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Published in Atlas Database: November 2014
Online updated version : http://AtlasGeneticsOncology.org/Genes/ING1ID40974ch13q34.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/62488/11-2014-ING1ID40974ch13q34.pdf
DOI: 10.4267/2042/62488

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Abstract
Review on ING1, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity
Other names: p24ING1c, p33, p33ING1, p33ING1b, p47, p47ING1a
HGNC (Hugo): ING1
Location: 13q34

DNA/RNA
Description
In 1996 Karl Riabowol's group identified a new Tumor Suppressor Gene (TSG) by using subtractive hybridization between cDNAs from normal mammary epithelial cells and mammary epithelial cells from tumor (breast cancer cell-lines). This experiment was followed by an in vivo screen for tumourigenesis. Using this method, the authors identified a new candidate TSG they named ING1 for INhibitor of Growth 1 (Garkavtsev et al., 1996).

Transcription
The ING1 gene has been mapped to chromosome 13 at locus 13q34 close to the telomeric region (Figure 1). Human ING1 is made of four exons, exon 1a, 1b, 1c, and 2, resulting in five transcribed variants (ING1a, ING1b, ING1c, and ING1d) (Figure 2).

Pseudogene
INGX is the pseudogene of ING1 (He et al., 2005).
ING1 (inhibitor of growth family, member 1)

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Figure 2. Structure and transcripts of Human ING1 genes. Coding regions are in dark blue and non-coding regions are represented in light blue. For ING1c v2 and v5: Exons E1d and E1balt (alternative) are represented in yellow. They both code for the same protein: ING1c (Figure 3).

Protein

Description

The amino acid sequence alignment of human ING proteins revealed several conserved regions in their C-terminal part: a Novel Conserved Region (NCR), a Nuclear Localization Signal (NLS), a Plant Homeo Domain (PHD) and a PolyBasic Region (PBR). The ING proteins are characterized by the presence of a highly conserved PHD in their C-terminal part. This domain is commonly found in proteins involved in chromatin modification (Bienz, 2006; Mellor, 2006). In the N-terminal part of ING1b isoform, which is the most abundant isoform with ING1a, there is also a PCNA Interacting Protein motif (PIP) and Partial Bromo Domain (PBD) (Figure 3).

Expression

ING1 is ubiquitously expressed in mammalian tissues (Walzak et al., 2008).

Localisation

The ING1 protein contains an NLS domain, so it is mainly located in the nucleus. Members of the 14-3-3 family are able to interact with ING1 phosphorylated on serine 199 leading to its relocation to the cytoplasm (Gong et al., 2006). Recently, it has been found that ING1b translocates also into mitochondria after UV or IR. This translocation is independent of p53 (Bose et al., 2013).

Function

ING1 has been identified as a tumor suppressor gene with Gatekeepers and Caretakers functions. Initially ING1 has been characterized as gatekeeper genes which are known to regulate the growth of tumors through senescence, apoptosis and cell cycle regulation. Indeed, ING1 is involved in the regulation of the cell cycle arrest, apoptosis and senescence.

Firtly, ING1b has been described to physically interact with the tumor suppressor protein p53 and being necessary for its transcriptional activity (Garkavtsev et al., 1998). ING1 is also involved in the regulation of p53 activity. Under stress, ING1b interact with PI5P in order to activate the p300 acetylase which acetylates p53 on lysine 382. This acetylation promotes the transcription of factors involved in cell cycle arrest, senescence and apoptosis (p21, Bax) (Garkavtsev et al., 1998; Kataoka et al., 2003).

ING1b is also able to interact with SIRT1, a histone deacetylase known for the p53 deacetylation. SIRT1 interaction with ING1b competitively inhibits its interaction with p53 leading to the maintenance of p53 activity (Kataoka et al., 2003). Moreover, ING1b is known to stabilize p53 by disrupting the regulation of p53 by MDM2 (Leung et al., 2002). ING1 functions can also be independent of p53 signaling pathways. ING1 has been described to interact with the Sin3/HDAC1/HDAC2/SAP30 complex through a direct interaction with SAP30 (Kuzmichev et al., 2002). ING1b has also been reported to increase the expression of Heat Shock Protein 70 (HSP70). This induction of HSP70 required the amino terminus of ING1b but not the PHD, PBR and NLS region (Feng et al., 2006). HSP70 is known to inhibit the NF-kB pathways (Ran et al., 2004; Shi et al., 2006). More recently, it has been shown that the C-terminal PHD of ING1 interacts with histone H3 trimethylated at Lys4 (H3K4me3). This interaction is involved in DNA repair and apoptotic activities of ING1 (Peña et al., 2008).
Under stress, ING1 interaction with H3K4me4 leads to the recruitment of the Sin3/HDAC1-2/SAP30 transcriptional repressor complex. Under UV stress, ING1b targets the nucleolus and stabilizes Cellular Senescence-Inhibited Gene (CSIG) through its Nucleolar Targeting Signal (NTS). This interaction is required for ING1b dependent apoptosis after UV stress (Li et al., 2012). More recently, ING1 has been characterized as a caretaker gene which controls the integrity of the genome. Indeed, ING1b is involved in DNA repair in response to UV. ING1b overexpression has been described to enhance repair of UV-damaged DNA in a p53 dependent manner. Furthermore, binding between ING1b and Growth Arrest and DNA Damage 45 (GADD45) has been reported. These findings suggest that ING1b cooperates with GADD45 and p53 in Nucleotide Excision Repair (NER) (Cheung Jr. et al., 2001). Interestingly, ING1b is also required for PCNA monoubiquitination in response to UV. More precisely, ING1b is necessary for the loading of the E3 ubiquitin ligase Rad18 at stalled replication forks through histone H4 acetylation (Wong et al., 2011).

**Homology**

The C-terminal part of ING proteins is highly-conserved through human to plant, especially the PHD-finger motif. There are five human ING genes (ING1, ING2, ING3, ING4, and ING5) which encode multiple isoforms via splicing.
Implicated in

**Various cancers**

For review: Ythier et al., 2008; Guérillon et al., 2014. Many genetics and epigenetics alterations of tumor suppressor genes (TSGs) or oncogenes contribute to cancerogenesis. Thus, the expression of tumor suppressor gene ING1 has been analysed in many tumor types.

**Adenocarcinoma of the esophagogastric junction**

Among 19 adenocarcinoma of the esophagogastric junction (AdEGJs), two tumors presented ING1b alterations (11%). One missense mutation at codon 147 had a G to T transition resulting in serine to isoleucine substitution. The second silent mutation at codon 173 had a G to A transition with no amino acid change. In addition, p33ING1b mRNA expression was reduced in 63% of AdEGJs samples compared to normal esophageal tissues. Moreover, ING1b protein expression was frequently decreased or not detected in tumor samples, while all normal gastric mucosa had ING1b protein expression (Hara et al., 2003).

**Ameloblastoma**

In the case of Ameloblastoma, a high frequency of loss of heterozygosity (33% - 72%) is found in the loci of each ING gene family member. In the case of ING1, 51% of 35 patients showed an allelic loss in the ING1 coding region. Different mechanisms, such as deletions, gene conversion or mitotic combination might be implicated to enhance INGs gene alterations and promote ameloblastoma tumorigenesis (Borkosky et al., 2010).

**Brain tumor**

In human brain tumors, ING1b mislocalization is observed in all of the analyzed glioma samples and in some glioma cell lines. However, mutations of ING1 seem to be infrequent. A single point mutation was detected in 29 brain tumor specimens (3.5%) manifested by an arginine to histidine substitution. This amino acid change is caused by a G-to-A transition. Furthermore, this mutation does not affect the expression of ING1b mRNA, but it leads to a cytoplasmic shift of p33ING1b, according to taking place in a sequence of ING1 reported to target p33ING1b to the nucleus (Vieyra et al., 2003).

**Breast cancer**

In breast cancer, expression of ING1 mRNA is decreased in 17 of 24 breast cancer tissues (71%), compared to normal tissues (Tokunaga et al., 2000). Furthermore, a downregulation of ING1 gene has been established in breast cancer cell lines. Mutations of ING1 are rare (1/377) in breast carcinomas (Toyama et al., 1999). In addition, 7 of 86 invasive breast carcinoma cases showed an increase of p33ING1b expression in the cytoplasm, while the 79 cases had a low p33ING1b cytoplasmic expression, with 33 of them lacking absolutely cytoplasmic expression of p33ING1b (Nouman et al., 2003).

**Childhood acute lymphoblastic leukemia**

In childhood Acute Lymphoblastic Leukemia (ALL), 78% of cases showed a decrease in the expression of nuclear p37ING1b which undergoes a shift to the cytoplasm. This translocation may attenuate the function of p37ING1b protein and plays an important role in pathogenesis of ALL (Nouman et al., 2002b).

**Colorectal carcinoma**

In colorectal carcinomas, no loss of alleles has been detected in ING1 gene. Neither Loss Of Heterozygosity (LOH) nor mutations have been observed in 29 sporadic colorectal carcinomas samples. It suggests that another mechanism might be implicated in the loss of gene. For example, hypermethylation of the promoter might be responsible for the inactivation of tumour suppressor genes (Sarela et al., 1999). In addition, the ratios of p33ING1b and p47ING1a mRNA expression were significantly lower in human sporadic colorectal tissue than those in normal tissues (Chen et al., 2005). Moreover, 32 % of patients with Dukes’A colorectal cancer display a low expression of p33ING1b protein (Ahmed et al., 2008).

**Esophageal squamous cell cancer**

In the case of Esophageal squamous cell cancer (ESCC), 59 % of 31 informative cases showed allelic loss at chromosome 1q33-34 where ING1 is mapped. Four somatic missense mutations are located between codons 214 and 270, and two somatic silent mutations are located between codons 219 and 223. All of the missense mutations found in ESCC were localised within the highly conserved PHD finger-like domain which is implicated in transcriptional regulation. Loss of ING1 protein expression has been observed in all ESCC samples, this absence of ING1 may contribute to ESCC carcinogenesis (Chen et al., 2001).

**Gastric cancer**

It has been demonstrated that 75% of tumor samples tested showed a significant decrease in p33ING1 expression associated with gastric carcinogenesis. Moreover, a silent mutation is detected in 2/12 gastric cancer cells with no amino acid change in codon 188, and only one cell line(1/12) had a missense mutation in codon 172 resulting in glutamine to lysine amino acid substitution. However, these findings suggest that p33ING1 acts...
as a tumor suppressor gene even if it is preserved in the majority of gastric cancer (Oki et al., 1999).

**Haematological malignancies**

49 patients with hematological malignancies and 5 normal patients showed a predominant expression of p33ING1b transcript, while p24/ING1 and p47/ING1 transcripts are very weakly expressed. In addition, no p33ING1b sequence variation has been observed in all of the 49 patients with hematological malignancies, suggesting that neither p33ING1b mutation, nor dysregulation of alternative splicing of ING1 gene are significantly responsible of hematological malignancies progression (Bromidge et al., 2002).

**Head and neck squamous cell carcinoma**

In Head and Neck Squamous Cell Carcinomas (HNSCC), a deletion is found on chromosome 13q33-34, where ING1 gene is mapped. A loss of heterozygosity has been detected in 68% of HNSCC cases. Six mutations were identified in the 23 tumors with allelic loss, 13% showed missense mutations with amino acid change and the other 3 samples showed an alteration at codon 173 of p33ING1b without an amino acid change. All these mutations are found in the PHD finger or NLS domain of ING1 (Gunduz et al., 2000). Another study showed that there is no somatic mutation in any of 20 primary tumors showing 13q LOH and in 5 head and neck cancer cell lines, suggesting an alternative factor implicated in this type of tumor (Sanchez-Cespedes et al., 2000).

**Hepatocellular carcinoma**

In hepatocellular carcinoma (HCC), p33ING1 gene is not frequently altered (7%). This mutation resulted in G to C transversion, in the codon 215, with an amino change (cysteine to serine) in ING1b gene. However, 42% of HCC tissus had an up-regulation of p33ING1b, whereas 58% showed a down-regulation of p33ING1b protein. Although the rate of mutation in ING1 is low, loss or inactivation of p33ING1b may be an important mechanism for malignant transformation of HCC (Zhu et al., 2005). Another study suggests that ING1 protein was expressed in either hepatocarcinoma cells or hepatocytes, and localized in the nuclei of hepatocarcinoma cells. In advanced stage group, the general expression level of ING1 was increased in the tumor tissue. Besides, transcriptional levels showed that ING1b is up-regulated in HCC tissus, whereas ING1a expression level remains unchanged (Chen et al., 2009).

**Lung cancer**

In lung cancer, 6/31 of primary lung cancer tumors and 1/30 human lung cancer cell lines showed a G to A transition that occurred in codon 173 located in the NLS domain of ING1. This substitution does not result in amino acid change. There is no point mutation detected in ING1b gene. An up-regulation of ING1b mRNA expression is detected in 7 of 8 lung cancer cell lines having a p53 mutation (Okano et al., 2006). In the case of non-small cell lung cancer (NSCLC), another study demonstrated that only 2% had point mutations in the coding regions of ING1b, but there was no missense mutation detected in ING1b coding region. In addition, 42% showed a reduction of ING1b gene expression associated with p53 mutations (Kameyama et al., 2003).

**Lymphoid malignancies**

In lymphoid malignancies, many deletions and chromosomal translocations were observed in 33-34 region of chromosome 13 where ING1 gene is mapped. A decrease in the expression of ING1 gene is found in 4/5 T-cell lines derived from acute lymphocytic leukemia. A decrease of ING1 expression was also found in 5/11 B-cell lines including 2 myeloma and 2 Burkitt lymphoma cell lines. Moreover, no point mutation or deletion was detected in the 9 cell lines having reduced ING1 expression. These findings suggest that transcriptional or post-transcriptional mechanisms are responsible of ING1 decreased expression which contributes to the development of lymphoid malignancies (Ohmori et al., 1999).

**Mantle cell lymphoma**

The genetic alteration of mantle cell lymphoma (MCL) is a translocation (11;14)(q13;q32), detected in all MCL cases. Additional aberrations are implicated in lymphoma malignancies, such as the inactivation of tumor suppressor genes. For example, the expression of ING1 is decreased in all the cell lines. No evidence of promotor methylation of ING1 gene has been found in MCL cell lines (Ripperger et al., 2007).

**Melanoma**

The p33ING1b protein expression level is significantly increased in 14 melanoma cell lines studied. An overexpression of p33ING1b mRNA was also found in all 6 melanoma cell lines examined, but it was absent in normal melanocytes. There is a low mutation rate (14%) in melanoma cell lines. Two silent nucleotide changes have been detected in exon 1a in Sk-mel-110, whereas, in Sk-mel-24, 8 nucleotide alterations in exon 2 were found. Seven alterations were silent and 1 missense alteration at codon 260 results in an asparagine to serine amino acid substitution. No mutation has been detected in 25 samples of healthy volunteers suggesting that these nucleotide alterations in the 2 melanoma cell lines are not due to a polymorphism. Thus, ING1 gene is not frequently mutated in melanoma cells. These findings demonstrated that
other mechanisms, such as DNA hypomethylation, may contribute to the overexpression of ING1 (Campos et al., 2002).

Another study showed a loss of nuclear p33ING1b expression associated with a strong or intermediate cytoplasmic p33ING1b expression in 35 % of the invasive malignant melanomas. Also, 47 % showed a complete loss of nuclear p33ING1b expression. The translocation or the shift to the cytoplasm may result in a loss of p33ING1b function (Nouman et al., 2002a).

Oral squamous cell carcinoma

Few studies about p33ING1b role and expression in oral squamous cell carcinoma (OSCC) have been published. In normal squamous cells, p33ING1b showed only a nuclear expression, whereas 24 % of OSCC showed a shift of p33ING1b protein from the nucleus to the cytoplasm with a weak nuclear staining, and 76 % of OSCC had a negative expression of p33ING1b. Furthermore, p33ING1b shift to the cytoplasm may result in an unfunctional protein that enhances oncogenesis of OSCC. Besides, it is demonstrated that the tumors with p33ING1b cytoplasmic expression are more aggressive, however the reason is still unknown (Zhang et al., 2008).

Ovarian cancer

Among the 111 cases of ovarian carcinoma analysed, 25 % are observed with a low or absent p33ING1b protein expression. Besides, a total of 32 cases showed a significant reduction of p33ING1b mRNA expression, with a decrease or absence of p33ING1b protein in 53 % of them. No mutation of p33ING1b has been detected in these samples. An allelic loss at the p33ING1b locus was detected in 25 % of the cases with a correlation between mRNA expression and p33ING1b protein decrease. Twenty four percent of 88 patients had p33ING1b promoter methylation. In addition, p33ING1b promoter methylation has been observed in two ovarian cancer cell lines, OVCA3 and SKOV3 (2/2). The absence of mutation suggests that other inactivation mechanisms can occur, such as histone deacetylation, and might contribute to p33ING1b silencing and tumorigenesis of ovarian cancer (Shen et al., 2005).

Pancreatic carcinoma

Only 15 % of pancreatic carcinoma specimens showed a reduction of p33ING1b protein expression. This low percentage demonstrates that there is no correlation between p33ING1b expression and clinicopathological factors. In addition, a single germline missense mutation located at codon 215 has been identified in 1/40 pancreatic carcinoma cases. This mutation is manifested by a cysteine to serine substitution at codon 215 localised in the PHD domain of ING1. However, p33ING1b protein expression is still normal in this sample. 60 % of tumor cases showed a loss of heterozygosity (LOH) with no mutation detected. In addition, 2 of these samples have an absence of p33ING1b protein expression. These findings suggested that mutations are not the only reason for the loss of function of p33ING1b in pancreatic carcinomas (Yu et al., 2004).

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This article should be referenced as such: