ING Genes in NSCLC

Subjects: Oncology

Contributor: Elisabeth Taucher

Carcinogenic mutations allow cells to escape governing mechanisms that commonly inhibit uncontrolled cell proliferation and maintain tightly regulated homeostasis between cell death and survival. Members of the inhibition of growth (ING) family act as tumor suppressors, governing cell cycle, apoptosis and cellular senescence. The molecular mechanism of action of ING genes, as well as their anchor points in pathways commonly linked to malignant transformation of cells, have been studied with respect to a variety of cancer specimens.

Keywords: Inhibition of growth ; ING ; lung cancer ; NSCLC

1. Introduction

In cells of healthy tissue, multiple mechanisms are necessary to control cell proliferation and maintain a balance between cell death and survival. Carcinogenic mutations in cancer genes allow cells to escape these governing mechanisms, permitting them to grow and proliferate indefinitely [1]. Within recent years, protein-coding and non-coding genes have been proposed as cancer drivers and tumor suppressors [2][3]. Tumor suppressor genes usually control growth-regulatory mechanisms, whilst genetic alterations affecting those tumor suppressor genes lead to metastatic spread [4][5][6]. Tumor protein p53 is one of the most frequently mutated tumor suppressors in human malignancies, however other genes exerting similar functions whose mutations also cause cellular control mechanisms to fail have recently been outlined: The inhibition of growth *(ING)* gene family is a powerful mediator of transcriptional regulation, regulation of the cell cycle, apoptosis, and cellular senescence [1]. All *ING* family members count among type II tumor suppressors since their inactivation leads to malignant transformation in different tissue types [7][8]. Until today, five human *ING* family genes *(ING1–5)* have been identified, with *ING1* being the most widely studied. Initially, *ING1* was isolated by means of subtractive hybridization between short segments of cDNAs [9]. The fragmented cDNAs were found to interfere with the activity of tumor suppressors by inhibiting protein synthesis through anti-sense sequences, or through truncated sense fragments that abrogate gene function in a dominant-negative fashion [9]. *ING2*, *ING3*, *ING4,* and *ING5* were outlined by homology search. *ING1* and *ING2* form a distinct subgroup, as they have been found to be evolutionally and functionally close [10]. Characteristic for the ING proteins is a high homology in their C-terminal domain, containing a nuclear localization sequence and a plant homeodomain featuring a high affinity to histone 3 tri-methylated on lysine 4 (H3K4Me3) [10]. H3K4-methylation has been reported to act primarily as a repressor of protein-coding genes, and this repressive function is ever-decreasing with age [11]. The methylation pattern of H3K4 has been linked to carcinogenesis, and may even have diagnostic and/or prognostic significance in numerous types of cancers [12]. All ING family proteins are involved in the control of cell growth, senescence, apoptosis, chromatin remodeling, and DNA repair. In recent years, *ING1* and *ING2* have been described as tumor suppressor genes in various human cancer types, and both *ING1* and *ING2* knockout mice were found to spontaneously develop malignant diseases such as B cell lymphomas and soft tissue sarcomas [13][14]. The highly conserved c-terminal plant homeodomain that is characteristic for the ING family, and is generally found in chromatin remodeling proteins, as well as the nuclear localization sequence and the nuclear conserved region directing the genes to the nucleus, allows the *ING* genes to interact with chromatin and possibly nucleosomes [15]. The N-termini differ between the *ING* members and define their specific functions, for instance, their antagonistic regulatory properties [16]. Moreover, it has been demonstrated that *ING* gene family members also exert their various functions on the epigenetic level [17]. They act as histone mark sensors, core components of histone deacetylases (HDACs) 1 and 2, and histone acetyltransferase (HAT) chromatin-modifying complexes. Thereby INGs affect the hallmarks of cancer by altering gene methylation patterns, acting mainly as tumor suppressors [17].

2. *ING1* and *ING2* Act as Tumor Suppressors in Human Lung Cancer

In an in-depth analysis of lung cancer tissue samples and cell lines, Okano et al. sought to identify whether *ING1* and *ING2* are aberrantly expressed in human lung cancer [18]. By means of PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequence analysis, the *ING2* gene was assessed. The expression

of *ING1b* and *ING2* was analyzed using quantitative real-time reverse transcription (RT)-PCR. Thirty-one primary lung cancer tissue samples were paired to matched controls. The samples consisted of 14 adenocarcinomas (AC), eight squamous cell carcinomas (SCC), six small cell lung cancers (SCLC), two large cell carcinomas and one adenosquamous cell carcinoma.

In addition, the authors investigated 15 human non-small cell lung cancer (NSCLC) cell lines as well as 15 human SCLC cell lines [19]. In the cell lines, PCR-SSCP analysis was carried out, and each of the exons of *ING1b* and *ING2* was amplified by means of PCR primers. Afterward, DNA sequence analysis was performed [18]. The results of this analysis revealed aberrant expression patterns of *ING1b* and *ING2* in six out of 31 lung cancer tissue samples, and in only one out of 30 lung cancer cell lines, i.e., in the NSCLC cell line NCI-H23. According to the DNA sequence analysis, the aberrant bands revealed a G to A substitution at codon 173, which does not lead to amino acid substitution. When matched with healthy control tissues, the same mutation was found in these as well, suggesting that it is due to gene polymorphism [18]. T to C substitution at codon 13 was detected in mutated bands in exon 1 of *ING2*, which occurred in 6 out of 31 lung cancer tissues. Moreover, this T to C substitution does not alter the amino acid encoded, and the authors conclude that also this aberration was due to polymorphism. In the cell lines, mRNA of *ING1b* and/or *ING2* was up-regulated in seven out of eight lung cancer cell lines, as compared to the normal bronchial epithelium cell line BETA2A. Interestingly, all seven cell lines that exhibited up-regulation of *ING1b* mRNA also featured *p53* mutations, while the remaining cell lines expressed wild-type *p53* [18]. In seven out of eight lung cancer cell lines, *ING2* mRNA was down-regulated, and among six of the seven cell lines that featured *p53* mutation, reduced mRNA expression of *ING2* was observed as well. Summing up these findings, *ING1* and *ING2* are evidently expressed differentially in human lung cancer, as compared to healthy lung tissue. Thus, these genes may serve as prognostic disease biomarkers for lung cancer in the future [18].

In immunohistochemical analysis, Zhao and colleagues analyzed expression profiles of the ING2 protein in normal versus cancer tissues [20]. Among the cancer specimens tested, there were 192 samples of human lung cancer. In addition, mouse tissues were analyzed for ING2 expression, comprising, among others, bronchial and alveolar tissue samples. Both tissue types were tested positive for cytoplasmic as well as nuclear ING2 protein expression. Interestingly, in human tissues, ING2 was universally expressed in the cytoplasm while only certain tissues featured nuclear ING2 expression as well [20]. According to this study, ING2 expression in lung cancer occurred infrequently, as compared to breast, ovarian, and endometrial cancer where the majority of cases expressed ING2.

ING1b has also been investigated with respect to NSCLC carcinogenesis. Therefore, tissue samples from 88 NSCLC patients who had undergone surgery were studied [21]. The samples comprised 35 ACs, 48 SCCs and five samples of large cell carcinoma. PCR-SSCP sequencing was performed for *ING1* and the *p53* gene mutation, as well as quantitative RT-PCR for *ING1b*, *p21* and Bcl-2 associated X, apoptosis regulator *(Bax)* gene expression. For p21 and Bax protein expression, immunohistochemistry was performed. It was found that only two (2.3%) out of 88 NSCLC samples studied featured point mutations of the coding regions of *ING1b*. Standardized gene expression was determined by two pathologists who had no knowledge of the patients' clinical data. In the 88 NSCLC samples studied, expression varied widely (0.768–0.404). In total, 37 carcinomas (42%) featured a diminished *ING1b* expression. No relation was found between *ING1b* expression and *p53* gene status [21]. With respect to *ING1b* gene expression, the standardized *p21* expression ratio tended to be higher in *ING1b*-positive tumors and was significantly lower in tumors that featured a reduced *ING1b* expression ($p < 0.0029$). Expression of the *Bax* gene also tended to be higher in *ING1b*-positive tumors and was significantly lower in tumor samples featuring a reduction in *ING1b* expression ($p < 0.0001$). The conclusion drawn from this analysis is an evident association of reduced *ING1b* gene expression with reduced *p21* and *Bax* gene expression in NSCLC [21]. This study was the first study to show this correlation. *ING1b* thus has a tumor-suppressive function in NSCLC. The mode of action is obviously connected to the expression of *p21* and *Bax*. However, the exact mechanism of this relationship still remains to be investigated in-depth.

In 2011, an interesting study was carried out by Luo and colleagues [22], investigating the role of *ING1* in lung carcinoma. Two lung cancer cell lines were transfected with recombinant *ING1b* plasmids. One cell line, A549, expressed wild-type *p53* whilst the other (SK-MES-1) featured *p53* mutation. Apoptotic rate, alterations in the cell cycle, cell growth rate and downstream p21waf1 protein expression were assessed in both transfected cell lines. Additionally, the p33ING1b-p53 complex was analyzed by means of co-immunoprecipitation. For the detection of gene aberrations and the expression pattern of *ING1*, 70 cases of fresh-frozen lung carcinomas and 217 cases of formalin-fixed paraffin-embedded (FFPE) lung cancer samples were examined for loss of heterozygosity (LOH), p33ING1b protein expression by PCR-SSCP, as well as immunohistochemistry. As a result, over-expression of *ING1b* was found to inhibit cell growth of both A549 and SK-MES-1 cells, induced cell cycle arrest and apoptosis. The p21waf1 protein was significantly up-regulated, and a complex of p33ING1b and wtp53 could be detected after the wtp53 lung cancer cells had been transfected by *ING1b.* LOH correlated inversely with the expression of p33ING1b, and p33ING1b expression was lost in the majority

(53%) of lung cancer specimens overall [22]. The authors conclude *ING1* being a potent inhibitor of lung cancer cell growth, inducing cell cycle arrest and apoptosis by forming a complex with the wtp53 protein, and up-regulating p21waf1 [22].

p33ING1, *p53* and the autophagy-related gene *Beclin1* were investigated in NSCLC in another study from 2011, to find out more about the correlation between the expression of these genes with clinico-pathological parameters [23]. The researchers used NSCLC tissue gathered from operation, along with non-cancerous healthy lung tissue. mRNA, as well as protein expression of *p33ING1*, *p53* and *Beclin1,* were detected by means of RT-PCR and Western blotting. mRNA and protein expression of *p33ING1* and *Beclin1* in NSCLC were significantly lower as compared to the surrounding healthy lung tissue ($p < 0.05$). Expression levels of *p33ING1* mRNA and protein expression were found to be diminished in NSCLC specimens with good or middle differentiation, whilst they were higher in poorly differentiated NSCLC samples [23]. Conversely, the expression levels of *p33ING1* and *Beclin1* were lower in the presence of lymph node metastasis and higher with no lymph node metastases. Both markers were also expressed at higher levels in patients with stage I-II disease as compared to stage III-IV disease ($p < 0.05$). Interestingly, protein expression of mutated *p53* in NSCLC was significantly higher in the lung cancer tissues than in the surrounding non-cancerous tissues [23]. On the basis of this data, it is not conclusive whether *p33ING1* and *Beclin1* act as tumor-suppressors or as tumor-promoters in NSCLC. Nevertheless, the combined detection of *p33ING1*, *p53,* and *Beclin1* genes is suggested by the authors as a potential diagnostic tool for early diagnosis and estimation of prognosis in NSCLC [23].

Furthermore, ING1 has recently been investigated in the context of micro RNAs (miRNAs) by Jinag et al. [24]. MiRNAs have repeatedly been shown to be involved in NSCLC carcinogenesis, acting either as tumor promoters, or as tumor suppressors. In this study, miR-500 and miR-628 expression profiles were assessed by quantitative RT-PCR. Migration, invasion, proliferation, cell adhesion, and apoptotic rate were analyzed in order to gain knowledge about the function of these micro RNAs in NSCLC. Luciferase reporter assay was used to validate the direct targeting of *ING1* by miR-500 as well as miR-628 [24]. In NSCLC tissues, miR-500 and miR-628 were expressed at higher levels in comparison to noncancerous tissues. Inhibition of miR-500 and miR-628 led to significant suppression of NSCLC cell proliferation, migration, invasion, and cell adhesion, and induced NSCLC cell apoptosis. In addition, it was shown that the *ING1* gene was a direct target both for miR-500 and miR-628. *ING1* over-expression inhibited NSCLC cell proliferation, migration, and invasion, and made cancer cells undergo apoptosis, according to this research study [24]. Conclusively, miR-500 and miR-628 act pro-carcinogenic in NSCLC, both targeting the tumor suppressor *ING1.*

An immunohistochemical study by Pan et al. was conducted in order to analyze the role of ING2 in lung carcinogenesis [25]. Sixty-four samples of NSCLC were tested for ING2 protein expression by means of immunohistochemistry, and the results were confirmed by Western blotting. Additionally, RT-PCR was used to evaluate *ING2* mRNA levels as well. ING2 protein expression was found to be significantly decreased in this cohort of Chinese NSCLC patients [25], when compared to samples of normal lung tissue. ING2 was more frequently lost in AC (45.8%) than in SCC (26.3%). In the lung cancer samples, there was a shift of ING2 expression from the nucleus to the cytoplasm. Moreover, this study revealed a significant association between ING2 expression, lymph node metastasis, and TNM stage, albeit only for SCC and not for AC. According to this research study, ING2 is aberrantly expressed in NSCLC and is likely to contribute to lung carcinogenesis [25]. Down-regulation of ING2 protein expression was also confirmed by immunohistochemical analysis on 120 NSCLC specimens [26]. In >50% of the investigated samples, ING2 was down-regulated, which was more frequent in AC (68%) as compared to SCC (45%). No association with the patients' gender, age, or five-years survival times were observed with respect to ING2. According to this study, no LOH or ING2 gene mutations were observed [26]. Notably, in 95% of the cancer samples examined, a silent single nucleotide polymorphism (SNP) was found. The authors of this study also investigated the promoter region of the *ING2* gene, however, no alterations in the methylation pattern were identified. Conversely to previous reports, no correlation between *p53* activity and *ING2* expression levels was seen in this study [26]. As a conclusion, also this data suggests an impact of *ING2* on NSCLC development.

Finally, it is also worth mentioning that *ING2* is the major target of the HDAC inhibitor vorinostat [27], which was proven efficient also in the treatment of NSCLC patients [28]. Interestingly, ING1 protein phosphorylation status impacts the cross-talk of the p33(ING1b) splicing isoform of ING1 with members of the 14-3-3 protein family [29]. The 14-3-3 proteins are expressed in human tissues universally and have the ability to interact with diverse signaling pathways, altering protein expression, function of kinases, phosphatases, and transmembrane receptors. Further, 14-3-3 binding resulted in a significant transfer of p33(ING1b) protein to the cytoplasm [29]. Hence, it is likely that 14-3-3 influences the activity of p33(ING1b) significantly by directing its subcellular localization [29]. A novel function of *ING2* was recently identified, i.e., the control of DNA replication and the maintenance of genome stability [30]. In small interfering RNA (siRNA) *ING2* cells, global replication rate was significantly reduced during normal S-phase of the cell cycle, which was demonstrated by DNA fiber spreading experiment. In accordance with this finding, *ING2* was also shown to interact with proliferating cell nuclear

antigen, regulating its amount to the chromatin fraction, which allows for normal cell replication and cell proliferation. A high proportion of siRNA *ING2* cells showed endo-reduplication of their genome and an increased ratio of sister chromatin exchange. The authors of this study thus propose that *ING2* exerts its tumor-suppressive function by directly maintaining DNA integrity [30].

3. *ING3* and Its Potential Role in Lung Cancer

To the best of our knowledge, no data on the impact of *ING3* in lung cancer have been published yet. According to a review article from 2017, high expression levels of *ING3* are found in healthy tissues that feature a rapid cell proliferation, such as the small intestine, bone marrow, or the epidermis [31][32]. It was also shown that *ING3* is expressed to a significantly higher degree in proliferating versus quiescent epithelial cells. Contrary to pre-existing data on the other *ING gene* family members, data on *ING3* suggests rather pro-carcinogenic effects of this particular gene, since high levels of *ING3* correlate with more rapid cell growth. On the other hand, down-regulation of *ING3* was observed in hepatocellular carcinoma and in colorectal adenocarcinoma [33][34]. In accordance with that, loss of *ING3* was found to be associated with head and neck carcinogenesis [35]. *ING3* induces germ cell apoptosis and embryonic death in a *p53*-dependent manner [36]. The link to tumor protein p53 has also been described for *ING1b* [21]. The function of the NuA4 histone acetyltransferase multi-subunit complex is linked *p53* as well and is also mediated by *ING3* [37]. NuA4 is essential for cell cycle proliferation and DNA-repair. Thus, *ING3* is likely to impact cell proliferation by regulating NuA4. Similarly to *ING4*, interaction with *p21* and *Bax* was demonstrated for *ING3* as well: it was shown that *ING3* induced the expression of *p21* and *Bax* which resulted in apoptosis and diminished cell proliferation as shown in the RKO colon carcinoma cell line [38]. Data specifically on the role of *ING3* in lung cancer is still very limited. Since previous research has shown controversial results, i.e., *ING3* having anti-proliferative effects, whilst enhancing proliferation in other studies, it would be interesting to outline its mechanism of action in lung cancer in the future.

The ING3 protein was investigated in the context of cell cycle arrest, p53-transactivated promoters of p21 and Bcl2-associated X protein [39]. Immunohistochemistry was used to characterize ING3 expression profiles in tissue microarrays comprising various cancer specimens. Amongst these were 192 samples of lung cancer. Additionally, mouse tissue was analyzed, and ING3 cytoplasmic, as well as nuclear expression, was detected in the murine healthy bronchial and alveolar epithelial cells. Similar to ING2 [20], also ING3 was expressed in some lung cancer samples but occurred most abundantly in gynecological cancer entities. The authors conclude ING3 being involved in the repair and regeneration of tissues and point out a particular role in gynecological carcinogenesis [39].

References

1. Gunduz, M.; Gunduz, E.; Rivera, R.S.; Nagatsuka, H. The inhibitor of growth (ING) gene family: Potential role in cancer therapy. Curr. Cancer Drug Targets 2008, 8, 275–284.
2. Schwarzenbacher, D.; Klec, C.; Pasculli, B.; Cerk, S.; Rinner, B.; Karbiener, M.; Ivan, C.; Barbano, R.; Ling, H.; Wulf-G oldenberg, A.; et al. MiR-1287-5p inhibits triple negative breast cancer growth by interaction with phosphoinositide 3-kin ase CB, thereby sensitizing cells for PI3Kinase inhibitors. Breast Cancer Res. 2019, 21, 20.
3. Stiegelbauer, V.; Vychytilova-Faltejskova, P.; Karbiener, M.; Pehserl, A.M.; Reicher, A.; Resel, M.; Heitzer, E.; Ivan, C.; Bullock, M.; Ling, H.; et al. MiR-196b-5p regulates colorectal cancer cell migration and metastases through interaction with HOXB7 and GALNT5. Clin. Cancer Res. 2017, 23, 5255–5266.
4. Hinds, P.W.; Weinberg, R.A. Tumor suppressor genes. Curr. Opin. Genet. Dev. 1994, 4, 135–141.
5. Al-Zoughbi, W.; Pichler, M.; Gorkiewicz, G.; Guertl-Lackner, B.; Haybaeck, J.; Jahn, S.W.; Lackner, C.; Liegl-Atzwanger, B.; Popper, H.; Schauer, S.; et al. Loss of adipose triglyceride lipase is associated with human cancer and induces mou se pulmonary neoplasia. Oncotarget 2016, 7, 33832–33840.
6. Gantenbein, N.; Bernhart, E.; Anders, I.; Golob-Schwarzl, N.; Krassnig, S.; Wodlej, C.; Brcic, L.; Lindenmann, J.; Fink-N euboeck, N.; Gollowitsch, F.; et al. Influence of eukaryotic translation initiation factor 6 on non-small cell lung cancer de velopment and progression. Eur. J. Cancer 2018, 101, 165–180.
7. Nouman, G.S.; Anderson, J.J.; Lunec, J.; Angus, B. The role of the tumour suppressor p33 ING1b in human neoplasia. J. Clin. Pathol. 2003, 56, 491–496.
8. Russell, M.; Berardi, P.; Gong, W.; Riabowol, K. Grow-ING, age-ING and die-ING: ING proteins link cancer, senescenc e and apoptosis. Exp. Cell Res. 2006, 312, 951–961.

9. Garkavtsev, I.; Kazarov, A.; Gudkov, A.; Riabowol, K. Suppression of the novel growth inhibitor p33ING1 promotes neo plastic transformation. Nat. Genet. 1996, 14, 415–420.
10. Guerillon, C.; Bigot, N.; Pedeux, R. The ING tumor suppressor genes: Status in human tumors. Cancer Lett. 2014, 345, 1–16.
11. Cruz, C.; Della Rosa, M.; Krueger, C.; Gao, Q.; Horkai, D.; King, M.; Field, L.; Houseley, J. Tri-methylation of histone H 3 lysine 4 facilitates gene expression in ageing cells. Elife 2018, 7, e34081.
12. Li, Q.; Jia, N.; Tao, X.; Hua, K.; Feng, W. The expression and significance of histone lysine methylation in endometrial c ancer. Oncol. Lett. 2017, 14, 6210–6216.
13. Kichina, J.V.; Zeremski, M.; Aris, L.; Gurova, K.V.; Walker, E.; Franks, R.; Nikitin, A.Y.; Kiyokawa, H.; Gudkov, A.V. Targ eted disruption of the mouse ing1 locus results in reduced body size, hypersensitivity to radiation and elevated incidenc e of lymphomas. Oncogene 2006, 25, 857–866.
14. Saito, M.; Kumamoto, K.; Robles, A.I.; Horikawa, I.; Furusato, B.; Okamura, S.; Goto, A.; Yamashita, T.; Nagashima, M.; Lee, T.L.; et al. Targeted disruption of Ing2 results in defective spermatogenesis and development of soft-tissue sarcom as. PLoS ONE 2010, 5, e15541.
15. Ragvin, A.; Valvatne, H.; Erdal, S.; Arskog, V.; Tufteland, K.R.; Breen, K.; ØYan, A.M.; Eberharter, A.; Gibson, T.J.; Beck er, P.B.; et al. Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300. J. Mol. Bio l. 2004, 337, 773–788.
16. Kataoka, H.; Bonnefin, P.; Vieyra, D.; Feng, X.; Hara, Y.; Miura, Y.; Joh, T.; Nakabayashi, H.; Vaziri, H.; Harris, C.C.; et al. ING1 represses transcription by direct DNA binding and through effects on p53. Cancer Res. 2003, 63, 5785–5792.
17. Tallen, G.; Riabowol, K. Keep-ING balance: Tumor suppression by epigenetic regulation. FEBS Lett. 2014, 588, 2728–2742.
18. Okano, T.; Gemma, A.; Hosoya, Y.; Hosomi, Y.; Nara, M.; Kokubo, Y.; Yoshimura, A.; Shibuya, M.; Nagashima, M.; Harri s, C.C.; et al. Alterations in novel candidate tumor suppressor genes, ING1 and ING2 in human lung cancer. Oncol. Re p. 2006, 15, 545–549.
19. Gemma, A.; Takenoshita, S.; Hagiwara, K.; Okamoto, A.; Spillare, E.A.; McMemamin, M.G.; Hussain, S.P.; Forrester, K.; Zariwala, M.; Xiong, Y.; et al. Molecular analysis of the cyclin-dependent kinase inhibitor genes p15INK4b/MTS2, p1 6INK4/MTS1, p18 and p19 in human cancer cell lines. Int. J. Cancer 1996, 68, 605–611.
20. Zhao, S.; Yang, X.F.; Gou, W.F.; Lu, H.; Li, H.; Zhu, Z.T.; Sun, H.Z.; Zheng, H.C. Expression profiles of inhibitor of growt h protein 2 in normal and cancer tissues: An immunohistochemical screening analysis. Mol. Med. Rep. 2016, 13, 1881–1887.
21. Kameyama, K.; Huang, C.L.; Liu, D.; Masuya, D.; Nakashima, T.; Sumitomo, S.; Takami, Y.; Kinoshita, M.; Yokomise, H. Reduced ING1b gene expression plays an important role in carcinogenesis of non-small cell lung cancer patients. Clin. Cancer Res. 2003, 9, 4926–4934.
22. Luo, Z.G.; Tang, H.; Li, B.; Zhu, Z.; Ni, C.R.; Zhu, M.H. Genetic alterations of tumor suppressor ING1 in human non-sm all cell lung cancer. Oncol. Rep. 2011, 25, 1073–1081.
23. Liu, J.; Lin, Y.; Yang, H.; Deng, Q.; Chen, G.; He, J. The expression of p33(ING1), p53, and autophagy-related gene Be clin1 in patients with non-small cell lung cancer. Tumor Biol. 2011, 32, 1113–1121.
24. Jiang, M.; Zhou, L.Y.; Xu, N.; An, Q. Down-regulation of miR-500 and miR-628 suppress non-small cell lung cancer prol iferation, migration and invasion by targeting ING1. Biomed. Pharmacother. 2018, 108, 1628–1639.
25. Pan, Y.Q.; Zhang, X.; Xu, D.P.; Bao, W.G.; Lin, A.F.; Xu, H.H.; Yan, W.H. Decreased expression of ING2 gene and its cli nicopathological significance in chinese NSCLC patients. Neoplasma 2014, 61, 468–475.
26. Ythier, D.; Brambilla, E.; Binet, R.; Nissou, D.; Vesin, A.; de Fraipont, F.; Moro-Sibilot, D.; Lantuejoul, S.; Brambilla, C.; Gazzeri, S.; et al. Expression of candidate tumor suppressor gene ING2 is lost in non-small cell lung carcinoma. Lung Cancer 2010, 69, 180–186.
27. Smith, K.T.; Martin-Brown, S.A.; Florens, L.; Washburn, M.P.; Workman, J.L. Deacetylase inhibitors dissociate the histo ne-targeting ING2 subunit from the Sin3 complex. Chem. Biol. 2010, 17, 65–74.
28. Ramalingam, S.S.; Kummar, S.; Sarantopoulos, J.; Shibata, S.; LoRusso, P.; Yerk, M.; Holleran, J.; Lin, Y.; Beumer, J. H.; Harvey, R.D.; et al. Phase I study of vorinostat in patients with advanced solid tumors and hepatic dysfunction: A nat ional cancer institute organ dysfunction working group study. J. Clin. Oncol. 2010, 28, 4507–4512.
29. Gong, W.; Russell, M.; Suzuki, K.; Riabowol, K. Subcellular targeting of p33ING1b by phosphorylation-dependent 14-3-3 binding regulates p21WAF1 expression. Mol. Cell Biol. 2006, 26, 2947–2954.

30. Larrieu, D.; Ythier, D.; Binet, R.; Brambilla, C.; Brambilla, E.; Sengupta, S.; Pedeux, R. ING2 controls the progression of DNA replication forks to maintain genome stability. EMBO Rep. 2009, 10, 1168–1174.
31. Nabbi, A.; Almami, A.; Thakur, S.; Suzuki, K.; Boland, D.; Bismar, T.A.; Riabowol, K. ING3 protein expression profiling in normal human tissues suggest its role in cellular growth and self-renewal. Eur. J. Cell Biol. 2015, 94, 214–222.
32. Zhang, R.; Jin, J.; Shi, J.; Hou, Y. INGs are potential drug targets for cancer. J. Cancer Res. Clin. Oncol. 2017, 143, 189–197.
33. Gou, W.F.; Sun, H.Z.; Zhao, S.; Niu, Z.; Mao, X.; Takano, Y.; Zheng, H. Downregulated inhibitor of growth 3 (ING3) expression during colorectal carcinogenesis. Indian J. Med. Res. 2014, 139, 561–567.
34. Lu, M.; Chen, F.; Wang, Q.; Wang, K.; Pan, Q.; Zhang, X. Downregulation of inhibitor of growth 3 is correlated with tumorigenesis and progression of hepatocellular carcinoma. Oncol. Lett. 2012, 4, 47–52.
35. Gunduz, M.; Ouchida, M.; Fukushima, K.; Ito, S.; Jitsumori, Y.; Nakashima, T.; Nagai, N.; Nishizaki, K.; Shimizu, K. Allelic loss and reduced expression of the ING3, a candidate tumor suppressor gene at 7q31, in human head and neck cancers. Oncogene 2002, 21, 4462–4470.
36. Luo, J.; Shah, S.; Riabowol, K.; Mains, P.E. The caenorhabditis elegans ing-3 gene regulates ionizing radiation-induced germ-cell apoptosis in a p53-associated pathway. Genetics 2009, 181, 473–482.
37. Doyon, Y.; Selleck, W.; Lane, W.S.; Tan, S.; Cote, J. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol. Cell Biol. 2004, 24, 1884–1896.
38. Nagashima, M.; Shiseki, M.; Pedeux, R.M.; Okamura, S.; Kitahama-Shiseki, M.; Miura, K.; Yokota, J.; Harris, C.C. A novel PHD-finger motif protein, p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. Oncogene 2003, 22, 343–350.
39. Gou, W.F.; Yang, X.F.; Shen, D.F.; Zhao, S.; Sun, H.; Luo, J.; Zheng, H. Immunohistochemical profile of ING3 protein in normal and cancerous tissues. Oncol. Lett. 2017, 13, 1631–1636.
