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Original contribution

The altered expression of ING5 protein is involved in gastric carcinogenesis and subsequent progression ☆

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Summary ING5 can interact with p53, thereby inhibiting cell growth and inducing apoptosis. To clarify the roles of ING5 in gastric tumorigenesis and progression, its expression was examined by immunohistochemistry on a tissue microarray containing gastric nonneoplastic mucosa (n = 119), dysplasia (n = 50), and carcinomas (n = 429), with its comparison with clinicopathologic parameters of the carcinomas. ING5 expression was analyzed in gastric carcinoma tissues and cell lines (MKN28, MKN45, AGS, GT-3 TKB, and KATO-III) by Western blot and reverse transcriptase-polymerase chain reaction. ING5 protein was found to distribute to the nuclei of gastric carcinoma cells with similar messenger RNA levels. An increased expression of *ING5* messenger RNA was observed in gastric carcinoma in comparison with paired mucosa ($P < .05$). Lower expression of nuclear ING5 was detected in gastric dysplasia and carcinoma than that in nonneoplastic mucosa ($P < .05$). Gastric nonneoplastic mucosa and metastatic carcinoma showed more expression of cytoplasmic ING5 than did gastric carcinoma and dysplasia ($P < .05$). Nuclear ING5 expression was negatively correlated with tumor size, depth of invasion, lymph node metastasis, and clinicopathologic staging ($P < .05$), whereas cytoplasmic ING5 was positively associated with depth of invasion, venous invasion, lymph node metastasis, and clinicopathologic staging ($P < .05$). Nuclear ING5 was more expressed in older than younger carcinoma patients ($P < .05$). There was a higher expression of nuclear ING5 in intestinal-type than diffuse-type carcinoma ($P < .05$), whereas it was the converse for cytoplasmic ING5 ($P < .05$). Survival analysis indicated that nuclear ING5 was closely linked to favorable prognosis of carcinoma patients ($P < .05$), albeit not independent. It was suggested that aberrant ING5 expression may contribute to pathogenesis, growth, and invasion of gastric carcinomas and could be considered as a promising marker to gauge aggressiveness and prognosis of gastric carcinoma.

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1. Introduction

Malignant transformation is genetically a complex process, featuring frequent genetic and epigenetic alterations activating oncogenes and inactivating tumor suppressor genes (TSGs) [1]. Among them, chromosomal deletion, mutation, or hypermethylation, which can lead to loss (class I) or inactivation (class II) of TSG causing immortality of cancer cells, has provided clues to the identification of genes critical for initiation, promotion, and development of tumors, such as the inhibitor of growth (ING) family [2].

ING proteins consist of 5 isoforms. Among them, *ING5* is localized to human chromosome 2q37.3, contains 8 exons and 7 introns, and encodes 5233-bp cDNA, whose 1068 nucleotides are translated into 28-kDa protein of 240 amino acids [2]. ING5 consists of several different domains from N-terminal to C-terminal such as leucine zipper like (LZL), novel conserved region (NCR), nuclear localization signal, and plant homeo domain. LZL domain has been shown to be important in DNA repair, apoptotic induction, and chromatin remodeling. NCR domain can bind to histone acetyl transferase (HAT) complexes during chromatin remodeling and regulation of gene expression [3,4]. ING5 interacts with histone H3K4me3 and is involved in the formation of 2 different HAT complexes, including a complex that binds to histone H4 through interaction between ING5, HBO1, and JADE, and the other complex that binds to histone H3 through an interaction with ING5 and contains MOZ, MORF, and BRPF [3-7]. ING5 protein can associate with minichromosome maintenance proteins, which play an essential role in DNA replication through the formation of a prereplicative complex at the origins of replication. It has been speculated that the HBO1-JADE-ING5 HAT complex has an important role during DNA replication in cooperation with the minichromosome maintenance complex because knockdown of ING5 completely abolishes DNA synthesis, and knockdown of HBO1 increases cells in S phase [8]. ING5 was reported to activate the cyclin-dependent kinase inhibitor p21/waf1 promoter to induce p21/WAF1 expression, enhance p53 acetylation at Lys-382 residues, and physically interact with p300, a member of HAT complexes, and p53 in vivo [9]. Taken together, it can be hypothesized that aberrant ING5 expression might be involved in carcinogenesis and subsequent progression.

Loss or down-regulation of ING protein function is frequently observed in different tumor types, many of which are resistant to apoptosis, thus warranting their classification as type II tumor suppressors [2-5,10-16]. Several different in vitro and in vivo models have explored the role of ING proteins in regulating apoptosis [2,5,17]. ING5 overexpression can result in a diminished colony-forming efficiency, a decreased cell population in S phase, and an induction of apoptosis in a p53-dependent manner [9]. Cengiz et al [18] found loss of heterozygosity of long arm of chromosome 2 in 85% (33/39) of oral carcinomas, where there is *ING5* gene, indicate a possible role of its deletion in oral carcinogenesis. Recently, reduced expression of *ING5* mRNA was detectable in 61% of oral squamous cell carcinoma with missense mutations located within LZL finger and NCR domains of ING5 protein [19]. The decrease in nuclear ING5 expression and the cytoplasmic translocation were observed in the tumorigenesis of neck squamous cell carcinoma (HNSCC). Nuclear ING5 may modulate the transactivation of target genes and promote apoptosis and cell cycle arrest by interacting with p300 and p21 proteins in HNSCC [20]. Our data indicated that the nuclear to cytoplasmic shift of ING5 protein occurs during colorectal carcinogenesis. The nuclear ING5 loss and its cytoplasmic overexpression were closely linked to the aggressive behaviors of colorectal carcinomas (CRCs) [21].

Despite a worldwide decline in incidence and mortality since the second half of the 20th century, gastric carcinoma (GC) still ranks as the fourth most common and the second most frequent cause of death from cancer, accounting for 10.4% of cancer deaths worldwide. It continues to be a major health concern because of a slow decrease in incidence in Asia and high mortality from diagnosed GC in the West, even though sophisticated diagnostic and operative techniques are widely applied in clinical practice [22,23]. Pathologic and genetic observations demonstrate that gastric dysplasia precedes the majority of carcinoma and could undergo malignant transformation [24,25]. However, the molecular mechanisms underlying gastric carcinogenesis are still poorly understood. In this article, *ING5* expression was examined in gastric nonneoplastic mucosa (NNM), dysplasia, carcinoma, and carcinoma cell lines and was compared with the clinicopathologic parameters of GC as well as prognosis to explore the clinicopathologic significance and molecular roles of ING5 expression in stepwise development of GC.

2. Materials and methods

2.1. Cell culture

GC cell lines, MKN28 (well-differentiated adenocarcinoma), MKN45 (poorly differentiated adenocarcinoma), AGS (moderately differentiated adenocarcinoma), KATO-III (poorly differentiated adenocarcinoma), and GT-3 TKB (undifferentiated adenocarcinoma) come from Japanese Physical and Chemical Institute. They were maintained in RPMI 1640 (MKN28, MKN45, and KATO-III), Dulbecco's modified Eagle's medium (GT-3 TKB), or HamS /F-12 (AGS) medium supplemented with 10% fetal bovine serum, 100 units/mL. All cells were harvested by centrifugation, rinsed with phosphate-buffered saline (PBS; pH 7.4), and subjected to total protein extraction by sonication in radioimmunoprecipitation assay lysis buffer (150 mmol/L NaCl, 5 mmol/L EDTA, 0.5% Nonidet P-40 (Sigma, St. Louis, MO, USA), 5 mmol/L