow NADPH + NAD^+$ [12]. Recently, our studies shows that disrupting G6PD- or ME2-mediated NADPH homeostasis enhances chemosensitivity in gastrointestinal cancer [13,14]. In humans and animals, NNT dysfunction usually leads to oxidative stress [15–17]. However, the effects of NNT on NADPH homeostasis and tumor malignant phenotypes in GC remain unclear.

Here, we identified that NNT is overexpressed in GC. Given that

Abbreviations: NNT, nicotinamide nucleotide transhydrogenase; ROS, reactive oxygen species; GC, gastric cancer; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; Ploy-HEMA, ploy-2-hydroxyethylmethacrylate

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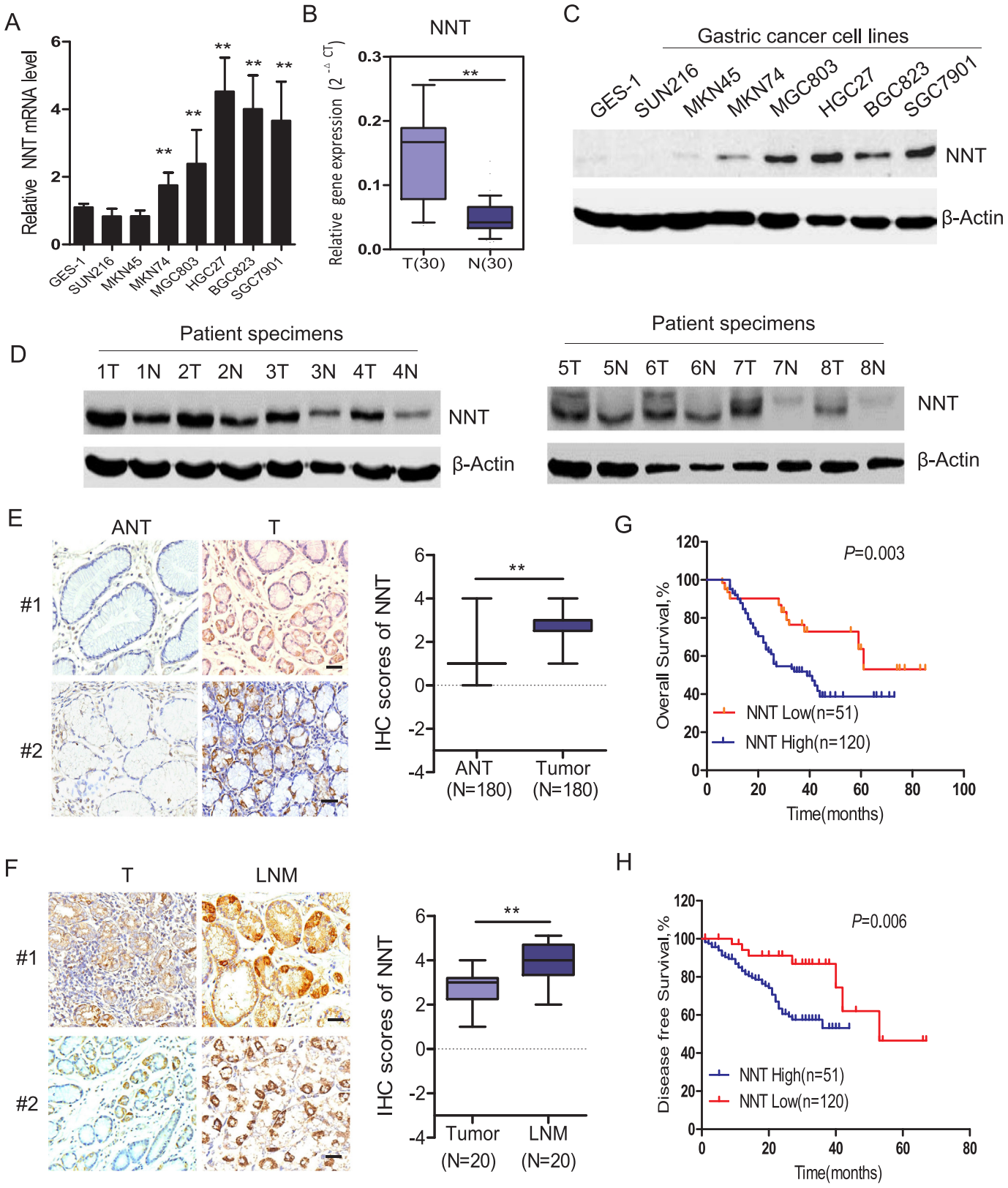

Fig. 1. Increased NNT expression is correlated with poor prognosis in GC. (A) qPCR analysis of NNT expression in a panel of gastric cancer (GC) cells and GES-1 epithelial cells. (B) qPCR analysis of NNT expression in 30 paired GC tissues obtained from our hospital. (C) Immunoblotting analysis of NNT protein levels in a panel of GC cells and GES-1 epithelial cells. (D) Immunoblotting analysis of NNT protein levels in 8 paired GC tissues. (E) Representative staining (left panel) and immuno-scoring of NNT (right panel, N = 180) showing low expression of NNT protein in adjacent normal tissues (ANT), positive staining in primary GC tumor tissues (scale bar: 100 μm). (F) Representative staining (left panel) and immuno-scoring of NNT (right panel) in primary GC tumor tissues and paired lymph node metastatic tissues (LNM, N = 20) (scale bar: 100 μm). Kaplan–Meier analysis of overall survival (G) or disease-free survival curves (H) for GC patients with low versus high expression of NNT (Kaplan–Meier analysis with the log-rank test). Data are presented as the mean ± SD. $^{**}P < 0.01$ for indicated comparison.