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Review Article

Grow-ING, Age-ING and Die-ING: ING proteins link cancer, senescence and apoptosis

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ABSTRACT

The INhibitor of Growth (ING) family of plant homeodomain (PHD) proteins induce apoptosis and regulate gene expression through stress-inducible binding of phospholipids with subsequent nuclear and nucleolar localization. Relocalization occurs concomitantly with interaction with a subset of nuclear proteins, including PCNA, p53 and several regulators of acetylation such as the p300/CBP and PCAF histone acetyltransferases (HATs), as well as the histone deacetylases HDAC1 and hSir2. These interactions alter the localized state of chromatin compaction, subsequently affecting the expression of subsets of genes, including those associated with the stress response (Hsp70), apoptosis (Bax, MDM2) and cell cycle regulation ($p21^{WAF1}$, cyclin B) in a cell- and tissue-specific manner. The expression levels and subcellular localization of ING proteins are altered in a significant number of human cancer types, while the expression of ING isoforms changes during cellular aging, suggesting that ING proteins may play a role in linking cellular transformation and replicative senescence. The variety of functions attributed to ING proteins suggest that this tumor suppressor serves to link the disparate processes of cell cycle regulation, cell suicide and cellular aging through epigenetic regulation of gene expression. This review examines recent findings in the ING field with a focus on the functions of protein–protein interactions involving ING family members and the mechanisms by which these interactions facilitate the various roles that ING proteins play in tumorigenesis, apoptosis and senescence.

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Introduction

The founding member of the INhibitor of Growth (ING) family of type II tumor suppressors, ING1, was discovered using PCR-mediated subtractive hybridization followed by selection of clones in a senescent cell library and a functional biological screen designed to identify factors that were differentially expressed in normal mammary epithelial cells compared to breast cancer cell lines [1]. Subsequently, ectopic overexpression of ING1 was found to promote G_1 arrest, while inhibition of ING1 expression with antisense RNA encouraged transformation in vitro and tumor formation in vivo [1–3]. ING1 has since been shown to impinge upon many aspects of cellular physiology, including both p53-dependent and -independent apoptosis, DNA damage repair, cell cycle regulation, senescence, and tumorigenesis [1–16].

Since the discovery of ING1, four additional ING genes, ING2-5, have been identified in humans and classified as ING family proteins based on sequence homology with ING1 (Fig. 1) [2,17,18]. Furthermore, homologues to different ING family members have also been identified in the mouse, rat, and cow genomes [19]. The highly conserved nature of the ING genes is apparent as clear homologues to human INGs have also been identified in other vertebrates, such as the frog *Xenopus* [20] as well as the zebrafish. In fact, phylogenetic analyses show that ING sequences are present in different kingdoms: the plants *Oryza satvia* and *Arabidopsis thaliana* as well as the fungi *S. cerevisiae*, *S. pombe* and *N. crassa* contain recognizable ING genes that exhibit a high degree of sequence conservation within the plant homeodomain (PHD) region [19,21].

ING family proteins: structure = function

All mammalian ING family proteins possess a highly conserved region containing a nuclear localization sequence (NLS) and a plant homeodomain (PHD) motif [2,18,19,22–24] (Fig. 1A). Although mutations in the ING NLS region are rare, loss of nuclear ING expression has been described for several human cancers, suggesting that attenuation of NLS-mediated nuclear import may play a role in the development of cancers where ING function is abrogated [25–29]. Within the NLS domain of ING1 lie two functional copies of a related functional motif, the nucleolar translocation signal (NTS) (Fig. 1D) [23], which have been shown to be both necessary and individually sufficient to mediate localization of p33ING1b to the nucleolus following UV irradiation in human fibroblasts, a process required for ING1-associated apoptosis following UV-induced DNA damage [23]. Furthermore, mutation of the ING1 NLS or NTS impairs the ability of human fibroblasts to undergo apoptosis following UV exposure, implicating the nuclear, and possibly subnuclear, localization of ING1 in DNA damage-induced apoptosis.

The plant homeodomain motif is a Cys4-His-Cys3 zinc finger that is structurally similar to RING and LIM domains (Figs. 1B and C). The most highly conserved feature of the ING family, the PHD, is known to be involved in protein–protein interactions and is commonly found in proteins associated with chromatin remodeling function [30–35]. A recent study showed that the rare lipid signaling molecule phosphatidylinositol-5-monophosphate (PtdIns-5-P), generated in response to DNA damage-induced cellular stress, binds the ING PHD region avidly, an activity necessary for activation of ING function [36]. These observations are consistent with the observed roles of ING in chromatin remodeling and stress-induced apoptosis, respectively, and provide a potential regulateable mechanism for ING proteins to respond differentially to cellular stresses (Fig. 2).

ING and p53: function-ING together

The 33 kDa splice isoform of ING1, p33ING1b, has been reported to sensitize cells to DNA damaging agents such as γ-radiation, etoposide and ultraviolet (UV) radiation in a p53-dependent fashion [4], suggesting that ING1 and p53 may act synergistically to activate or repress specific DNA damage response pathways. Moreover, p33ING1b, p33ING2, p29ING4 and p28ING5 can induce G_1-phase cell cycle arrest or apoptosis in a p53-dependent manner following DNA damage [2,4,5,11,15,37,38], further implying at least a functional association in response to genotoxic insult.

Several reports suggest that in some systems, p53 and p33ING1b require the presence of the other protein for activity [4]; however, subsequent studies have indicated that p33ING1b can induce apoptosis in response to DNA damage independent of p53 status [20]. At present, the precise mechanism by which

Fig. 1 – Structural features of the ING proteins. (A) Schematic illustration of the ING family of proteins showing alignment relative to the highly conserved PHD motif and NCR region. PHD = plant homeodomain; NLS = nuclear localization sequence; PIP = PCNA-interacting protein motif; PB = partial bromodomain; NCR = novel conserved region; LZL = leucine zipper-like. (B) Amino acid sequence alignment of the ING PHD motif (modified from [19]). Highlighted residues correspond to the characteristic spacing of the zinc-binding cysteine and histidine residues that defines the PHD motif. (C) Structural comparison of the related zinc-binding RING, LIM, and PHD domains. Note the different spacing of the cysteine and histidine residues involved in zinc chelation, resulting in differential folding of the motifs and thus the unique properties of each domain. (D) Amino acid sequence alignment of the conserved bipartite NLS regions from each ING protein. Highlighted residues correspond to the related nucleolar translocation sequences (NTS) that are required for nuclear and, in the case of p33ING1b, nucleolar localization [19,23].