Functions of the Tumor Suppressor ING Family Genes in Head and Neck Cancer and Their Future Applications in Cancer Gene Therapy

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Abstract:ING1 gene, the founding member of the ING tumor suppressor family, was originally identified through subtractive hybridization between normal mammary epithelial cells and breast cancer cell lines, and subsequent in vivo selection of genetic suppressor element that displayed oncogenic features. The four additional members of the ING family (ING2-5) were recently identified and all the gene products contain a highly conserved plant homeodomain (PHD) finger motif in the carboxy (C)-terminal end. Although exact functions of ING family genes have not been clarified, the gene products are involved in transcriptional regulation, apoptosis, cell cyle, angiogenesis and DNA repair through p53-dependent and –independent pathways and constituting complexes with histone acetyltransferases (HAT) and histone deacetylases (HDAC).

Introduction

Two major groups of tumor-associated genes, oncogenes and tumor suppressor genes (TSGs), have been implicated in the carcinogenic process. Tumor suppressor genes are defined as genetic elements whose loss or mutational inactivation allows cell to display one or more phenotypes of neoplastic growth. The five members of the ING family were recently identified and all contain a highly conserved plant homeodomain (PHD) finger motif in the carboxy (C)-terminal end of the proteins. Although exact functions of ING family genes have not been clarified, the gene products are involved in transcriptional regulation, apoptosis, cell cycle, angiogenesis and DNA repair through p53-dependent and –independent pathways and constituting complexes with histone acetyltransferases (HAT) and histone deacetylases (HDAC). Within the members, ING1 is the founding member and thus most information about the family genes comes from the researches on ING1.

Isolation and characterization of the prototype of human ING family genes, ING1

We showed that ING1 gene produced at least 4 mRNA variants from 3 different promoters. Two of these variants, p33ING1b consisting of exons 1a and 2, and p24ING1c consisting of a truncated p47ING1a message including the first ATG codon in exon 2, are expressed majorly, while p47ING1a consisting exon 1b and exon 2, was not detected in head and neck tissues[1]. All ING1 gene mRNA variants encode nuclear proteins that contain a PHD zinc finger motif and a nuclear localization signal in their COOH-terminal half [1].

Analysis of ING1 in human tumors

First clinical studies on ING1 gene demonstrated rearrangement in one neuroblastoma cell line and reduced expression in primary cancers and cell lines. The deletion of ING1 locus at 13q33-34 was reported in head and neck cancer. At the time, ING1 gene was not known to be responsible for this deletion. Later in a comprehensive study, we showed tumor specific missense mutations in ING1 gene and frequent deletion at 13q33-34 region for the first time in head and neck cancers [1]. These mutations were found in the PHD zinc finger domain and putative nuclear localization signal, which may abrogate the normal function of ING1 protein. Moreover, the majority of breast cancers showing decreased ING1 expression had metastasized to regional lymph nodes whereas only a small subset of cancers with elevated ING1 expression as compared to adjacent normal tissues were metastatic.

Rare missense mutations in ING1 have been detected in some other studies. Most of these studies used primers for mRNA expression analysis in the common exon 2 of ING1 gene since alternative splicing variants of ING1 gene were not intensively investigated in that time. The products of different splicing variants of ING1 gene were shown to have different functions and relationship with p53 gene both in mouse and human, recent researches focused on the expression of the each variant in human cancer. Such a study, which examined the p24ING1c and p33ING1b expressions specifically, demonstrated that the p33ING1b is the major variant and exceeds the combined expression of p24ING1c and p47ING1a in haematological malignancies. In our analysis, we could not detect the p47ING1a splicing variant, while p24ING1c and p33ING1b expressions were equally detected in head and neck tissues[1]. Specific mRNA expression analysis of p24ING1c and p33ING1b demonstrated various patterns, mostly a decrease of p24ING1c and an increase of p33ING1b in head and neck cancer samples as compared to their normal counterparts (unpublished data). Since ING1 has 3 different promoters, the difference in expression pattern may be related with carcinogenesis of head and neck cancer through methylation of different promoters or abnormalities of upstream or downstream genes.

Most tumor suppressors contain nuclear transport signals that facilitate their shuttling between the nucleus and the cytoplasm. This type of dynamic intracellular movement not only regulates protein localization, but also often impacts on function. Therefore, it is possible that nucleo-cytoplasmic modifications of the ING family proteins could occur during carcinogenic process.

p53–mediated gene therapy has been used to treat several kinds of cancers[2-4]. However, most of these patients did not respond enough or at all to the therapy. Requirement of ING1 for p53 function led the combined gene therapy of these genes. Resistance of tumors against p53 gene therapy may be simply due to lack of the partner gene, ING1. In fact, two in vitro studies recently showed the effectiveness of such combined therapy [2,3]. Although the single adenovirus-mediated transfer of p33ING1b or p53 did not induce apoptosis in glioma cells (U251 and U-373 MG), coinfection of adenovirus-p33ING1b and adenovirus-p53

dramatically enhanced apoptosis in both cell lines[2]. In another similar study, synergistic effect between p33ING1 and p53 in the induction of apoptosis of human esophageal carcinoma cells were shown[3]. These studies suggested the combined gene therapy of ING1 and p53 as a promising method for treatment of human cancers.

ING family genes

Since the isolation of ING1, four additional members of ING family named ING2-5 have been discovered.

We recently identified a novel member of ING family, ING3, as a candidate tumor suppressor gene in head and neck cancer [6]. We characterized the genomic structure of ING3 gene, which included 12 exons spanning over 25 kb genomic interval and encoding 418 aminoacids. ING3 was mapped at 7q31 region where frequent loss of heterozygosity was detected in various cancers including breast, renal, colon and head and neck carcinomas. ING3 protein also harbors the PHD zinc finger domain highly homologous among ING family members. Northern Blot analysis revealed a 1.9 kb ING3 transcript which expressed in human heart, skeletal muscle, thymus, spleen, kidney, liver, placenta, and peripheral blood leukocytes[5]. We also found an alternatively spliced form of ING3 message by cDNA cloning from human normal tissue. This variant form was devoid of the exon 3 but the coding frame was not shifted. Although only one missense mutation of the ING3 was found, half of the tumors demonstrated reduced or no expression of the gene as compared with that of matched normal samples in head and neck squamous cell carcinomas, suggesting the ING3 as a class 2 tumor suppressor gene[5].

ING4 is localized to chromosome 12p13.31 region and harbors the PHD domain highly homologous among ING family proteins. We analyzed loss of heterozygosity at 12p12-13 region in 50 head and neck squamous cell carcinomas by using six highly polymorphic microsatellite markers and found allelic loss in 66% (33/50) of the informative cases[6]. Quantitative real-time RT-PCR analysis demonstrated decreased expression of ING4 mRNA in 76% of primary tumors as compared with that of matched normal samples, suggesting ING4 as a strong candidate tumor suppressor gene[6].

Frequent abnormalities of ING4 in various cancer and its roles in cellular functions such as cell cycle, apoptosis, transcriptional regulation as well as anti-angiogenesis put it into an important place within ING family members.

Conclusions

The ING family is involved in various cellular processes implicated in growth regulation, senescence, DNA repair, oncogenesis, apoptosis and angiogenesis (Figure 1). Since the cloning of ING1 gene, it was established as a tumor suppressor gene. In addition to their clear relationship and partnership with p53, some ING family members are also components of HAT and HDAC complexes, which regulate gene expression through modulation of chromatin structure. Different splicing variants of ING1 were shown to have various functions, including G1-cell cycle arrest, apoptosis, chemosensitivity, DNA repair, transcriptional repression and activation. Recently new members of ING family (ING2-5) were identified as a negative regulator of cell growth or a candidate tumor suppressor gene. Progress on the knowledge of functions of ING family genes as well as relationship with p53 and other unknown binding molecules will facilitate our understanding of the biochemical mechanisms by which the ING proteins regulate growth, proliferation, senescence and stress response and will help to clarify the functional significance of altered ING family functions in tumorigenesis and cancer progression

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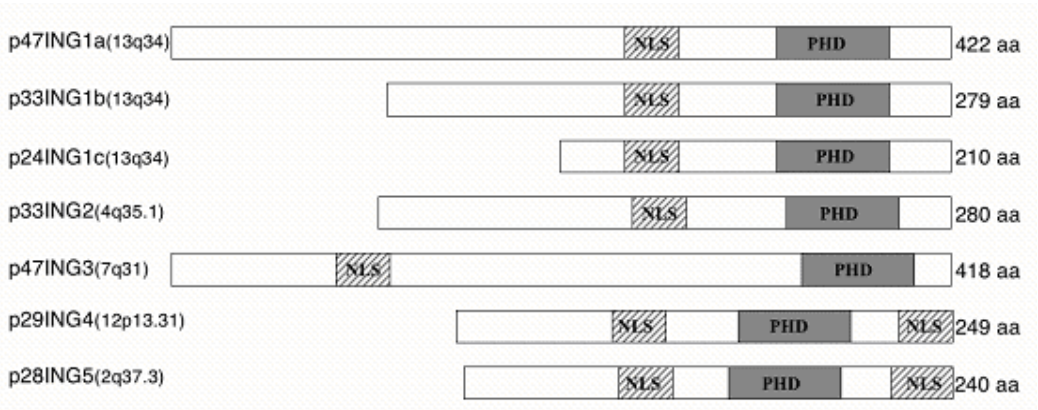

Fig.1