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Gene Section
Review

ING4 (inhibitor of growth family, member 4)

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Identity

Other names: MGC12557; my036; p29ING4

HGNC (Hugo): ING4

Location: 12p13.31

DNA/RNA

Description

ING4 belongs to family of highly homologous five members containing PHD domain and has been identified through a computational sequence homology search for expressed tag clones with a PHD finger motif (Shiseki et al., 2003). ING4 gene is located on chromosome 12p13.31 and consists of eight exons encoding a 29-kDa protein expressed in multiple human tissues.

Transcription

Multiple alternatively spliced transcript variants have been observed using different splice sites in the coding region; transcript variants span from 1461 bp to 1313 bp.
Wobble splicing events have been described at exon 4 and 5 boundary.

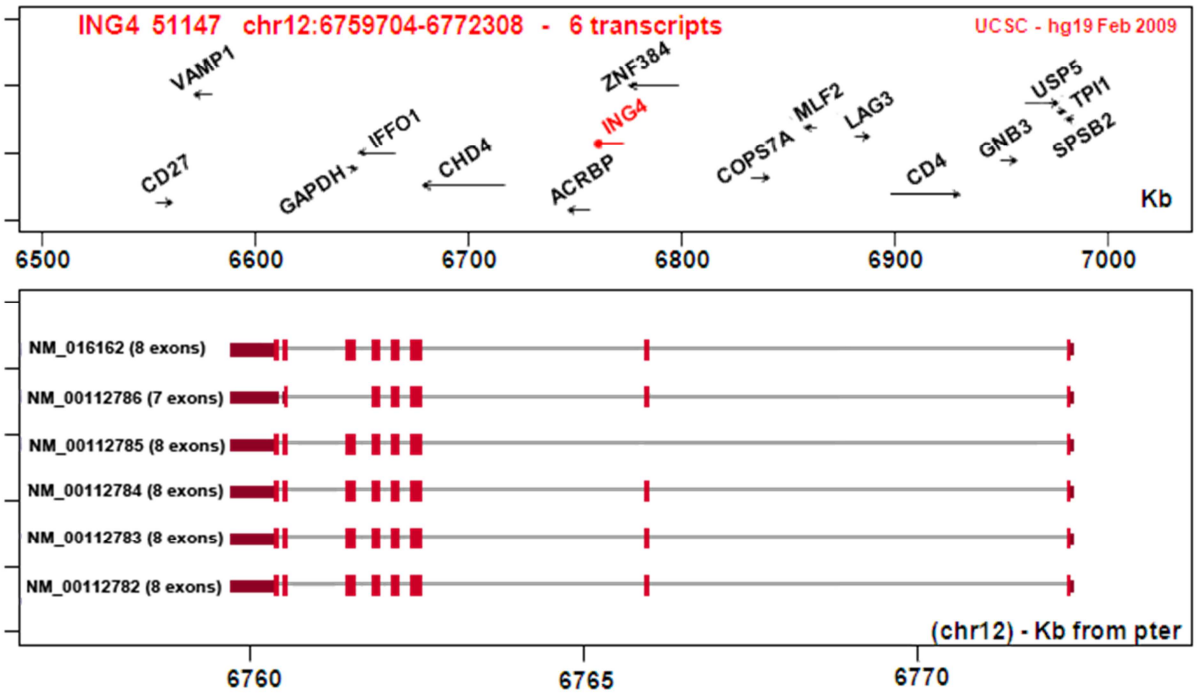


Figure adapted from Atlas of Genetics and Cytogenetics in Oncology and Haematology.

Different splicing variants have been identified (among them -v1, -v2, -v3 and -v4/Δ4AA) involving 12 bp (379-390) and resulting in in frame deletions of one to four aminoacids in NLS (Tsai and Lin, 2006).
Several splicing variants have been described lacking exon 2, 3 and 6 (entirely or in part), and named ING4-ΔEx2, -ΔEx3, -ΔEx6A and -ΔEx6B, respectively (Raho et al., 2007).
Splicing variants have been detected in all tissues analysed, indicating that are not tissue specific (Tsai and Lin, 2006; Raho et al., 2007).
More recently five novel spliced variants of ING4-v1 and -v2 were identified, causing codon frame shift and eventually deletion of NLS or PHD domains. Increased expression of these variants was found in gastric adenocarcinomas compared to normal tissue (Li M et al., 2009).

Protein

Note
249 aminoacids, 29 kDa protein.

Description

ING4 protein contains several conserved regions: i) a leucine zipper-like (LZL) domain, probably involved in protein interactions, located at the N-terminus; ii) a functional bipartite nuclear localization signal (NLS1); iii) a C-terminal plant homeo-domain (PHD), a Cys4-His-Cys3 zinc finger motif spanning 50-80 residues, found in many nuclear proteins, such as transcription factors and proteins regulating chromatin structure; iv) a non functional NLS located at the C-terminal end.

Expression

Ubiquitous.

Localisation

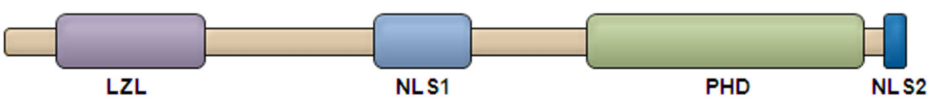
p29ING4 is a nuclear protein. It possesses a bipartite nuclear localization signal. ING4 splicing variants have been described involving the NLS1 domain; most/all of them retains nuclear localization. Furthermore ING4-v1 is translocated to the nucleolus and such subcellular localization is modulated by two wobble-splicing events at the exon 4-5 boundary, causing displacement from the nucleolus to the nucleus.

Function

ING4 was also isolated through a screening for genes able to suppress loss of contact inhibition, thus suggesting its tumor suppressor role.
ING4 is a nuclear protein participating to a variety of cellular functions, such as apoptosis, cell-cycle regulation, chromatin remodeling, and regulation of gene expression. Several ING4 partners have been described. Similarly to the other ING members, ING4 was described to interact with p53 and to modulate p53 transcriptional activity (Shiseki et al., 2003). The interaction of ING4 with p53 is mediated by the bipartite ING4 nuclear localization signal (NLS) (Zhang et al., 2005) and drives an increase of p53 acetylation at lysine 382 (Shiseki et al., 2003). ING4 is a critical regulator of chromatin acetylation required for gene expression. In particular, ING4 associates with the HAT complex HBO1 and it is required for the majority of histone 4 acetylation and for normal progression through S phase (Doyon et al., 2006; Shi et al., 2006). Recently a critical role for specific recognition of histone H3 trimethylated at lysine 4 (H3K4me3) by the ING4 PHD finger in mediating ING4 gene expression and tumor suppressor functions has been shown (Hung et al., 2009).
ING4 can also function as repressor of factors mediating angiogenesis. It was demonstrated that ING4 plays an inhibitory role on NF-kappaB activity by interaction with p65NF-kappaB and that the lack of inhibition of the NF-kappaB pathway by ING4 results in increased angiogenesis in glioblastomas (Garkavtsev et al., 2004). More recently, it has been described that physiologic levels of ING4 govern innate immunity in mice by regulating the levels of IkappaB and NF-kappaB proteins and the activation of select cytokine promoters (Coles et al., 2010).
ING4 was also described to repress the ability of hypoxia inducible factor (HIF)-1 to activate transcription of its downstream target genes by interacting with the HPH-2 prolyl hydroxylase. Under hypoxic conditions, ING4 may act as an adapter protein recruiting transcriptional repressors to mediate HIF activity (Ozer et al., 2005).
Involvement of ING4 in regulation of apoptosis has been demonstrated in several cellular systems. Its overexpression can induce apoptosis through the downregulation of Bcl-2 and the upregulation of p21 and Bax expression (Shiseki et al., 2003; Yu et al., 2007; Li X et al., 2009b; Cai et al., 2009).

Homology

ING4 protein shares homology with other ING family members with respect to the following regions: i) a leucine zipper-like (LZL) domain, probably involved in interaction with proteins, located at the N-terminus of all the ING proteins except for ING1; ii) a nuclear localization signal (NLS); iii) a C-terminal plant homeo-domain (PHD) involved in chromatin.



LZL: leucine zipper-like; NLS1: nuclear localization signal 1; PHD: plant homology domain; NLS2: nuclear localization signal 2.

Mutations

Note

The following ING4 point mutations have been found in lung adenocarcinoma and small cell lung carcinoma (H23 and H28, respectively) human cancer cell lines.

N214D: it alters ING4 capability of inhibition of proliferation, anchorage independent cell migration reducing protein stability by proteasome mediated degradation.

Y121N: it does not alter ING4 functions (Moreno et al., 2010).

Implicated in

Breast cancer

Cytogenetics

Analysis of CGH data revealed that 10-20% of primary breast tumors present deletions in 12p13. The deletions appear to affect only one copy of the gene; no genomic mutations were found in the remaining allele of ING4 (Kim et al., 2004).

Head and neck squamous cell carcinoma (HNSCC)

Cytogenetics

LOH of 12p13.

Oncogenesis

Loss of heterozygosity at 12p12-13 region was found in 66% (33/50) of head and neck squamous cell carcinomas by using six highly polymorphic microsatellite markers. No mutations of the ING4 gene were found.

Quantitative real-time RT-PCR analysis demonstrated decreased expression of ING4 mRNA in 76% of primary tumors compared to matched normal samples (Gunduz et al., 2005).

Glioma

Oncogenesis

Expression of ING4 is significantly reduced in gliomas as compared with normal human brain tissue, and the extent of reduction correlates with the progression from lower to higher grades of tumours. ING4 regulates brain tumour angiogenesis through transcriptional repression of NF-kB-responsive genes (Garkavtsev et al., 2004).

Astrocytoma

Prognosis

A potential of role of ING4 as a biomarker for the prediction of the grade of astrocytic neoplasms has been suggested (Klironomos et al., 2010).

Oncogenesis

Significantly reduced levels of ING4 were observed in human astrocytomas compared to normal brain tissue, suggesting that down-regulation of this protein might be involved in the pathogenesis of human astrocytic tumors. Decreased ING4 expression correlated significantly with tumor progression, with lower expression levels of ING4 observed in cases of high-grade neoplasms. A statistical significant negative correlation between expression of ING4 and expression of nuclear p65 was noticed (Klironomos et al., 2010).

Hepatocellular carcinoma (HCC)

Prognosis

Survival and metastasis analysis indicated that HCC patients with lower ING4 expression had poorer overall and disease-free survival than those with high expression. Multivariable Cox regression analysis revealed that the ING4 expression level was an independent factor for prognosis (Fang et al., 2009).

Oncogenesis

The ING4 mRNA and protein levels were significantly lower in HCC than paracarcinomatous liver tissue. ING4 expression level correlates with prognosis and metastatic potential, suggesting that ING4 as a candidate prognostic marker of HCC (Fang et al., 2009).

Multiple myeloma (MM)

Prognosis

MM patients with high IL-8 production and microvascular density (MVD) have significantly lower ING4 levels compared with those with low IL-8 and MVD.

Oncogenesis

ING4 suppression in MM cells up-regulated IL-8 and OPN under hypoxic conditions, increasing the hypoxia inducible factor-1alpha (HIF-1alpha) activity and its target gene NIP-3 expression. ING4 suppression in MM cells significantly increased vessel formation in vitro, blunted by blocking IL-8 or OPN (Colla et al., 2007).

Lung cancer

Oncogenesis

Reduced ING4 nuclear and cytoplasmic expression were both revealed in lung cancer and associated with tumour grade. ING4 expression in the cytoplasm was found higher than in the nucleus in a high percentage of tumors. Nuclear ING4 inhibition correlated with the tumour stage and lymph node metastasis, thus suggesting that ING4 is involved in the initiation and progression of lung cancers (Wang et al., 2010).

Gastric cancer

Oncogenesis

ING4 RNA and protein were drastically reduced in stomach adenocarcinoma cell lines and tissues, significantly less in female than male patients. Novel spliced forms of ING4-v1 and -v2 were identified in both normal and tumor tissue; increased expression of the novel spliced variants was observed in tumors; however no correlation with clinical parameters was observed (Li M et al., 2009).

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