CMLS, Cell. Mol. Life Sci. 61 (2004) 2597–2613
1420-682X/04/202597-17
DOI 10.1007/s00018-004-4199-4

CMLS Cellular and Molecular Life Sciences

Review

Biological functions of the ING family tumor suppressors

E. I. Campos, M. Y. Chin, W. H. Kuo and G. Li*

Department of Medicine, Division of Dermatology, University of British Columbia, Vancouver, British Columbia, V6H 3Z6 (Canada)
Mailing address: Jack Bell Research Centre, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6 (Canada), Fax: +1 604 875 4497, e-mail: gangli@interchange.ubc.ca

Received 7 May 2004; received after revision 17 June 2004; accepted 8 July 2004

Abstract. Early studies of the inhibitor of growth 1 (*ING1*) gene, the founding member of the *ING* tumor suppressor family, demonstrated that this gene plays an important role in apoptosis and cellular senescence. Four other related genes have since been identified and found to be involved in various biological activities, including cell cycle arrest, regulation of gene transcription, DNA repair and apoptosis. The biochemical functions of ING proteins as histone acetyltransferases and histone deacetylase co-factors ties this new tumor suppressor family to the regulation of transcription, cell cycle checkpoints, DNA repair and apoptosis. This review is aimed at summarizing the known biological functions of the ING tumor suppressors and the signalling pathways that they involve.

Key words. ING; p53; tumor suppressor; cell cycle; DNA repair; apoptosis; histone acetylation.

Introduction

The *ING1* gene was originally identified through subtractive hybridization between normal human mammary epithelial cells and seven breast cancer cell lines, and subsequent in vivo selection of genetic suppressor elements that displayed oncogenic characteristics [1]. Three alternatively spliced transcripts of the *ING1* gene have been found, and they encode protein variants with a predicted size of 47, 33 and 24 kDa [2]. In addition to *ING1*, four additional related genes (*ING2*, *ING3, ING4, ING5*) have since been identified. Cloning of *ING2* (formally known as *ING1L*) was done through a homology search of $p33^{ING1b}$ complementary DNA (cDNA) sequence with the Otsuka cDNA database [3]. *ING2* encodes a 33-kDa protein, and $p33^{ING2}$ shares 58.9% homology with $p33^{ING1b}$, while the nucleotide sequences between these two genes shows 60% identity [3]. $p33^{ING2}$ contains a unique leucine zipper domain that is thought to mediate hydrophobic protein-protein interactions [4]. *ING3* was subsequently identified through a sequence homology search [5], and *ING4* and *ING5* were identified through a computational search of expressed sequence tag (EST) clones showing homology to *ING1* and *ING2*. Both protein products, $p29^{ING4}$ and $p28^{ING5}$, are highly homologous, sharing 72.8% of identity to one another [6]. All ING proteins further share a highly conserved carboxy-terminal plant homeodomain (PHD) (a C4HC3 zinc finger motif implicated in chromatin remodelling) and a nuclear localization sequence (see previous reviews [4, 7, 8] for *ING* gene and protein structures).

ING1 messenger RNA (mRNA) is found ubiquitously expressed in various human tissues [3]. Northern analysis of specific *ING1* multiplex polymerase chain reaction (PCR) products confirmed that $p33^{ING1b}$ is the predominant form expressed in normal tissues, with varying degrees of expression in different types of tissues. The expression of

* Corresponding author.

p24^{ING1c} is lower than *p33*^{ING1b}, and all tissues examined express little if any *p47*^{ING1a} [2]. Northern analysis of murine ING1 homologs, *p31*^{ING1} and *p37*^{ING1}, also demonstrates ubiquitous expression in all adult tissues, with highest levels in thymus [9]. Furthermore, both isoforms were expressed during all stages of embryonal development, with differing peak levels [9]. p33^{ING2} and p47^{ING3} expression levels were found to be ubiquitous in normal human tissues, with varying expression levels in different tissue types. For example, the mRNA expression level of *ING2* is high in testis but low in lung and undetectable in kidney [3], whereas *ING3* is highly expressed in spleen in which *ING2* expression is undetectable, but less expressed in lung and brain [5]. *ING4* is expressed in multiple human tissues with the highest expression in testis [10].

Early studies quickly linked negative regulation of cellular growth and sensitization to apoptosis as biological functions of the *ING1* gene. Flow cytometry analysis of normal fibroblasts overexpressing *ING1* demonstrated the capability of this gene to initiate a G_1/G_0 cell cycle block [1], while overexpression of *ING1* was reported to enhance apoptosis in various cultured cell models [1, 11–15]. This review presents ING proteins as evolutionary conserved co-factors of histone acetyltransferases (HATs) and histone deacetylase (HDAC) that mediate acetylation of essential cellular components, including core histones and the p53 tumor suppressor. Moreover, it is the acetylation of these components that tightly links ING proteins to the regulation of transcription, cell cycle checkpoints, DNA repair, apoptosis and ultimately tumor suppression.

Cell cycle regulation

Maintenance of genomic integrity upon genotoxic stress relies on the ability of cells to halt during the cell cycle to allow proper cellular repair or apoptosis, thus preventing the propagation of genetic alterations. Activation of the p53 tumor suppressor to prevent cellular G_1/S transition is a key mechanism utilized by cells to mediate cellular growth control [16]. Various lines of evidence indicate that ING proteins can also influence cell cycle progression and are actively involved in cellular checkpoints. One candidate mechanism that was first proposed as responsible for *ING1*-mediated growth suppression is the cooperation of p24^{ING1c} with the p53 tumor suppressor to enhance transcription of the cyclin-dependent kinase inhibitor (CDKI) p21^{Waf1} [17]. Waf1 (also know as Cip1 and Sdi1) is a potent mediator of the G_1 cellular checkpoint that binds and inactivates cyclin-Cdk complexes, resulting in reduced phosphorylation of retinoblastoma protein (Rb), E2F sequestration and cell cycle arrest at the G_1/S transition [18]. Reduced growth inhibition is observed when either p53 or p24^{ING1c} is suppressed, and p24^{ING1c} expression is required to obtain efficient transcriptional activation of the p21^{Waf1} promoter. Recent experiments utilizing luciferase gene constructs fused with the p21^{Waf1} promoter further demonstrated that p33^{ING2} and p47^{ING3} enhance while p47^{ING1a} represses the p21^{Waf1} promoter activity [19]. p33^{ING2} and p47^{ING3} involvement in negative regulation of cell growth is further evidenced by p33^{ING2} or p47^{ING3}-mediated G_1 cell cycle arrest and inhibition of colony formation in RKO colorectal carcinoma cells carrying wild-type (wt) p53 [5, 20]. Chronic overexpression of p47^{ING3} also suppresses colony formation in NIH 3T3 cells [5]. Furthermore, Western analysis of uninhibited viable clones showed loss of p47^{ING3} expression [21]. In addition, overexpression of p29^{ING4} and p28^{ING5} resulted in a decrease in the S-phase cell population and an increase in both the G_1/S- and G_2/M-phase cell populations in wt p53 RKO cells but not in p53-depleted RKO-E6 cells, suggesting that these two ING members also mediate cell cycle arrest in a p53-dependent manner [6]. As expected, both p29^{ING4} and p28^{ING5} enhanced transcriptional regulation of p21^{Waf1} in a p53-dependent manner [6].

Recent evidence suggests different mechanisms associated with the initiation of a G_2 cell cycle arrest and its maintenance. Initiating a G_2 arrest requires phosphorylation of the cyclin-dependent kinase 1 (CDK1) and is p53-independent. p53 can, however, promote the maintenance of this cellular G_2/M transition halt [22–24] by oppressing cyclin B1 mRNA and protein levels [24–26]. Cyclin B1 is the regulatory subunit of CDK1 and is required for mitotic onset [27]. Microarray analysis of p33^{ING1b}-regulated genes coincidentally identified *CCNB1* (cyclin B1 gene) as a downregulatory target of p33^{ING1b} [28], suggestive of p53-dependent involvement of ING proteins in cellular G_2/M checkpoint. Such involvement was substantiated by observations made in a few studies. First, overexpression of p33^{ING1b} was observed to enhance adriamycin-induced G_2 arrest in the p53-null H1299 non-small cell lung carcinoma cell line [29]. The antiproliferative effects of p33^{ING1b} were further evidenced by an increased cellular doubling time upon p53 overexpression compared to severely compromised proliferation upon expression of both recombinant p53 and p33^{ING1b} proteins [29]. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of p53-deficient SAOS2 cells infected with p33^{ING1b} also resulted in a slight decrease in cyclin B1 mRNA levels after 72 h. However, infection of both p53 and p33^{ING1b} constructs caused a marked reduction of cyclin B1 mRNA 24 h post-infection, suggesting that p33^{ING1b} repression of cyclin B1 may be enhanced in the presence of p53 [28]. It is conceivable that certain ING members may also indirectly enhance G_2 arrest by promoting the acetylation of p53, as a recent report suggested that cells expressing p53 lacking c-terminal lysine residues had a minimal effect on cyclin B1 and could undergo G_1 but not G_2 cycle arrest [30]. It is interesting to note that p24^{ING1c} protein level is inversely correlated to

cyclin B1 level. While $p24^{ING1c}$ protein level increases in late G_1 and decreases in G_2 in normal murine mammary gland epithelial (NMuMG) cells [31], cyclin B1 expression is normally maximal during the G_2/M phase of the cell cycle and is rapidly degraded at the end of mitosis [27]. Collectively, these observations imply a role of ING proteins in the regulation of cell cycle checkpoints.

Other studies have further implied ING proteins in cell cycle regulation. Cyclin E is involved in G_1/S phase progression in the cell cycle and is used as a marker for cell proliferation. Overexpression of cyclin E can induce acceleration of the G_1 phase of the cell cycle and may be linked with various malignancies [32]. In a recent study, cyclin E was immunoprecipitated from human hepatocellular carcinoma (HCC) protein lysates and assayed in vitro for the ability to phosphorylate recombinant Rb. The authors found cyclin E-associated kinase activity to be significantly higher in HCC biopsies with negative immunological $p33^{ING1b}$ staining compared to HCC samples positive for $p33^{ING1b}$ [33]. Further exploration of the relation between ING proteins and CDKIs and cyclins may provide useful information for understanding the role of ING proteins in cell cycle regulation and deregulation in malignancies. More recently, $p33^{ING1b}$ was shown to interact with the DNA methyltransferase 1 (DNMT1)-associated protein 1 (DMAP1) complex and a core Sin3-HDAC1/2 complex to maintain histone methylation and hypoacetylation in pericentric heterochromatin during late S phase of the cell cycle [34]. ING proteins may also be involved in mitosis and meiosis, as suggested by Choy and colleagues [35]. Experiments in budding yeast have demonstrated that acetylation of histone 4 (H4) by the NuA4 complex requires Yng2p (a yeast ortholog of $p47^{ING3}$) and is necessary to correctly progress through mitosis [36]. In fact, treatment of *Yng2* mutant cells with trichostatin A (TSA), a histone deacetylase inhibitor, was sufficient to restore H4 acetylation and allow a nearly normal mitotic progression [35]. Yng2 was later found to be required for in vitro NuA4 HAT activity [37], possibly by participating in NuA4-dependent H4 acetylation to promote the transcription of specific genes, and/or facilitating genome-wide acetylation to ensure proper chromatin arrangement during mitosis, and helping mediate acetylation of other non-histone substrates that regulate cell cycle progression. This was an interesting finding and has been followed up in a recent study by Doyon and colleagues [21] which demonstrates that a NuA4 complex homolog does exist in humans, consisting of the $p47^{ING3}$ ING family member (see section on acetylation).

Apoptosis

Initial evidence indicative of a role of *ING1* in apoptosis came from the observation of prominent *ING1* expression in regressing tails of *Xenopus* tadpoles but absence from growing hind limbs, the induced expression of *ING1* in serum-starved P19 teratocarcinoma cells [11, 38], as well as anchorage-independent growth and neoplastic transformation associated with the suppression of *ING1* [1]. Consistent with these observations, senescent human fibroblasts were shown to express 10-fold more *ING1* mRNA compared to early passage fibroblasts, while antisense expression of *ING1* resulted in prolonged proliferative life span of these cells [31]. Early passage but not senescent fibroblasts were also able to upregulate $p33^{ING1b}$ expression and enter apoptosis upon growth factor deprivation [39].

As in cell cycle regulation, cooperation with p53 was quickly recognized as essential for *ING1*-mediated sensitization to apoptosis. First, $p24^{ING1c}$ was found to physically bind to p53 by immunoprecipitation [17]. More important, *ING1*-expressing fibroblasts were shown to have little effect on cell survival in the absence of p53, while both *ING1* and p53 were required to suppress colony formation [17]. In agreement with this study, adenoviral delivery of both $p33^{ING1b}$ and p53 was shown to synergistically induce apoptosis in cultured human glioma cells [12]. Similar observations were reported upon overexpression of both p53 and $p33^{ING1b}$ in T.Tn human esophageal carcinoma cells [40]. Together, both tumor suppressor proteins inhibited anchorage-dependent cell growth, reduced cell viability and enhanced apoptosis more efficiently than with p53 alone [40]. The same was true for transcriptional upregulation of the p53-downsteam targets $p21^{Waf1}$ and MDM2 [40]. We provided further evidence supporting the involvement of *ING1* in the p53-mediated intrinsic apoptotic pathway by demonstrating that $p33^{ING1b}$ enhances transactivation of the proapoptotic Bcl-2 family protein Bax upon ultraviolet (UV) irradiation and consequently promotes change in mitochondrial membrane potential [15]. As expected, enhancement of UV-induced apoptosis of melanoma cells by $p33^{ING1b}$ was dependent on wt p53 function [15]. This p53 dependency in apoptosis was further illustrated by an experiment in which p53-null H1299 cells expressing both p53 and $p33^{ING1b}$ tumor suppressors were found to be more sensitive to adryamicin compared to either one alone [29].

A few observations hint at different mechanisms through which different ING proteins likely enhance apoptosis. The interrelation between p53 and different ING members is apt to influence the apoptotic response (see section on p53 dependency). However, the interaction of ING proteins with the proliferating cell nuclear antigen (PCNA) and histone-modifying factors may also play a large part. A recent study demonstrated that the ectopic expression of $p33^{ING1b}$, but not $p47^{ING1a}$, sensitized early passage human fibroblasts to apoptosis [39]. These differences are reflective of the distinctive amino-terminal

sequences of ING proteins. For example, $p33^{ING1b}$, but not $p47^{ING1a}$, contains a PCNA-interacting protein (PIP) box which permits $p33^{ING1b}$ to physically interact with PCNA following UV exposure and enhance apoptosis [13]. UV irradiation of $p33^{ING1b}$-transfected cells increased baseline levels of sub-G_1 cells compared to mock-transfected cells. However, overexpression of PIP-mutant $p33^{ING1b}$ efficiently protected cells from UV, as these cells showed a similar sub-G_1 DNA content distribution compared with non-irradiated controls [13]. Part of this ING-mediated enhancement of the apoptotic response is also likely mediated by ING transcriptional activity and histone acetylation linked to ING proteins. ING proteins contain a nucleolar targeting sequence (NTS) and are translocated to the nucleolus, a transcriptionally active area of the nucleus, upon UV irradiation [4, 14]. $p33^{ING1b}$ has been shown to downregulate the expression of various genes involved in cellular growth, such as the *Cyclin B1* gene, and upregulate genes that will promote a cell cycle halt and apoptosis, such as *Waf1* and *Bax*, presumably by association with HATs and HDACs. Furthermore, $p33^{ING1b}$ mutants lacking a functional NTS motif were restricted in their ability to appropriately promote apoptosis [14]. Antagonistic effects are also seen between different ING family members. For example, while $p33^{ING1b}$ and $p47^{ING1a}$ possessed antagonistic effects on histone acetylation both in vitro and in vivo, ectopic overexpression of $p33^{ING1b}$, but not $p47^{ING1a}$, resulted in an increased sub-G_1 peak, indicative of an apoptotic component [39]. This observation is also consistent with previous studies reporting a link between increased histone acetylation and apoptosis [41, 42].

Other ING members are also shown to be actively, but differentially involved in apoptosis. The expression of $p33^{ING2}$ was found to be specifically induced by etoposide or neocarzinostatin, but not by gamma irradiation, doxorubicin, *cis*-platinum, or bleomycin in a normal lymphoblastoid cell line [20]. This induction of $p33^{ING2}$ was independent of ATM or p53 function. Unlike $p33^{ING2}$, $p47^{ING3}$ expression level was not altered by various genotoxic stresses [5]. However, overexpression of both $p33^{ING2}$ and $p47^{ING3}$ induced apoptosis and G_1-phase cell cycle arrest in RKO cells, but not in a p53-deactivated, RKO-E6 cell line [5], suggesting overlapping functions between various ING family members in negative regulation of cell growth and apoptosis as well as a dependent relation between various ING members and p53. The role of *ING2* in hormone-dependent developmental events was studied using *Xenopus* tadpole metamorphosis as a model system, in which a single stimuli thyroid hormone (TH) initiates tissue-specific proliferation, apoptosis and remodelling simultaneously. Thyroid receptors (TRs) selectively repress or activate gene transcription in response to absence or presence of TH [38]. It is shown that *Xenopus* ING2 (xING2) transcript levels increase in response to TH-induced metamorphosis and during spontaneous metamorphosis in *X. laevis* tadpole tissues [38]. It is interesting to note that in the tail and brain, which apoptose extensively during metamorphosis, xING2 induction follows TRs expression pattern; whereas in the legs, which proliferate and grow, xING2 expression is not induced, similar to that of TRs, suggesting the role of xING2 in hormonally regulated nuclear transcription factor-mediated apoptotic response. *ING3* may also be activated upon cellular exposure to reactive oxygen species, as it was found to be differentially expressed in an mRNA differential display screen comparing cells overexpressing mitochondrial phospholipids hydroperoxide glutathione peroxidase (mtPHGPx) with control cells [43].

DNA repair

The p53 tumor suppressor is well known to play an essential role in cellular stress response to UV irradiation, including DNA repair and promotion of apoptosis in cases where the extent of DNA damage is too great [44–46]. The fact that $p33^{ING1b}$ physically binds to p53 and shares similar biological functions with p53 led us to hypothesize that ING proteins may also directly participate in the cellular stress response to DNA damage**.** In a previous study, our group showed that UV irradiation induced a cell type-specific $p33^{ING1b}$ expression at both mRNA and protein levels in a time- and dose-dependent manner independent of p53 **[**47, 48**]**. We further demonstrated that overexpression of the $p33^{ING1b}$ protein enhanced nucleotide excision repair (NER) of UVC-damaged exogenous plasmid DNA and UVB-damaged genomic DNA. Using a host-cell-reactivation assay where a UV-damaged reporter plasmid was transfected into a melanoma cell line, $p33^{ING1b}$ overexpression led to an increased repair efficiency of the UV-damaged plasmid DNA by two- to fourfold compared to vector and antisense controls. Likewise, the levels of the major UVB-induced photoproduct, cyclobutane pyrimidine dimers (CPDs), were monitored in global genomic repair using a radioimmunoassay (RIA). Human melanoma MMRU cells overexpressing $p33^{ING1b}$ repaired CPDs at a rate nearly twice as fast as vector-transfected control cells 24 h after UV irradiation. Furthermore, $p33^{ING1b}$-mediated enhancement of DNA repair was dependent on wt p53. However, unlike p53, $p33^{ING1b}$ was found incapable of interacting with the XPA and XPB DNA repair proteins. More recently, our group found that repeated mutations occur at codons 102 and 260 of the *ING1b* gene in human cutaneous melanoma biopsies [49]. Using the same host-cell reactivation assay, we have found these mutations to be detrimental to $p33^{ING1b}$-enhanced NER, as both R102L and N260S mutants abolished $p33^{ING1b}$ enhancement in NER. RIA using specific antibodies against pyrimidine

(6-4) pyrimidinone photoproducts (6-4PPs) confirmed this observation. While cells overexpressing wt $p33^{ING1b}$ repaired 47% of 6-4PPs 4 h following UVB irradiation, cells transfected with R102L and N260S mutants only repaired 18.6 and 18.5% of 6-4PPs, respectively. Both $p33^{ING1b}$ mutants displayed a similar phenotype of reduced NER as a truncated $p33^{ING1b}$ mutant lacking the PHD domain, suggesting that this zinc finger is required for proper DNA repair functions. Preliminary data from our laboratory further links other ING family members to DNA repair [unpublished data].

ING proteins may also be involved in early events in the cellular responses to DNA lesions. Early mobilization of p53 involves acetylation of a series of lysine residues at its C-terminal regulatory domain by CBP/p300. The resulting conformational changes play a role in the ubiquitination and degradation of MDM2 [50], enhance the ability of p53 to bind DNA and upregulate transcription of various genes required for DNA repair [51, 52]. Recent studies showed that $p33^{ING1b}$, $p33^{ING2}$, $p29^{ING4}$ and $p28^{ING5}$ can cooperate with p300 to promote the acetylation of p53 at Lys-382 [5, 6, 19, 20], suggesting that ING proteins may act early in NER upon DNA damage. Since there are multiple acetylation sites present in p53, it is likely that there may be potential subtle differences between ING family members in acetylating p53, which will assist in defining specificity of their modulating activities in DNA repair.

While the exact molecular mechanism that links ING proteins to DNA repair is still not fully understood, a few noteworthy points may clarify the involvement of ING proteins in the DNA repair machinery. PCNA is a highly conserved protein deemed essential for viability. This protein forms a homo-trimeric complex that can encircle DNA as part of larger multi-protein complexes involved in DNA replication and repair. Several PCNA-interacting proteins contain a common octapeptide motif (QXX [ILM]XXF[FY]) called PIP-domain, through which they bind competitively to PCNA [53]. Since $p33^{ING1b}$ binds to PCNA upon UV irradiation and competes with $p21^{Waf1}$ for binding with PCNA [13], it has been proposed that $p33^{ING1b}$ may inhibit DNA replication resulting from $p21^{Waf1}$ dissociation with PCNA [54], thus favoring DNA repair over DNA replication [13]. Alternatively, but not mutually exclusively, the observation of p300 association with PCNA-$p33^{ING1b}$ [55] has also been proposed to potentially link chromatin remodelling to DNA repair, since both PCNA and p300 have been shown to play a role in chromatin remodelling following DNA damage [56, 57]. Furthermore, chromatin immunoprecipitation experiments (ChIP) demonstrated that $p33^{ING1b}$ can interact with chromatin [55], further implying a role of ING proteins in chromatin remodelling. The role of different ING proteins in DNA repair is currently under investigation in our laboratories.

ING proteins may also be involved in the repair of double-strand breaks. The chemotherapeutic agent adriamycin is a topoisomerase II inhibitor that generates double-strand DNA breaks [58]. In a study by Tsang and colleagues [29], a prominent G_2/M phase arrest was observed upon exposure to adriamycin when $p33^{ING1b}$ was expressed in p53-null H1299 cells. Interestingly, this G_2/M arrest was not observed upon treatment with cisplatin and UV exposure, both of which generate intrastrand cross-links [59, 60]. Reintroduction of p53 restored the G_1 arrest after UV irradiation, suggesting that in the absence of p53, $p33^{ING1b}$ can respond to double-stand breaks but not to intrastrand cross-links. Further investigation should be conducted to verify whether ING proteins are involved in homologous repair or non-homologous end joining (NHEJ) repair pathways.

Transcription

The gene expression profile of mouse mammary epithelial (NMuMG) cells infected with a viral construct expressing antisense *ING1b* was studied using microarray technology and subsequently confirmed by RT-PCR. Suppression of *ING1b* led to a minimal 1.5-fold upregulation of 14 genes (0.6% of monitored genes) and a minimal 1.5-fold downregulation of 5 genes (0.2% of monitored genes) [28]. In accordance with its role as a tumor suppressor, overexpression of $p33^{ING1b}$ resulted in downregulation of α-fetoprotein (AFP), a tumor-specific marker of hepatocellular carcinoma [61]; cyclin B1, which is correlated with poor prognosis and is overexpressed in various cancers including non-small cell lung cancer, colorectal cancer, squamous cell carcinoma of the head and neck, esophagous, tongue, larynx, breast cancer cells and malignant breast lesions [62–69]; 12-*O*-tetradecanoylphorbol-13-acetate-inducible sequence 11 (TIS11), a member of the CCCH zinc finger protein family, which consists of growth factor-inducible nuclear proteins and functions as a positive transcriptional regulator reported to influence growth and/or survival pathways [70, 71]; DEK, a chromatin-associated proto-oncogene initially identified as part of a fusion protein with the nucleoporin CAN/Nup214 in a subtype of acute myeloid leukemia [72, 73] overexpressed in various cancers including acute myeloid leukemia, bladder cancer and human hepatocellular carcinoma [74–76], and thought to be involved in certain autoimmune disorders; testicular tumor differentially expressed (TDE) which is overexpressed in lung tumors [77]; and finally the downregulation of osteopontin, a protein thought to be involved in cancer metastasis [78, 79]. It is, however, puzzling to note that $p33^{ING1b}$ downregulates the insulin-like growth factor 2 receptor (M6P/IGF2R), a multifunctional receptor involved in cytotoxic T cell-induced

apoptosis and tumor suppression [28]. Also intriguing is the RT-PCR-based observation that low expression of $p33^{ING1b}$ within a short time period (up to 12 h) caused downregulation of the translationally controlled tumor protein (TPT1/TCTP), a protein associated with cell growth and malignant transformation [80]. However, higher expression levels of $p33^{ING1b}$ in NMuMG cells observed at longer time points clearly upregulated the expression of TPT1 protein [28]. The upregulation of various ribosomal proteins (S7, S11, S29, L12) and Int-6 (a component of the eukaryotic translation initiation factor-3) by $p33^{ING1b}$ [28] further link $p33^{ING1b}$ to the translation machinery. Some of these components, such as S29 ribosomal protein, are known to help modulate apoptosis [81, 82].

Recently, a study was carried out to investigate the mechanisms by which ING family members contribute to the downregulation of the AFP gene. It was found that, in contrast to upregulation of the *Waf1* promoter, $p33^{ING1b}$ and $p33^{ING2}$ strongly repressed human *AFP* promoter activity, possibly through binding to the *AFP* AT motif, in wt p53 hepatocellular carcinoma HepG2 cells [19]. Contrarily, $p47^{ING3}$ was found to have no effect on the *AFP* promoter, although it upregulated *Waf1* promoter activity [5, 19]. This repressive effect on the *AFP* promoter was also observed to a lesser extent in a p53-null cell line, suggestive of plausible p53-dependent and p53-independent regulatory mechanisms. $p33^{ING1b}$ was found to physically associate with hSir2 [19], an HDAC family member that binds and deacetylates Lys-382 of the p53 protein [52, 83]. The authors therefore proposed a gene regulation model in which $p33^{ING1b}$ represses *AFP* transcription by binding to the AT motif, thus excluding HNF1 (the primary *AFP* positive transcriptional regulatory factor) binding, and by increasing p53 acetylation through binding and inhibition of hSir2 [19].

Using an MDM2 promoter-luciferase reporter, both $p33^{ING1b}$ and $p24^{ING1c}$ enhanced transcription in a p53-dependent manner [84]. However, this was not observed when ING1 proteins were co-expressed with p53 mutants lacking the N-terminal transactivation domain [84], raising the possibility that $p33^{ING1b}$- and $p24^{ING1c}$-mediated activation of p53 through acetylation could account for or at least enhance transcription of p53 gene targets such as bax, MDM2 and $p21^{Waf1}$. Murine $p31^{ING1}$ was also found to have similar biological functions to its $p33^{ING1b}$ human counterpart. However, unlike $p31^{ING1}$, overexpression of murine $p37^{ING1}$ inhibited UV-induced accumulation of p53 and repressed p53-mediated gene transactivation [9]. Interestingly, $p31^{ING1}$ but not $p37^{ING1}$ was found to associate with p53 through immunoprecipitation [9]. This differential role is consistent with studies examining *Waf1* promoter activity, which revealed that while $p33^{ING1b}$, $p47^{ING3}$, $p29^{ING4}$ and $p28^{ING5}$ enhanced *Waf1* promoter activity in a p53-dependent manner, this promoter was repressed by $p47^{ING1a}$ [5, 6, 17, 19, 20]. Like $p33^{ING1b}$, $p33^{ING2}$ and $p47^{ING3}$ were also found to enhance the promoter activities of Bax in wt p53 colorectal carcinoma RKO cells [5, 19]. It is interesting to note that in non-small cell lung cancer, both *Waf1* and *Bax* gene expression levels were significantly lower in $p33^{ING1b}$-reduced tumors than in the $p33^{ING1b}$-positive tumors [85]. The p53-dependent transcriptional activity of $p29^{ING4}$ and $p28^{ING5}$ seems to be weaker than that of $p33^{ING2}$ [6]. Moreover, $p33^{ING2}$ was significantly more effective in enhancing these p53 transcriptional-transactivation activities compared to $p33^{ING1b}$ [20]. Similarly, it was found that the transcriptional activities of the p53-related $p63\alpha$ and $p73\alpha$ proteins were enhanced in the presence of $p33^{ING1b}$ but not to the extent observed with p53 [29]. It would be interesting to examine whether ING members can modulate p53-related proteins via protein acetylation. Garkavtsev and colleagues [10] recently showed that $p29^{ING4}$ co-immunoprecipitated with the transcription factor NF-κB and attenuated NF-κB transcriptional activity. $p29^{ING4}$ was found to attenuate the expression of various angiogenic factors, including interleukins IL-6 and IL-8, prostaglandin-endoperoxide synthase 2 and colony-stimulating factor 3, thus inhibiting angiogenesis of brain tumors in mice. The author demonstrated that $p29^{ING4}$ directly associates with and represses the transcriptional activities of NF-κB in the gel mobility shift assay. Furthermore, $p29^{ING4}$ was shown to negatively regulate the expression of vascular endothelial growth factor (VEGF).

The exact mechanism(s) that account for ING-mediated gene regulation remain to be elucidated. All ING proteins contain a highly evolutionarily conserved plant homeodomain zinc finger within their carboxyl moiety, a motif found in various chromatin-regulating proteins, including HATs and transcription factors [86]. Evidence from electromobility shift assay (EMSA) and ChIP suggest that $p33^{ING1b}$ is capable of binding DNA either directly or indirectly [19, 55]. It remains unknown whether ING proteins interact directly with DNA though the PHD motif or indirectly via other accessory proteins. However, interaction of various ING proteins with different HAT/HDAC components (see table 1) strongly argues a role of ING protein as co-activator/repressors of gene transcription. A few clues have emerged on events that lead to ING-mediated transcriptional activation. First, UV treatment of HS68 cells, a stress event leading to the transcription of appropriate response genes, resulted in a clear translocation of $p33^{ING1b}$ protein into the nucleolus of cells where immunofluorescent imaging of high levels of transcription was evidenced by using anti-BrdU antibodies [14]. ING-mediated transcriptional response may also be modulated and help modulate responses to endocrine factors. TH was shown to differentially affect the expression of different ING members in *Xenopus laevis*

Table 1. ING protein association with various components of histone acetyltransferase and histone deacetylase complexes.

Protein	Associated components	Complex involved	Species	Methods	References
$p33^{ING1b}$	RBP1, Sin3, HDAC1/2, RbAp48, RbAp46, Sap30, Brg1, BAF155, p42, p35	Sin3-HDAC	human	MS, AP	[101, 102]
	TRAPP		human	IP	[55]
	p300/CBP, PCNA		human	IP	[55]
	P/CAF		human	IP	[55]
	HSir2		human	IP	[19]
$p47^{ING1a}$	HDAC1		human	IP	[55]
$p33^{ING2}$	Sin3, HDAC1/2, RbAp48, RbAp46, Sap30	MSin3	human	MS, AP	[102]
$p29^{ING4}$	Tip60, TRAPP, BAF53a, RUVBL1/2, EPC1, MRG15, DMAP1, hDomino, Brd8, Actin	hNuA4	human	IP, AP, MS	[21]
	p300, p53		human	IP	[6]
$p28^{ING5}$	p300, p53		human	IP	[6]
Yng1	Sas3, Anc1/Taf30	NuA3	yeast	IP, MS, AP	[98, 99]
Yng2	Tra1, Esa1, Epl1, Arp4, Act1, Eaf1, Eaf2, Eaf3, Eaf4, Eaf5, Eaf6, p53[a]	NuA4	yeast	Y2H, IP, GST, AP	[35, 36, 97, 99]
Pho23	Rpd3, Sap30, Sin3	Rpd3-Sin3	yeast	AP	[97]

[a] Mammalian recombinant p53.
Actual HATs and HDACs are underlined. Eafs (Esa1-associated factors) 1–6 are previously unidentified polypeptides. Y2H, yeast-two-hybrid; IP, immunoprecipitation; MS, mass spectrometry; GST, glutathione-*S*-transferase pull-down assay; AP, affinity purification.

and correlate with apoptosis during development [38]. Such observations suggest the possibility that ING family members may be involved in transducing signals initiated by certain endocrine factors such as TH. More recently, studies using an estrogen response element luciferase construct found that $p33^{ING1b}$ is able to increase estrogen receptor α expression in a dose-dependent manner via the AF2 domain [87, 88]. The modes of action of ING family members and the different stressed cellular contexts in which they synergize with p53 might attribute to the variations in the effectiveness of facilitating p53 transcriptional activities.

Modes of action of ING proteins

Although ING family members are involved in various biological functions, they possess no known enzymatic activity. It is therefore reasonable to assume that ING proteins may act by facilitating specific protein-protein, possibly protein-DNA and even protein-phospholipid interactions. Some of these interactions have been deemed crucial for ING tumor suppressive properties. With the exception of the anti-apoptotic $p47^{ING1a}$, and perhaps $p47^{ING3}$, all other mammalian family members are known to interact with or promote posttranslational modifications that activate the p53 tumor suppressor protein. Furthermore, various functions of ING proteins seem to depend on p53 status and vice versa, indicative of an important interrelation. The $p33^{ING2}$ protein is also known to interact with phosphoinositides through its PHD zinc finger domain. This interaction results in altered subcellular distribution of the $p33^{ING2}$ tumor suppressor, altered p53 interactions and cellular susceptibility to undergo apoptosis. Finally, ING members are known to differentially associate with HAT and HDAC components. These chromatin-regulative interactions are vital to ING-mediated transcriptional regulation, apoptosis and probably DNA repair.

p53 dependency

First evidence suggesting an interrelation between ING proteins and p53 came from the observation that the growth-inhibitory effect of $p24^{ING1c}$ was suppressed by the simian virus 40 (SV40) large T-antigen [31]. Demonstration that the growth suppressive effect of $p24^{ING1c}$ is only observed in wt but not p53-null or mutant p53 cells [17] was indeed the direct evidence for such interrelation between these two tumor suppressors. Various studies have since shown that ING proteins help sensitize various cell types to apoptosis in a p53-dependent manner [12, 15, 29, 40]. Although ING enhancement of apoptosis is dependent on p53 status, *ING1* transcription is independent of p53, as $p33^{ING1c}$ expression is unaffected in p53

null mice and upregulated by UV radiation in both wt and p53-deficient keratinocytes [47]. This may be true for various, if not all, ING products because the $p33^{ING2}$ expression level was found to be induced by etoposide in the p53-null lung cancer cell line Calu-6 after etoposide treatment, suggesting that accumulation of $p33^{ING2}$ is independent of p53 function [20].

Two main mechanisms may help explain how ING proteins enhance the p53 response: association of ING members with p53 and ING-mediated acetylation of p53. In a report by Leung and colleagues [84] $p33^{ING1b}$ was proposed to compete with MDM2 for binding to p53. In experiments using cycloheximide to block de novo protein synthesis, $p33^{ING1b}$ overexpression was found to increase the half-life of the p53. Furthermore, while MDM2 expression reduced p53 stability, this reduction was abrogated by $p33^{ING1b}$ in a dose-dependent manner [84]. Most important, both MDM2 and $p33^{ING1b}$ were found to compete for the same p53 binding site in a mutually exclusive fashion. Both proteins seem to bind the N-terminal transactivation domain of p53. Interestingly, a p53 mutant that does not allow MDM2 binding (L22Q_W23S) also showed significantly reduced binding with $p33^{ING1b}$ [84]. $p33^{ING1b}$, $p29^{ING4}$ and $p28^{ING5}$ are now known to co-precipitate with p53, while $p33^{ING2}$ and $p47^{ING3}$ do not [5, 6, 20]. Furthermore, $p33^{ING1b}$ was also found to associate with the p53-related p63α and p73α tumor suppressors [29], suggestive of interrelation and interdependency between different members of p53- and ING-family tumor suppressors. Although certain ING proteins do not physically associate with p53, they may modulate p53 activity by enhancing protein stability. Overexpression of $p33^{ING2}$ was found to induce increased acetylation of p53 at Lys-382 in the colorectal carcinoma cell line RKO [5, 20]. Posttranslational modifications such as acetylation and phosphorylation within the C-terminal region of p53 are believed to facilitate activation and stimulate p53 sequence-specific DNA binding activity [89, 90]. It was reported that p53 acetylation at Lys-382 in normal lymphoblastoid cell line C3ABR after exposure to DNA-damaging agents, etoposide or neocarzinostatin correlated with an increased expression level of $p33^{ING2}$. Furthermore, it was shown that antisense *ING2* expression in osteosacorma OsACL cells reduced the levels of endogenous $p33^{ING2}$ and acetylated p53 proportionally following exposure to etoposide [20]. Similarly, small interfering RNA (siRNA)-directed disruption of *ING2* resulted in resistance to etoposide- and hydrogen peroxide-induced apoptosis in HT1080 human fibrosarcoma cells and caused significant reduction of p53 acetylation [91]. However, $p33^{ING2}$ expression did not alter the phosphorylation of p53 at either Ser-15 or Ser-392 or Fas-mediated apoptosis [20, 91]. On the other hand, $p47^{ING3}$ did not affect posttranslational modifications of p53 [5], and its relationship with p53 remains to be clarified. The $p29^{ING4}$ and to a lesser extent the $p28^{ING5}$ proteins are also known to facilitate acetylation of p53 on Lys-382 [6]. Conflicting results are, however, presented for $p33^{ING1b}$, as this variant did not induce p53 acetylation at Lys-382 in RKO cells [6] but increased p53 acetylation in HCT-116 colorectal carcinoma cells [19]. These differences could be due to different model systems or the antibodies used, as in the latter study the antibody did not discriminate between acetylated Lys-373 and Lys-382. Since both p63 and p73 can be acetylated [92], it would be of interest to further study the diverse interactions between different ING and p53 family members, including the anti-apoptotic ΔNp73. Taken together, ING association with p53 and ING-mediated acetylation of p53 present diverse mechanisms through which ING proteins may stabilize p53 and enhance the p53 cellular responses to genotoxic stresses and apoptotic stimuli.

Interaction with phosphoinositides

Gozani and colleagues [91] were the first and the only group to identify $p33^{ING2}$ as a phosphoinositide-binding module through a library expression screening using PtdlnsP-affinity resins. It was found that $p33^{ING2}$ interacts with Ptdlns(3)P and Ptdlns(5)P through its PHD finger motif, which alone is sufficient for this binding. The importance of the PHD finger motif in this interaction was further evidenced by the findings that the TPEN zinc chelator and mutations of the basic residues at the C-terminal of the PHD finger of $p33^{ING2}$ greatly reduced the binding affinity of $p33^{ING2}$ with Ptdlns(3)P and Ptdlns(5)P. In fact, it was shown that the PHD finger motif was a general PtdlnsPs binding domain and that the general structure for PtdlnsPs binding was conserved through evolution. Therefore, we speculate that other ING family members might be phosphoinositide-interacting partners as well, with different specificity and binding affinity.

Ptdlns(5)Ps can modulate the subcellular localization of $p33^{ING2}$. This is evidenced by the finding that generation of Ptdlns(5)Ps at the plasma membrane by lipid phosphatase IpgD, which dephosphorylates Ptdlns(4,5)P_2 to Ptdlns(5)P, recruited $p33^{ING2}$ PHD finger to plasma membrane and caused loss of diffuse $p33^{ING2}$ cytoplasmic distribution [91]. Furthermore, reducing nuclear Ptdlns(5)P levels by overexpressing PIKIIβ, which phosphorylates Ptdlns(5)P to form Ptdlns(4,5)P_2 [93], reduced $p33^{ING2}$ association with the chromatin/nuclear matrix and increased $p33^{ING2}$ levels in the cytosol, without changing the total $p33^{ING2}$ protein levels [91]. In addition, mutant PHD that abrogated Ptdlns(5)P binding disrupted the ability of $p33^{ING2}$ to enhance p53 acetylation and p53-dependent apoptosis. Overexpression of a PHD zinc finger peptide demonstrates dominant-negative effects on

$p33^{ING2}$ functions. Taken together, these observations suggest that Ptdlns(5)P recruits or stabilizes $p33^{ING2}$ in the chromatin and/or interaction with Ptdlns(5)P might lead to allosteric activation of $p33^{ING2}$.

Histone acetylation

The folding of DNA into higher-order chromatin is a highly dynamic process influenced by multiple covalent reversible modifications of chromatin-associated proteins. Acetylation of histones is a highly conserved mechanism that has evolved to remodel eukaryotic chromatin structure and facilitate various biological functions, including gene transcription regulation, cell cycle progression and DNA repair. This process is carried by HAT enzymes that catalyze the transfer of the acetyl moiety of an acetyl-co-enzyme A (Co-A) cosubstrate to ε-NH_3^+ groups of lysine residues on the N-terminal extremities of histone proteins. Such posttranslational modifications are believed to attenuate positive charges on histones and increase their hydrophobicity to reduce the affinity between histones and DNA, thus alleviating the level of chromatin density. Histone acetylation may further induce interactions with proteins such as transcription activators that contain a bromodomain, an acetyl-lysine-interacting structure. HATs can be divided into five families: Gcn5-related acetyltransferases (GNATs), the MYST-related HATs, the p300/CBP HATs, general transcription factor HATs and nuclear hormone-related HATs [94–96]. ING proteins have so far been associated with MYST-related HATs, GNATs complexes and p300/CBP.

The involvement of ING proteins in histone acetylation was first noted in yeast by Loewith and colleagues [36]. Through database searching, three *Saccharomyces cerevisae* (Yng1, Yng2 and Pho23) and two *Schizosaccharomyces pombe* (Png1 and Png2) proteins were found to contain high homology to human $p33^{ING1b}$ [36]. Deletion of Yng2 led to pleiotropic phenotypes, including slow growth and increased sensitivity to UV irradiation that was rescued by the expression of either human $p33^{ING1b}$ or *S. pombe* Png1, indicative of highly conserved functional properties [36]. Two subsequent reports identified Yng2 as part of the nucleosomal acetyltransferase of histone 4 (NuA4) complex, a yeast multi-subunit complex composed of the MYST Esa1 protein, which is involved in the acetylation of histones 2A and 4 and is linked to cell cycle progression [35, 96, 97]. Through yeast two-hybrid screen and immunoprecipitation, Yng2 was further found to interact with Tra1, an ATM/phosphatidylinositol 3 (PI-3)-kinase-related homolog of the human TRRAP cofactor that acts as an accessory subunit to various HAT complexes [35]. Furthermore, all three ING-related *S. cerevisae* proteins and the human $p33^{ING1b}$ homolog were found to mediate HAT activity in yeast [35]. In an independent report published the same month, Nourani and colleagues [97] also identified Yng2p (a 32-kDa variant of the *Yng2* gene) as a co-eluted component of purified NuA4 from yeast extracts that is required for NuA4-associated HAT activity. Like the work by Choy and colleagues [35], this study reported that Yng2 was required for normal cell growth as its deletion was associated with slow growth, an observation that is in agreement with the normal function of NuA4 in yeast. However, both mutant and wt cells had similar G_0/G_1 and G_2/M phase distributions [97]. Expression of truncated Yng2 lacking the PHD domain did, however, rescue the aberrant growth phenotype of *Yng2*-deficient cells, demonstrating that the PHD motif is not required for normal cell growth [97]. A yeast-based system was further used to analyze the requirement of Yng2p/NuA4 for p53-dependent transcriptional activation in vivo. Using a reporter plasmid carrying p53 binding sites, p53 transactivation of the $p21^{Waf1}$ promoter was found to require Yng2p/NuA4 [97]. The authors further showed that p53 binds NuA4 via its transcriptional activation domain in a functional interaction resulting in NuA4 recruitment for targeted chromatin acetylation of histone 4 but not histone 3 and subsequent transcriptional activation. This exciting observation suggests an evolutionarily conserved p53/ING molecular relationship. Interestingly, p53-dependent transcriptional activation was crippled in cells expressing only the Yng2 PHD finger domain region, while NuA4 complexes containing truncated Yng2 lacking the PHD-finger motif could bind p53 but exhibited crippled p53 transactivation [97]. The authors therefore proposed that the Yng2 PHD domain is perhaps involved in transcriptional activation in an aspect that does not involve the recruitment of NuA4 HAT activity.

The Yng1p protein was also found to be a stable component of the NuA3 complex (yeast complex comprised of the MYST Sas3 HAT that is involved in the acetylation of histones 3 and 4) and to be required for proper HAT activity [98]. Although Yng1p is an integral component of NuA3, it was not required for proper integrity of a stable NuA3 complex [98]. Similar to Yng2/NuA4, Yng1 was required to maintain HAT activity in the NuA3 complex. Like Yng2 in NuA4, the PHD domain of Yng1 was not required for the function of the HAT complex. It is also of interest to note that unlike Yng2 in NuA4, Yng1p was incapable of interacting with p53 in the context of NuA3. The authors suggested caution when interpreting this result, however, since it is possible that the recombinant p53 used in the experiment may lack proper modification required for such interaction. To further study this complex, the authors reconstituted an array of nucleosomes bound to paramagnetic beads and incubated them with partially purified NuA3 isolated from either wt or Yng1-deficient strains. Yng1p deletion severely compromised HAT activity on free histones. However, the absence of Yng1p in

NuA3 resulted in the incapability of acetylating nucleosomes. Severely compromised Sas3p interactions with nucleosomes in the absence of Yng1p suggests that certain ING proteins may facilitate interactions between HAT complexes and chromatin [98].

It is very likely that ING associations with HAT and HDAC complexes may mediate ING biological functions. This notion is supported by Western and mass spectrometry analysis of *S. cerevisae* of ING homologs, showing that Yng1, Yng2 and Pho23 are solely associated with NuA3, NuA4 acetyltransferase and Rpd3/Sin3 deacetylase complexes, respectively [99]. Yng2 and Pho23 were found to possess antagonistic effects on p53-dependent transactivation of a reporter $p21^{waf1}$ construct in yeast [99]. However, Yng1 also demonstrated HAT-dependent repression of transcription [99], which is the first evidence of negative regulation of transcription by the NuA3 complex. Furthermore, Yng1 and Yng2 deletions are found to be detrimental to NuA3 and NuA4 activities but do not disrupt complex formation [97, 98]. More recently, $p47^{ING3}$ was identified as a component of a Tip60-based complex, a human equivalent to the yeast NuA4 HAT complex [21, 100]. Purification of the complex identified multiple components homologous to the yeast NuA4 complex. It is interesting to note that $p47^{ING3}$ was the closest human ING member to the Yng2 yeast counterpart. Although Tip60 could not acetylate p53, this human NuA4 (hNuA4) complex strongly influenced p53-dependent transcription, while a defective hNuA4 complex was detrimental to p53-depedendent transcription [21]. In fact, a trimeric complex composed of recombinant Tip60, EPC1 and $p47^{ING3}$ was found to sufficiently acetylate nucleosomal histones in vitro [21].

However, not all ING proteins are created equal. Both agonistic and antagonistic effects have been observed when it comes to histone acetylation. Immunoprecipitation of ING1 proteins from SNB19 cell lysates co-immunoprecipitated various HAT components, including TRRAP (accessory subunit to MYST HAT complexes), PCAF (subunit of the human Gcn5 GNAT HAT complex involved in histone 3 acetylation) and CBP (involved in acetylation of histones 2a, 2b, 3 and 4) [55]. Yet it appears that different ING members can associate with different HAT/HDAC components resulting in different biological effects. Immunoprecipitation from human fibroblast lysates overexpressing ING1 proteins showed that while both $p33^{ING1b}$ and $p47^{ING1a}$ bound CBP, $p33^{ING1b}$ appeared to bind it much more avidly. On the other hand, $p47^{ING1a}$ could not be co-precipitated with the closely related p300 protein [55]. Like $p33^{ING1b}$, $p29^{ING4}$ and $p28^{ING5}$ proteins have since been shown to immunoprecipitate with p300, suggesting a role of ING proteins in targeting of HATs.

Mass spectrometry of purified immunoprecipitates also found that $p33^{ING1b}$ was capable of associating with known components of the mSin3 transcriptional corepressor complex, including mSin3, HDAC1/2 histone deacetylases, RbAp48, RbAp46 and SAP30, whereas $p24^{ING1c}$ was not, suggesting that $p33^{ING1b}$ may also mediate histone deacetylation. This observation was substantiated by the findings that $p33^{ING1b}$ was functionally associated with HDAC-dependent transcriptional repression both in an in vivo reporter gene expression assay and from in vitro histone deacetylation assay [101]. Interactions between ING proteins and HDAC were further characterized in a more recent study. Mass spectrometry analysis confirmed the presence of $p33^{ING1b}$ and $p33^{ING2}$ in human Sin3-HDAC complexes isolated from HeLa cells [101]. Furthermore, $p33^{ING1b}$ was found to physically interact with SAP30 and be an integral component of two of three biochemically distinct Sin3 complexes, all of which included Sin3, SAP30 and HDAC1 [101]. $p33^{ING1b}$ interacts with SAP30 through its N-terminus, an area that the authors termed SAID for SAP30-interacting domain. Based on a colony formation assay, the deletion of $p33^{ING1b}$ SAID was found to be detrimental to $p33^{ING1b}$-mediated cell growth regulation. However, caution should be taken when interpreting these results, as SAID was defined to the first 125 amino acids of $p33^{ING1b}$ (45% of the protein), and deletion of this area included the PIP domain and a partial bromodomain. It is of interest to note that a computational search revealed that $p33^{ING1b}$, $p33^{ING2}$, $p47^{ING3}$ and the uncharacterized proteins AAD48585 (named ING1 homolog, or ING1h) and HSPC301 possess a SAID domain. Furthermore, deletion of the PHD domain, which is dispensable for the interaction with the Sin3 complex, did not reduce but enhanced the growth suppression effect of $p33^{ING1b}$ [102].

Vieyra and colleagues [55] confirmed the association between $p33^{ING1b}$ and HDACs in human fibroblast. The authors demonstrated a more robust physical interaction between $p47^{ING1a}$ and HDAC1 than that between $p33^{ING1b}$ and HDAC1. Such observations are consistent with the recovery of greater amounts of HAT activity in immunoprecipitates of $p33^{ING1b}$-overexpressing cells compared with cells transfected with empty vector and reduced HAT activity from cells overexpressing $p47^{ING1a}$. In fact, immunofluorescent analysis of cells microinjected with $p33^{ING1b}$ expression vector demonstrated increased staining for acetylated histones H4 and H3, while cells microinjected with $p47^{ING1a}$ constructs exhibited decreased staining for acetylated H4. On the other hand, downregulation of $p33^{ING1b}$ resulted in decreased histone acetylation, indicating a direct correlation between HAT activity and ING levels [55]. It is also interesting to note that $p33^{ING1b}$ represses the expression of DEK [28], a protein implicated in chromatin remodelling involving nucleosomal core histones [73, 103]. Consistent with such observations, DEK was further found to co-fractionate with Daxx, a transcriptional co-repressor, and HDACII [104], and may therefore antagonize $p33^{ING1b}$. Such observations provide support to the perception that different ING pro-

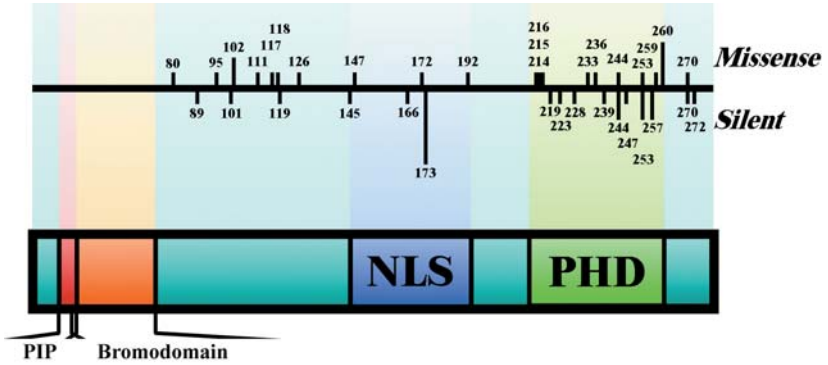

Figure 1. Summary of reported missense and silent mutations of the *ING1* gene in human malignancies. Numbers refer to p33^{ING1b} codons. PIP, PCNA-interacting protein domain; NLS, nuclear localization sequence; PHD, plant homeodomain zinc finger motif. Bromodomain refers to a partial bromodomain.

Table 2. Summary of reported *ING* expression levels and gene alterations in human malignancies.

Gene	Tumor type	Expression			Mutation[a]	References
		↓ or ↑	Rate (%)	Methods	(%)	
ING1	basal cell carcinoma	↑	25.0	IHC	1.9	[119]
	bladder cancer	↓[b]	ND	IHC	ND	[76]
	brain tumor	↓[b]	100	RT-PCR; WB; ISH; IHC	3.4	[105]
	brain tumor	↓	ND	RT-PCR	0.0	[120]
	brain tumor cell lines	↑	80.0	RT-PCR	ND	[106]
	breast cancer	↓	43.8	RT-PCR; ISH; WB	0.3	[121]
	breast cancer	↓	70.8	Q-PCR	ND	[122]
	breast cancer	↓[b]	80.2	IHC	ND	[8]
	breast cancer cell lines	↓	100	RT-PCR, WB	0.0	[121]
	colorectal carcinoma	ND	ND	ND	0.0	[123]
	esophageal SCC	↓[b]	54.8	RT-PCR	12.9	[124]
	esophagogastric junction adenocarcinoma	↓	63.2	RT-PCR	5.3	[125]
	gastrointestinal cancers	↓	75.0	RT-PCR	ND	[126]
	gastrointestinal carcinoma cell lines	ND	ND	ND	8.3	[126]
	head and neck SCC	ND	ND	ND	13.0	[127]
	head and neck SCC	ND	ND	ND	0.0	[128]
	haematological malignancy	NC	ND	QC-PCR	0.0	[129]
	hepatocarcinoma	↓[b]	54.7	IHC	ND	[33]
	lymphoblastic leukemia	↓	76.5	IHC	ND	[113]
	lymphoid cancer cell lines	↓	56.3	RT-PCR	0.0	[112]
	malignant melanoma	↑↓[c]	80.0/47.5	IHC	ND	[116]
	malignant melanoma	ND	ND	IHC	19.6	[49]
	melanoma cell lines	↑	100	WB	ND	[130]
	melanoma cell lines	↑	100	WB; RT-PCR	7.1	[131]
	myeloid leukemia	ND	ND	ND	0.0	[132]
	non-small cell lung cancer	↓	42.0	Q-PCR	0.0	[133]
	oral SCC	↑	93.0	RT-PCR	ND	[134]
	oral SSC	ND	ND	ND	0.0	[135]
	ovarian cancer	ND	ND	ND	0.0	[121]
ING2	various cancer cell lines	↓	41.7	WB	ND	[20]
ING3	head and neck SSC	↓↑	50.0/12.5	Q-RT-PCR	2.0	[118]
ING4	glial tumor	↓[b]	100	RT-PCR	ND	[10]

[a] Mutation rates include somatic missense and nonsense mutations.
[b] Associated with advancing clinical stages.
[c] Increased cytoplasmic expression in 80% and reduced nuclear expression in 47.5% of melanoma cases.
IHC, immunohistochemistry; WB, Western blot; RT-PCR, reverse transcriptase polymerase chain reaction; ISH, in situ hybridization; ND, not determined; NC, no change.

teins may contribute to the regulation and targeting of different HAT/HDAC complexes and their catalytic activities. However, more studies are required to investigate in which systems and under what circumstances different ING members associate with different HAT/HDAC complexes. Table 1 summarizes reported associations between HAT and HDAC components with ING members.

ING genes and human malignancies

Various reports reveal infrequent gene alterations but prevalent dowregulation of *ING1* gene expression in various human malignancies. Many of these reports have been summarized in previous reviews [4, 7, 8]. Table 2 recapitulates published altered *ING* expression levels in human malignancies. An abridgment of all reported *ING1* gene mutations is also illustrated in figure 1. The following section will therefore focus on recently published data.

Consistent with a general loss of ING expression in cancer, recent studies further link the loss of ING expression and cancer progression. In a study by Vieyra and colleagues [105] semi-quantitative RT-PCR analysis of *ING1* mRNA expression levels in human brain tumors showed significantly higher expression in pilocytic astrocytomas (WHO grade I) and in diffuse astrocytomas (WHO grade II) than in anaplastic astrocytomas and glioblastoma (WHO grades III and IV), suggesting a role of *ING1* downregulation in the progression of low-grade astrocytomas to higher grades of malignancy. However, high *ING1* expression was also shown to correlate with cellular chemoresistance in brain cancer cell lines [106]. *ING1* was also differentially expressed in early-stage and invasive bladder tumors. Similar to brain tumors, *ING1* expression was detected in normal urothelium and most early-stage transitional cell carcinoma of the bladder but became less evident with advancing cancer progression [76]. Interestingly, $p33^{ING1b}$ expression was found significantly associated with patient survival, as patients with higher $p33^{ING1b}$ expression had a poorer outcome than those expressing low $p33^{ING1b}$ levels [76]. Various factors can lead to chemoresistance, ranging from initial drug entry into the cell to intracellular resistance mechanisms that prevent drug interaction with DNA or DNA damage signals from activating the apoptotic machinery [107]. Studies demonstrate that chronic DNA lesions induced by anti-cancer drugs correlate directly with cytotoxicity [107–110]. Reducing the extent of DNA damage through DNA repair factors has been reported as a mechanism that increases resistance, and can lead to apoptotic inhibition. In fact, increased rates of DNA repair often correlate with an inhibition of drug-induced cytotoxicity in several cultured malignancies [107]. Inhibition of certain DNA repair proteins has been shown to increase chemo- and radiosensitivity of certain cultured cancer cells [107, 111, 112]. It is therefore plausible that loss of ING may be involved in malignancy progression, but that high ING levels may constrain cancer chemo- and radiotherapies. This idea is consistent with a report demonstrating that loss of $p33^{ING1b}$ nuclear expression is correlated with better prognosis in childhood acute lymphoblastic leukemia [113]. It is therefore intriguing to note that *ING1* mutations in malignant melanoma negatively affect patient outcome [49]. Although chemoresistance is a multifactorial concept, further evaluation of ING prognostic value and predicted chemoresponse in cancer may help explain the reported correlation of higher ING levels with poorer prognosis.

Although loss of ING expression may lead to progression of certain malignancies, we have shown no correlation between loss of *ING1* expression and invasiveness in melanoma cells [114]. No difference was observed in MMP-1, MMP-2 and MMP-9 matrix metalloproteinase expression or in the expression of the angiogenesis-related proteins VEGF, Flt-1 and Flk-1 in cells transfected with vector, $p33^{ING1b}$ and antisense $p33^{ING1b}$. Furthermore, no difference was observed in human umbilical vein endothelial cells (HUVEC) cells cultured with medium derived from $p33^{ING1b}$-transfected melanoma compared to controls, providing support to the lack of functional role of $p33^{ING1b}$ in angiogenesis [114]. Previous reports have also demonstrated loss of nuclear localization and increased cytoplasmic localization of $p33^{ING1b}$ in certain malignancies [49, 113, 115, 116]. Consistent with the inclusion of a nuclear localization sequence (NLS) [4], both $p33^{ING1b}$ and $p33^{ING2}$ proteins were found to be mainly localized in the nucleus [14, 91, 117].

Unlike $p33^{ING1b}$, protein levels of $p33^{ING2}$ in cancer cell lines were found to be highly variable, with undetectable expression in some cell lines. It was reported that the expression profile of $p33^{ING2}$ did not correlate with the mutational status of p53. However, 3 out of 12 cell lines which express the most abundant $p33^{ING2}$ contained either null or mutant p53 [20]. *ING2* mRNA expression was significantly higher in colon cancers, where p53 abnormalities occur very frequently, compared to the adjacent normal tissues. Importantly, its expression levels increased with tumor progression, as poorly differentiated carcinoma expressed stronger *ING2* levels than differentiated adenocarcinoma [3]. Allelic loss of *ING3* was reported by Gunduz and colleagues [118] in 48% of a total of 49 head and neck squamous cell carcinoma (HNSSC) cases analyzed. Furthermore, *ING3* mRNA expression was found decreased in 50% of the primary tumors and 75% of tumor-derived cell lines, whereas 5% of the tumor tissues analyzed exhibited complete loss of *ING3* mRNA expression and 12% of the tumor samples showed increased *ING3* expression compared to the paired normal tissues. Mutational analysis of 49 HNSCC by PCR-SSCP (single-

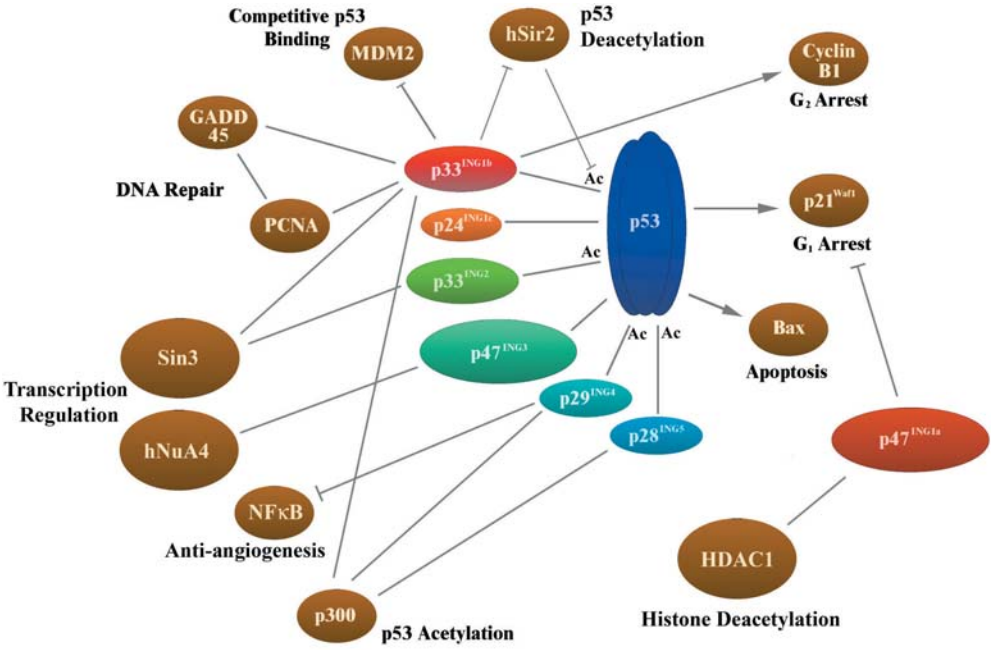

Figure 2. Protein-protein interactions, gene regulation and biological functions of ING proteins. ING family members interact with tumor suppressors, transcription factors, DNA repair and replication factors, HATs and HDACs to induce cell cycle arrest, enhance DNA repair, promote apoptosis and inhibit angiogenesis.

strand DNA conformational polymorphism) and direct sequencing detected a tumor-specific missense mutation of *ING3* at codon 20 with an amino acid substitution from aspartic acid to glycine [118]. It was reported that there was no significant correlation between *ING3* mRNA levels and clinicopathological features such as tumor stage, recurrence and metastasis. There was, however, a tendency towards mortality in cases that had tumors with reduced *ING3* expression compared to cases with tumors expressing high or normal *ING3* expression. Moreover, there was a location preference of decreased *ING3* expression, as 64% of tongue tumor and 63% of larynx tumor showed decreased *ING3* expression compared to 17 and 20% for hypopharynx and oropharynx tumors, respectively [118].

In spite of the recent study reporting that *ING4* expression is reduced in gliomas correlating with the extent of tumour progression from low to high grades [10], no studies have so far been done to investigate the expression levels of $p28^{ING5}$ protein in various organs and in normal and cancerous cellular states. Such study along with mutational status analyses will provide further understanding of the functional importance of these *ING* family genes.

Conclusion

Although ING proteins have not been found to possess any enzymatic activity, they exert their biological functions by associating with various cellular components (fig. 2). They are involved in various cellular responses to stress, including cell cycle arrest, DNA repair and apoptosis. With the exception of $p47^{ING1a}$, ING proteins operate with p53 to promote upregulation of $p21^{Waf1}$, resulting in reduced phosphorylation of Rb, E2F sequestration and a G_1/G_0 cell cycle arrest. $p33^{ING1b}$ further facilitates G_2/M cell cycle arrest by repressing the expression of cyclin B1, which is required for mitotic onset. ING proteins are transcriptional regulators; they may help target various HAT/HDAC components to induce local acetylation/ deacetylation of histones and regulate gene expression. With the association of p300, certain ING members may further contribute to p53 activation and stability by promoting acetylation of its regulatory domain and by competitively binding to MDM2, thus preventing its association with p53. ING proteins can also upregulate the pro-apoptotic Bcl-2 member Bax to promote apoptosis and interact with various DNA repair factors and facilitate the removal of UV-induced DNA lesions. ING proteins are silenced in various human malignancies or become excluded from the nucleus, where they are required to exert tumor suppressive functions. Mutations of the *ING* genes may also be detrimental to nucleotide excision repair. However, recent studies suggest that ING proteins could negatively affect chemotherapy. Further studies are therefore required to assess ING prognostic values in predicting patient chemotherapeutical responses.

Acknowledgements. This work is supported by grants from the National Cancer Institute of Canada. E.I.C. is a recipient of the trainee award from the Michael Smith Foundation for Health Research/ VGH and UBC Hospital Foundation. G.L. is a recipient of the Research Scientist Award from the National Cancer Institute of Canada supported with funds provided by the Canadian Cancer Society.

1 Garkavtsev I., Kazarov A., Gudkov A. and Riabowol K. (1996) Suppression of the novel growth inhibitor p33ING1 promotes neoplastic transformation. Nat. Genet. **14:** 415–420
2 Saito A., Furukawa T., Fukushige S., Koyama S., Hoshi M., Hayashi Y. et al. (2000) p24/ING1-ALT1 and p47/ING1-ALT2, distinct alternative transcripts of p33/ING1. J. Hum. Genet. **45:** 177–181
3 Shimada Y., Saito A., Suzuki M., Takahashi E. and Horie M. (1998) Cloning of a novel gene (ING1L) homologous tumor suppressor. Cytogenet. Cell Genet. **83:** 232–235
4 Feng X., Hara Y. and Riabowol K. T. (2002) Different HATS of the ING1 gene family. Trends Cell Biol. **12:** 532–538
5 Nagashima M., Shiseki M., Pedeux R. M., Okamura S., Kitahama-Shiseki M., Muira K. et al. (2003) A novel PHD-finger motif protein, p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. Oncogene **22:** 343–350
6 Shiseki M., Nagashima M., Pedeux R. M., Kitahama-Shiseki M., Miura K., Okamura S. et al. (2003) p29ING4 and p28ING5 bind to p53, and enhance p53 activity. Cancer Res. **63:** 2373–2378
7 Cheung K. J. Jr and Li G. (2001) The tumor suppressor ING1: structure and function. Exp. Cell Res. **268:** 1–6
8 Nouman G. S., Anderson J. J., Lunec J. and Angus B. (2003) The role of the tumour suppressor p33ING1b in human neoplasia. J. Clin. Pathol. **56:** 491–496
9 Zeremski M., Hill J. E., Kwek S. S., Grigorian I. A., Gurova K. V., Garkavtsev I. V. et al. (1999) Structure and regulation of the mouse ing1 gene. Three alternative transcripts encode two phd finger proteins that have opposite effects on p53 function. J. Biol. Chem. **274:** 32172–32181
10 Garkavtsev I., Kozin S. V., Chernova O., Xu L., Winkler F., Brown E. et al. (2004) The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature **428:** 328–332
11 Helbing C. C., Veillette C., Riabowol K., Johnston R. N. and Garkavtsev I. (1997) A novel candidate tumor suppressor, ING1, is involved in the regulation of apoptosis. Cancer Res. **57:** 1255–1258
12 Shinoura N., Muramatsu Y., Nishimura M., Yoshida Y., Saito A., Yokoyama T. et al. (1999) Adenovirus-mediated transfer of p33ING1 with p53 drastically augments apoptosis in gliomas. Cancer Res. **59:** 5521–5528
13 Scott M., Bonnefin P., Vieyra D., Boisvert F. M., Young D., Bazett-Jones D. P. et al. (2001) UV-induced binding of ING1 to PCNA regulates the induction of apoptosis. J. Cell Sci. **114:** 3455–3462
14 Scott M., Boisvert F. M., Vieyra D., Johnston R. N., Bazett-Jones D. P. and Riabowol K. (2001) UV induces nucleolar translocation of ING1 through two distinct nucleolar targeting sequences. Nucleic Acids Res. **29:** 2052–2058
15 Cheung K. J. Jr and Li G. (2002) p33(ING1) enhances UVB-induced apoptosis in melanoma cells. Exp. Cell Res. **279:** 291–298
16 Levine A. J. (1997) p53, the cellular gatekeeper for growth and division. Cell **88:** 323–331
17 Garkavtsev I., Grigorian I. A., Ossovskaya V. S., Chernov M. V., Chumakov P. M. and Gudkov A. V. (1998) The candidate tumour suppressor p33ING1 cooperates with p53 in cell growth control. Nature **391:** 295–298
18 Harper J. W., Adami G. R., Wei N., Keyomarsi K. and Elledge S. J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell **75:** 805–816
19 Kataoka H., Bonnefin P., Vieyra D., Feng X., Hara Y., Miura Y. et al. (2003) ING1 represses transcription by direct DNA biding ad through effects on p53. Cancer Res. **63:** 5787–5792
20 Nagashima M., Shiseki M., Miura K., Hagiwara K., Linke S. P., Pedeux R. et al. (2001) DNA damage-inducible gene *p33ING2* negatively regulates cell proliferation through acetylation of p53. Proc. Natl. Acad. Sci. USA **98:** 9671–9676
21 Doyon Y., Selleck W., Lane W. S., Tan S. and Côté J. (2004) Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol. Cell. Biol. **24:** 1884–1896
22 Agarwal M. L., Agarwal A., Taylor W. R. and Stark G. R. (1995) p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proc. Natl. Acad. Sci. USA **92:** 8493–8497
23 Stewart N., Hicks G. G., Paraskevas F. and Mowat M. (1995) Evidence for a second cell cycle block at G2/M by p53. Oncogene **10:** 109–115
24 DeSimone J. N., Bengtsson U., Wang X., Lao X. Y., Redpath J. L. and Stanbridge E. J. (2003) Complexity of the mechanisms of initiation and maintenance of DNA damage-induced G2-phase arrest and subsequent G1-phase arrest: TP53-dependent and TP53-independent roles. Radiat. Res. **159:** 72–85
25 Innocente S. A., Abrahamson J. L., Cogswell J. P. and Lee J. M. (1999) p53 regulates a G2 checkpoint through cyclin B1. Proc. Natl. Acad. Sci. USA **96:** 2147–2152
26 Taylor W. R., DePrimo S. E., Agarwal A., Agarwal M. L., Schonthal A. H., Katula K. S. et al. (1999) Mechanisms of G2 arrest in response to overexpression of p53. Mol. Biol. Cell **10:** 3607–3622
27 Porter L. A. and Donoghue D. J. (2003) Cyclin B1 and CDK1: nuclear localization and upstream regulators. Prog. Cell Cycle Res. **5:** 335–547
28 Takahashi M., Seki N., Ozaki T., Kato M., Kuno T., Nakagawa T. et al. (2002) Identification of the p33(ING1)-regulated genes that include cyclin B1 and proto-oncogene DEK by using cDNA microarray in a mouse mammary epithelial cell line NMuMG. Cancer Res. **62:** 2203–2209
29 Tsang F. C., Po L. S., Leung K. M., Lau A., Siu W. Y. and Poon R. Y. (2003) ING1b decreases cell proliferation through p53-dependent and -independent mechanisms. FEBS Lett. **553:** 277–285
30 Nakamura S., Gomyo Y., Roth J. A. and Mukhopadhyay T. (2002) C-terminus of p53 is required for G(2) arrest. Oncogene **21:** 2102–2107
31 Garkavtsev I. and Riabowol K. (1997) Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33ING1 candidate tumor suppressor. Mol. Cell. Biol. **17:** 2014–2019
32 Donnellan R. and Chetty R. (1999) Cyclin E in human cancers. FASEB J. **13:** 773–780
33 Ohgi T., Masaki T., Nakai S., Morishita A., Yukimasa S., Nagai M. et al. (2002) Expression of p33(ING1) in hepatocellular carcinoma: relationships to tumour differentiation and cyclin E kinase activity. Scand. J. Gastroenterol. **37:** 1440–1448
34 Xin H., Yoon H. G., Singh P. B., Wong J. and Qin J. (2004) Components of a pathway maintaining histone modification and heterochromatin protein 1 binding at the pericentric heterochromatin in Mammalian cells. J. Biol. Chem. **279:** 9539–9546
35 Choy J. S., Tobe B. T., Huh J. H. and Kron S. J. (2001) Yng2p-dependent NuA4 histone H4 acetylation activity is required for mitotic and meiotic progression. J. Biol. Chem. **276:** 43653–43662

36 Loewith R., Meijer M., Lees-Miller S. P., Riabowol K. and Young D. (2000) Three yeast proteins related to the human candidate tumor suppressor p33(ING1) are associated with histone acetyltransferase activities. Mol. Cell. Biol. **20:** 3807–3816

37 Boudreault A. A, Cronier D., Selleck W., Lacoste N., Utley R. T., Allard S. et al. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. Genes Dev. **17:** 1415–1428

38 Wagner M. J., Gogela-Spehar M., Skirrow R. C., Johnston R. N., Riabowol K. and Helhbing C. C. (2001) Expression of novel ING variants is regulated by thyroid hormone in the *Xenopus laevis* tadpole. J. Biol. Chem. **276:** 47013–47020

39 Vieyra D., Toyama T., Hara Y., Boland D., Johnston R. and Riabowol K. (2002) ING1 isoforms differentially affect apoptosis in a cell age-dependent manner. Cancer Res. **62:** 4445–4452

40 Shimada H., Liu T. L., Ochiai T., Shimizu T., Haupt Y., Hamada H. et al. (2002) Facilitation of adenoviral wild-type p53-induced apoptotic cell death by overexpression of p33(ING1) in T.Tn human esophageal carcinoma cells. Oncogene **21:** 1208–1216

41 Ikura T., Ogryzko V. V., Grigoriev M., Groisman R., Wang J., Horikoshi M. et al. (2000) Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell **102:** 463–473

42 Rosato R. R. and Grant S. (2003) Histone deacetylase inhibitors in cancer therapy. Cancer Biol. Ther. **2:** 30–37

43 Kitahara J., Chiba N., Sakamoto H. and Nakagawa Y. (2003) Alteration of gene expressions by the overexpression of mitochondrial phospholipid hydroperoxide glutathione peroxidase (mtPHGPx). Gene Expr. **11:** 77–83

44 Li G. and Ho V. C. (1998) p53-dependent DNA repair and apoptosis respond differently to high- and low-dose ultraviolet radiation. Br. J. Dermatol. **139:** 3–10

45 Bernstein C., Bernstein H., Payne C. M. and Garewal H. (2002) DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat. Res. **511:** 145–178

46 Adimoolam S. and Ford JM. (2003) p53 and regulation of DNA damage recognition during nucleotide excision repair. DNA Repair **2:** 947–954

47 Cheung K. J. Jr, Bush J. A., Jia W. and Li G. (2000) Expression of the novel tumour suppressor p33(ING1) is independent of p53. Br. J. Cancer **83:** 1468–1472

48 Cheung K. J. Jr, Mitchell D., Lin P. and Li G. (2001) The tumor suppressor candidate p33(ING1) mediates repair of UV-damaged DNA. Cancer Res. **61:** 4974–4977

49 Campos E. I., Martinka M., Mitchell D. L., Dai D. L. and Li G. (2004) Mutations of the ING1 tumor suppressor gene detected in human melanoma abrogate nuclear excision repair. Int. J. Oncol. **25:** 73–80

50 Li M., Luo J., Brooks C. L. and Gu W. (2002) Acetylation of p53 inhibits its ubiquitination by Mdm2. J. Biol. Chem. **277:** 50607–50611

51 Lakin N. D. and Jackson S. P. (1999) Regulation of p53 in response to DNA damage. Oncogene **18:** 7644–7655

52 Luo J., Nikolaev A. Y., Imai S., Chen D., Su F., Shiloh A. et al. (2001) Negative control of p53 by Sir2_ promotes cell survival under stress. Cell **107:** 137–148

53 Warbrick E. (1998) PCNA binding through a conserved motif. Bioessays **20:** 195–199

54 Waga S., Hannon G. J., Beach D. and Stillman B. (1994) The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature **369:** 574–578

55 Vieyra D., Loewith R., Scott M., Bonnefin P., Boisvert F. M., Cheema P. et al. (2002b) Human ING1 proteins differentially regulate histone acetylation. J. Biol. Chem. **277:** 29832–29839

56 Hasan S., Hassa P. O., Imhof R. and Hottiger M. O. (2001) Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. Nature **410:** 387–391

57 Rubbi C. P. and Milner J. (2003) p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. EMBO J. **22:** 975–986

58 Tewey K. M., Rowe T. C., Yang L., Halligan B. D. and Liu L. F. (1984) Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science **226:** 466–468

59 Fichtinger-Schepman A. M., van der Veer J. L., den Hartog J. H., Lohman P. H. and Reedijk J. (1985) Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: formation, identification and quantitation. Biochemistry **24:** 707–713

60 Cadet J., Anselmino C., Douki T. and Voituriez L. (1992) Photochemistry of nucleic acids in cells. J. Photochem. Photobiol. B. **15:** 277–298

61 Caruso M. L. and Valentini A. M. (1999) Overexpression of p53 in a large series of patients with hepatocellular carcinoma: a clinicopathological correlation. Anticancer Res. **19:** 3853–2856

62 Keyomarsi K. and Pardee A. B. (2003) Redundant cyclin overexpression and gene amplification in breast cancer cells. Proc. Natl. Acad. Sci. USA **90:** 1112–1116

63 Kawamoto H., Koizumi H. and Uchikoshi T. (1997) Expression of the G2-M checkpoint regulators cyclin B1 and cdc2 in nonmalignant and malignant human breast lesions: immunocytochemical and quantitative image analyses. Am. J. Pathol. **150:** 15–23

64 Dong Y., Sui L., Watanabe Y., Sugimoto K. and Tokuda M. (2002) Clinical relevance of cyclin B1 overexpression in laryngeal squamous cell carcinoma. Cancer Lett. **177:** 13–19

65 Takeno S., Noguchi T., Kikuchi R., Uchida Y., Yokoyama S. and Muller W. (2002) Prognostic value of cyclin B1 in patients with esophageal squamous cell carcinoma. Cancer **94:** 2874–2881

66 Yoshida T., Tanaka S., Mogi A., Shitara Y. and Kuwano H. (2004) The clinical significance of Cyclin B1 and Wee1 expression in non-small-cell lung cancer. Ann. Oncol. **15:** 252–256

67 Hassan K. A., Ang K. K., El-Naggar A. K., Story M. D., Lee J. I., Liu D. et al. (2002) Cyclin B1 overexpression and resistance to radiotherapy in head and neck squamous cell carcinoma. Cancer Res. **62:** 6414–6417

68 Li J. Q., Kubo A., Wu F., Usuki H., Fujita J., Bandoh S. et al. (2003) Cyclin B1, unlike cyclin G1, increases significantly during colorectal carcinogenesis and during later metastasis to lymph nodes. Int. J. Oncol. **22:** 1101–1110

69 Hassan K. A., El-Naggar A. K., Soria J. C., Liu D., Hong W. K. and Mao L. (2001) Clinical significance of cyclin B1 protein expression in squamous cell carcinoma of the tongue. Clin. Cancer Res. **7:** 2458–2462

70 Murata T., Hikita K. and Kaneda N. (2000) Transcriptional activation function of zinc finger protein TIS11 and its negative regulation by phorbol ester. Biochem. Biophys. Res. Commun. **274:** 526–532

71 Johnson B. A., Geha M. and Blackwell T. K. (2000) Similar but distinct effects of the tristetraprolin/TIS11 immediate-early proteins on cell survival. Oncogene **19:** 1657–1664

72 von Lindern M., Fornerod M., van Baal S., Jaegle M., de Wit T., Buijs A. et al. (1992) The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, DEK and CAN and the expression of a chimeric, leukemia-specific DEK-CAN mRNA. Mol. Cell. Biol. **12:** 1687–1697

73 Waldmann T., Baack M., Richter N. and Gruss C. (2003) Structure-specific binding of the proto-oncogene protein DEK to DNA. Nucleic Acids Res. **31:** 7003–7010

74 Kondoh N., Wakatsuki T., Ryo A., Hada A., Aihara T., Horiuchi S. et al. (1999) Identification and characterization of

genes associated with human hepatocellular carcinogenesis. Cancer Res. **59:** 4990–4996
75 Casas S., Nagy B., Elonen E., Aventin A., Larramendy M. L., Sierra J. et al. (2003) Aberrant expression of HOXA9, DEK, CBL and CSF1R in acute myeloid leukemia. Leuk. Lymphoma **44:** 1935–1941
76 Sanchez-Carbayo M., Socci N. D., Lozano J. J., Li W., Charytonowicz E., Belbin T. J. et al. (2003) Gene discovery in bladder cancer progression using cDNA microarrays. Am. J. Pathol. **163:** 505–516
77 Bossolasco M., Lebel M., Lemieux N. and Mes-Masson A. M. (1999) The human TDE gene homologue: localization to 20q13.1-13.3 and variable expression in human tumor cell lines and tissue. Mol. Carcinog. **26:** 189–200
78 Furger K. A., Menon R. K., Tuckl A. B., Bramwelll V. H. and Chambers A. F. (2001) The functional and clinical roles of osteopontin in cancer and metastasis. Curr. Mol. Med. **1:** 621– 632
79 Denhardt D. T., Mistretta D., Chambers A. F., Krishna S., Porter J. F., Raghuram S. et al. (2003) Transcriptional regulation of osteopontin and the metastatic phenotype: evidence for a Ras-activated enhancer in the human OPN promoter. Clin. Exp. Metast. **20:** 77–84
80 Tuynder M., Susini L., Prieur S., Besse S., Fiucci G., Amson R. et al. (2002) Biological models and genes of tumor reversion: cellular reprogramming through tpt1/TCTP and SIAH-1. Proc. Natl. Acad. Sci. USA **99:** 14976–14981
81 Khanna N., Reddy V. G., Tuteja N. and Singh N. (2000) Differential gene expression in apoptosis: identification of ribosomal protein S29 as an apoptotic inducer. Biochem. Biophys. Res. Commun. **277:** 476–486
82 Khanna N., Sen S., Sharma H. and Singh N. (2003) S29 ribosomal protein induces apoptosis in H520 cells and sensitizes them to chemotherapy. Biochem. Biophys. Res. Commun. **304:** 26–35
83 Vaziri H., Dessain S. K., Ng Eaton E., Imai S. I., Frye R. A., Pandita T. K. et al. (2001) hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell **107:** 149–159
84 Leung K. M., Po L. S., Tsang F. C., Siu W. Y., Lau A., Ho H. T. et al. (2002) The candidate tumor suppressor ING1b can stabilize p53 by disrupting the regulation of p53 by MDM2. Cancer Res. **62:** 4890–4893
85 Kameyama K., Huang C. L., Liu D., Masuya D., Nakashima T., Sumitomo S. et al. (2003) Reduced ING1b gene expression plays an important role in carcinogenesis of non-small cell lung cancer patients. Clin. Cancer Res. **9:** 4926–4934
86 Aasland R., Gibson T. J. and Stewart A. F. (1995) The PHD finger: implications for chromatin mediated transcriptional regulation. Trends Biochem. Sci. **20:** 56–59
87 Toyama T., Iwase H., Yamashita H., Hara Y., Sugiura H., Zhang Z. et al. (2003) p33(ING1b) stimulates the transcriptional activity of the estrogen receptor alpha via its activation function (AF) 2 domain. J. Steroid. Biochem. Mol. Biol. **87:** 57–63
88 Toyama T. and Iwase H. (2004) p33ING1b and estrogen receptor (ER) alpha. Breast Cancer **11:** 33–37
89 Gu W. and Roeder R. G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:** 595–606
90 Sakagushi K., Herrera J. E., Saito S., Miki T., Bustin M., Vassilev A. et al. (1998) DNA damage activates p53 through a phosphorylation-acetylation cascade. Gene Dev. **12:** 2831–2841
91 Gozani O., Karuman P., Jones D. R., Ivanov D., Cha J., Lugovskoy A. A. et al. (2003) The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. Cell **114:** 99–111
92 Maisse C., Guerrieri P. and Melino G. (2003) p73 and p63 protein stability: the way to regulate function? Biochem. Pharmacol. **66:** 1555–1561
93 Rameh L. E., Tolias K. F., Duckworth B. C., and Cantley L. C. (1997) A new pathway for synthesis of phosphatidylinositol-4,5-bisphosphate. Nature **390:** 192–196
94 Carrozza M. J., Utley R. T., Workman J. L. and Côté J. (2003) The diverse functions of histone acetyltransferase complexes. Trends Genet. **19:** 321–329
95 Hasan S. and Hottiger M. O. (2002) Histone acetyl transferases: a role in DNA repair and DNA replication. J. Mol. Med. **80:** 463–474
96 Sterner D. E. and Berger S. L. (2000) Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:** 435–459
97 Nourani A., Doyon Y., Utley R. T., Allard S., Lane W. S. and Côté J. (2001) Role of an ING1 growth regulator in transcriptional activation and targeted histone acetylation by the NuA4 complex. Mol. Cell. Biol. **21:** 7629–7640
98 Howe L., Kusch T., Muster N., Chaterji R., Yates J. R. 3rd and Workman J. L. (2002) Yng1p modulates the activity of Sas3p as a component of the yeast NuA3 histone acetyltransferase complex. Mol. Cell. Biol. **22:** 5047–5053
99 Nourani A., Howe L., Pray-Grant M. G., Workman J. L., Grant P. A. and Côté J. (2003) Opposite role of yeast ING family members in p53-dependent transcriptional activation. J. Biol. Chem. **278:** 19171–19175
100 Cai Y., Jin J., Tomomori-Sato C., Sato S., Sorokina I., Parmely T.J. et al. (2003) Identification of new subunits of the multiprotein mammalian TRRAP/TIP60-containing histone acetyltransferase complex. J. Biol. Chem. **278:** 42733–42736
101 Skowyra D., Zeremski M., Neznanov N., Li M., Choi Y., Uesugi M. et al. (2001) Differential association of products of alternative transcripts of the candidate tumor suppressor ING1 with the mSin3/HDAC1 transcriptional corepressor complex. J. Biol. Chem. **276:** 8734–8739
102 Kuzmichev A., Zhang Y., Erdjument-Bromage H., Tempst P. and Reinberg D. (2002) Role of the Sin3-histone deacetylase complex in growth regulation by the candidate tumor suppressor p33(ING1). Mol. Cell. Biol. **22:** 835–848
103 Alexiadis V., Waldmann T., Andersen J., Mann M., Knippers R. and Gruss C. (2000) The protein encoded by the proto-oncogene DEK changes the topology of chromatin and reduces the efficiency of DNA replication in a chromatin-specific manner. Genes Dev. **14:** 1308–1312
104 Hollenbach A. D., McPherson C. J., Mientjes E. J., Iyengar R. and Grosveld G. (2002) Daxx and histone deacetylase II associate with chromatin through an interaction with core histones and the chromatin-associated protein Dek. J. Cell Sci. **115:** 3319–3330
105 Vieyra D., Senger D. L., Toyam T., Muzik H., Brasher P. M., Johnston R. N. et al. (2003) Altered subcellular localization and low frequency of mutations of ING1 in human brain tumors. Clin. Cancer Res. **9:** 5952–5961
106 Tallen G., Riabowol K. and Wolff J. E. (2003) Expression of p33ING1 mRNA and chemosensitivity in brain tumor cells. Anticancer Res. **23:** 1631–1635
107 Siddik Z. H. (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene **22:** 7265–7279
108 Valenzuela M. T., Nunez M. I., Villalobos M., Siles E., Olea N., Pedraza V. et al. (1995) Relationship between doxorubicin cell sensitivity, drug-induced DNA double-strand breaks, glutathione content and P-glycoprotein in mammalian tumor cells. Anticancer Drugs **6:** 749–757
109 Fry A. M., Chresta C. M., Davies S. M., Walker M. C., Harris A. L., Hartley J. A. et al. (1991) Relationship between topoisomerase II level and chemosensitivity in human tumor cell lines. Cancer Res. **51:** 6592–6595
110 Epstein R. J. (1990) Drug-induced DNA damage and tumor chemosensitivity. J. Clin. Oncol. **8:** 2062–2084
111 Collis S. J., Swartz M. J., Nelson W. G. and DeWeese T. L. (2003) Enhanced radiation and chemotherapy-mediated cell

killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. Cancer Res. 63: 1550–1554
112 Ohmori M., Nagai M., Tasaka T., Koeffler H. P., Toyama T., Riabowol K. et al. (1999) Decreased expression of p33ING1 mRNA in lymphoid malignancies. Am. J. Hematol. **62:** 118–119
113 Nouman G. S., Anderson J. J., Wood K. M., Lunec J., Hall A. G., Reid M. M. et al. (2002) Loss of nuclear expression of the p33(ING1b) inhibitor of growth protein in childhood acute lymphoblastic leukaemia. J. Clin. Pathol. **55:** 596–601
114 Cheung K. J. Jr and Li G. (2002) The tumour suppressor p33ING1 does not regulate migration and angiogenesis in melanoma cells. Int. J. Oncol. **21:** 1361–1365
115 Nouman G. S., Anderson J. J., Crosier S., Shrimankar J., Lunec J. and Angus B. (2003) Downregulation of nuclear expression of the p33(ING1b) inhibitor of growth protein in invasive carcinoma of the breast. J. Clin. Pathol. **56:** 507–511
116 Nouman G. S., Anderson J. J., Mathers M. E., Leonard N., Crosier S., Lunec J. et al. (2002) Nuclear to cytoplasmic compartment shift of the p33ING1b tumour suppressor protein is associated with malignancy in melanocytic lesions. Histopathology **40:** 360–366
117 Garkavtsev I., Boland D., Mai J., Wilson H., Veillette C. and Riabowol K. (1997) Specific monoclonal antibody raised against the p33ING1 tumor suppressor. Hybridoma **16:** 537–540
118 Gunduz M., Ouchida M., Fukushima K., Ito S., Jitsumori Y., Nakashima T. et al. (2002) Allelic loss and reduced expression of *ING3*, a candidate tumor suppressor gene at 7q31, in human head and neck cancers. Oncogene **21:** 4462–4470
119 Chen B., Campos E. I., Crawford R., Martinka M. and Li G. (2003) Analyses of the tumour suppressor ING1 expression and gene mutation in human basal cell carcinoma. Int. J. Oncol. **22:** 927–931
120 Tallen G., Kaiser I., Krabbe S., Lass U., Hartmann C., Henze G. et al. (2004) No ING1 mutations in human brain tumours but reduced expression in high malignancy grades of astrocytoma. Int. J. Cancer **109:** 476–479
121 Toyama T., Iwase H., Watson P., Muzik H., Saettler E., Magliocco A. et al. (1999) Suppression of ING1 expression in sporadic breast cancer. Oncogene **18:** 5187–5193
122 Tokunaga E., Maehara Y., Oki E., Kitamura K., Kakeji Y., Ohno S. et al. (2000) Diminished expression of ING1 mRNA and the correlation with p53 expression in breast cancers. Cancer Lett. **152:** 15–22
123 Sarela A. I., Farmery S. M., Markham A. F. and Guillou P. J. (1999) The candidate tumour suppressor gene, ING1, is retained in colorectal carcinomas. Eur. J. Cancer **35:** 1264–1267
124 Chen L., Matsubara N., Yoshino T., Nagasaka T., Hoshizima N. and Shirakawa Y. et al. (2001) Genetic alterations of candidate tumor suppressor ING1 in human esophageal squamous cell cancer. Cancer Res. **61:** 4345–4349
125 Hara Y., Zheng Z., Evans S. C., Malatjalian D., Riddell D. C., Guernsey D. L. et al. (2003) ING1 and p53 tumor suppressor gene alterations in adenocarcinomas of the esophagogastric junction. Cancer Lett. **192:** 109–116
126 Oki E., Maehara Y., Tokunaga E., Kakeji Y. and Sugimachi K. (1999) Reduced expression of p33(ING1) and the relationship with p53 expression in human gastric cancer. Cancer Lett. **147:** 157–162
127 Gunduz M., Ouchida M., Fukushima K., Hanafusa H., Etani T., Nishioka S. et al. (2000) Genomic structure of the human ING1 gene and tumor-specific mutations detected in head and neck squamous cell carcinomas. Cancer Res. **60:** 3143–3146
128 Sanchez-Cespedes M., Okami K., Cairns P. and Sidransky D. (2000) Molecular analysis of the candidate tumor suppressor gene ING1 in human head and neck tumors with 13q deletions. Genes Chromosomes Cancer **27:** 319–322
129 Bromidge T. and Lynas C. (2002) Relative levels of alternative transcripts of the ING1 gene and lack of mutations of p33/ING1 in haematological malignancies. Leuk. Res. **26:** 631–635
130 Kichina J. V., Rauth S., Das Gupta T. K. and Gudkov A. V. (2003) Melanoma cells can tolerate high levels of transcriptionally active endogenous p53 but are sensitive to retrovirus-transduced p53. Oncogene **22:** 4911–4917
131 Campos E. I., Cheung K. J. Jr, Murray A., Li S. and Li G. (2002) The novel tumour suppressor gene ING1 is overexpressed in human melanoma cell lines. Br. J. Dermatol. **146:** 574–580
132 Ito K., Kinjo K., Nakazato T., Ikeda Y. and Kizaki M. (2002) Expression and sequence analyses of p33(ING1) gene in myeloid leukemia. Am. J. Hematol. **69:** 141–143
133 Kameyama K., Huang C. L., Liu D., Masuya D., Nakashima T., Sumitomo S. et al. (2003) Reduced ING1b gene expression plays an important role in carcinogenesis of non-small cell lung cancer patients. Clin. Cancer Res. **9:** 4926–4934
134 Hoque M. O., Kawamata H., Nakashiro K., Omotehara F., Hino S., Uchida D. et al. (2002) Dysfunction of the p53 tumor suppressor pathway in head and neck cancer. Int. J. Oncol. **21:** 119–126
135 Krishnamurthy J., Kannan K., Feng J., Mohanprasad B. K., Tsuchida N. and Shanmugam G. (2001) Mutational analysis of the candidate tumor suppressor gene ING1 in Indian oral squamous cell carcinoma. Oral Oncol. **37:** 222–224