

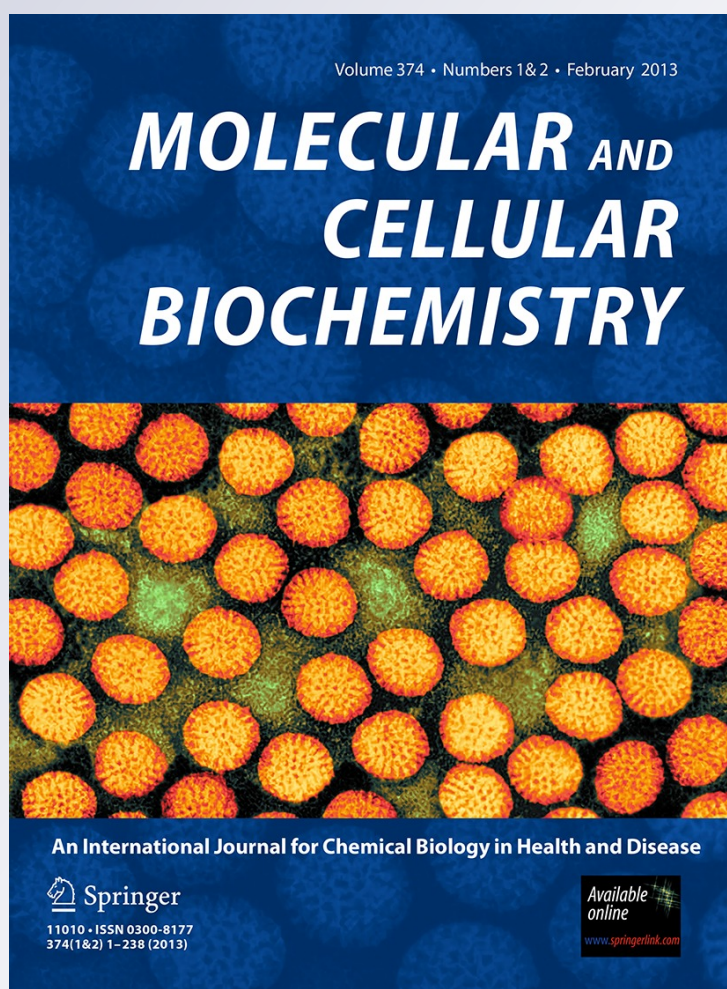
*INMAP, a novel truncated version
of POLR3B, represses AP-1 and p53
transcriptional activity*

**Zhou Yunlei, Chen Zhe, Lei Yan, Wang
Pengcheng, Zheng Yanbo, Sun Le &
Liang Qianjin**

Molecular and Cellular Biochemistry
An International Journal for Chemical
Biology in Health and Disease

ISSN 0300-8177
Volume 374
Combined 1-2

Mol Cell Biochem (2013) 374:81-89
DOI 10.1007/s11010-012-1507-4



INMAP, a novel truncated version of POLR3B, represses AP-1 and p53 transcriptional activity

Zhou Yunlei · Chen Zhe · Lei Yan ·
Wang Pengcheng · Zheng Yanbo · Sun Le ·
Liang Qianjin

Received: 15 August 2012 / Accepted: 25 October 2012 / Published online: 4 November 2012
© Springer Science+Business Media New York 2012

Abstract INMAP was first identified as an interphase nucleus and mitotic apparatus-associated protein that plays essential roles in the formation of the spindle and cell-cycle progression. Here, we report that INMAP might be conserved from prokaryotes to humans, is a truncated version of the RNA polymerase III subunit B POLR3B, and is up-regulated in several human cancer cell lines including HeLa, Bel-7402, HepG2 and BGC-823. Deletion analysis revealed that the 209–290 amino-acid region is necessary for the punctate distribution of INMAP in the nucleus. Furthermore, over-expression of INMAP inhibited the transcriptional activities of p53 and AP-1 in a dose-dependent manner. These results suggest that INMAP may function through the p53 and AP-1 pathways, thus providing a possible link of its activity with tumourigenesis. Integrating our data and those in previous studies, it can be

concluded that INMAP plays dual functional roles in the coordination of mitotic kinetics with gene expression as well as in cell-fate determination and proliferation.

Keywords INMAP · POLR3B · p53 · AP-1

Introduction

Three nuclear DNA-dependent RNA polymerases (Pol I, Pol II and Pol III) in eukaryotes share the task of transcribing the information contained in genes into RNA entities [1, 2]. Pol III transcribes a diverse group of small noncoding RNAs, including tRNAs, 5S rRNA, U6 snRNA, 7SL RNA and others [3, 4]. These RNAs participate in the regulation of transcription, splicing and translation. After transcription, Pol III molecules are either directly degraded or modified for participation in the regulation and execution of processes in the nucleus and the cytoplasm including transcription regulation, RNA processing, ribosome assembly, translation and others which ultimately leads to protein synthesis. Pol III is highly conserved from yeast to humans and is composed of 17 subunits. Its two largest subunits, Rpc1 (~160 kDa) and Polr3B (~130 kDa), provide a large surface area for the interaction with many of the other subunits [4]. Transcription by Pol III is precisely regulated in normal cells, but this regulation is lost during tumourigenesis [5]. Previous studies have revealed that Pol III transcription is negatively regulated in normal cells by tumour suppressor gene products (e.g. Rb, p53, BRCA1, Maf1 or PTEN) or other factors (e.g. MAF1 and EGCG), and its transcription is activated via multiple signal transduction cascades including the MAP kinase and the PI3 kinase pathways [5–8].

Electronic supplementary material The online version of this article (doi:10.1007/s11010-012-1507-4) contains supplementary material, which is available to authorized users.

Z. Yunlei · C. Zhe · L. Yan · L. Qianjin (✉)
Key Laboratory of Cell Proliferation and Regulation Biology of
Ministry of Education, College of Life Sciences, Beijing Normal
University, Beijing 100875, People's Republic of China
e-mail: liangqianjin0059@163.com

W. Pengcheng · L. Qianjin
Beijing Key Laboratory, College of Life Sciences, Beijing
Normal University, Beijing 100875, People's Republic of China

Z. Yanbo
The Institute of Medical Biotechnology (IMB) of the Chinese
Academy of Medical Sciences, Beijing 100050,
People's Republic of China

S. Le
AbMax Biotechnology Co., Ltd, Haidian, Beijing 100085,
People's Republic of China

Recessive mutations in *POLR3B* cause a rare hypomyelinating leukodystrophy in humans [9], and a deficiency of Pol III subunit C11, which mediates Pol III RNA cleavage activity and is important for the termination of transcription, disrupts zebrafish digestive development [10].

AP-1 (activator protein-1) is a heterodimeric transcription factor composed of proteins belonging to the c-Fos, c-Jun, and ATF (activating transcription factor) families. It directly binds to a DNA motif with the sequence 5'-TGAGTCA-3' in promoters [11]. Both cells and mice deficient in AP-1 proteins exhibit abnormal cell proliferation, neoplastic transformation and apoptosis [12–16]. AP-1 proteins control cell proliferation and death through their ability to regulate the expression of cell-cycle regulators, such as cyclin D1, p53, p21^{cip1/waf1}, p19^{ARF} and p16 [17–23]. Numerous studies have suggested that p53 is an important tumour suppressor protein [24, 25]. The *p53* gene is mutated in approximately half of human tumours [26–32]. Furthermore, p53 is a transcription factor whose activity gives rise to a variety of cellular outcomes, mainly cell-cycle arrest and apoptosis [33–36].

We previously cloned the gene encoding INMAP, an interphase nucleus and mitotic apparatus-associated protein, and found that it is highly homologous with the 3'-terminal sequence of *POLR3B* through bioinformatics analysis [37]. In addition, INMAP plays an essential role in spindle formation and cell-cycle progression [37]. However, the molecular mechanism by which INMAP is involved in cell-cycle progression and its relationship with *POLR3B* has not been clarified.

In this study, we demonstrated that INMAP is a novel truncated version of *POLR3B*, might be evolutionarily conserved from prokaryotes to humans, and is over-expressed in several cancer cell lines. We found that the 209–290 amino-acid (aa) region is responsible for the nuclear localisation of INMAP. Moreover, over-expression of INMAP in HEK293T cells repressed AP-1 and p53 transcriptional activity in a dose-dependent manner.

Materials and methods

Cell culture, reagents and antibodies

HeLa, HEK293T, A375, MCF-7, BGC823, HepG-2 and Bel-7402 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco-BRL, USA) supplemented with 10 % (v/v) foetal bovine serum (Invitrogen, New Zealand) in a CO₂ (5 %) incubator at 37 °C. DAPI, penicillin and streptomycin were purchased from Sigma. Other reagents included the high-efficiency transfection reagent Vigofect (Vigorse, Beijing, China), geneticin G418 (Merck, USA)

as well as anti-FLAG, anti-tubulin and anti-GAPDH monoclonal antibodies (Proteintech Group, Inc., USA). The anti-INMAP monoclonal antibody was obtained as previously described [37]. Alkaline phosphatase-conjugated goat anti-rabbit IgG and goat anti-mouse IgG antibodies were obtained from Vector Laboratories, Peterborough, UK. Oligonucleotides were synthesised by Sangon (Shanghai Sangon Biotechnology, Shanghai, China).

Plasmid construction

3XFLAG-INMAP was generated by PCR using pEGFP-C3-INMAP as a template and the following pair of primers: 5' primer (GGAATTC CATGGGGCCCATGTTG) and 3' primer (CGGGATCCTTCATTGTACTTGGACAG) containing restriction site for *Eco*R1 and *Bam*H1, respectively (underlined). The PCR fragment was then cloned into the p3XFLAG-CMV14 expression vector (Sigma, St. Louis, MO) [37]. The PCR reaction programme was 94 °C for 5 min followed by 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min for 30 cycles, with a final extension at 72 °C for 10 min.

For the synthesis of digoxigenin-labelled INMAP and *POLR3B* RNA probes, *INMAP* and *POLR3B* DNAs were obtained by PCR from plasmids p3Xflag-INMAP and pEGFP-C3-POLR3B, respectively. The PCR products were then cloned into the pGEM-T vector containing SP6 and T7 promoters (Promega, China).

pEGFP-C3-truncated *INMAP* plasmids were constructed by PCR using pEGFP-C3-INMAP as a template with *Eco*R1-linked 5' primers (Table S1 in supplement) and *Bam*H1-linked 3' primers (Table S1 in supplement) and then cloned into the p3XFLAG-CMV14 expression vector.

Northern blot analysis

Total RNA extraction from HeLa cells was performed using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Digoxigenin-labelled anti-sense RNA probes for human *INMAP* (nucleotides 138–643) and human *POLR3B* (nucleotides 448–849) were synthesised using the DIG Northern Starter kit (Roche Molecular Biochemicals, Indianapolis, Indiana, USA). In brief, 2 µg of total RNA was separated on a 1.5 % (w/v) denaturing agarose gel (MP-Agarose, Boehringer-Mannheim, Germany) in 1 × MOPS buffer containing 4 % (v/v) formaldehyde. After electrophoresis, the gels were immersed and gently shaken in 20 × SSC (3 M NaCl, 0.3 M citric acid, pH 7.0) for 30 min at room temperature. Capillary transfer of RNAs was conducted with 20 × SSC for 12 h to a positively charged nylon membrane (Boehringer-Mannheim, Germany), and RNAs were cross-linked to the membrane

by exposure to UV (254 nm, 125 mJ). The membranes were prehybridised in 0.05 mL/cm² DIG Easy Hyb solution (Boehringer-Mannheim, Germany) at 68 °C. For hybridisation, the solution was replaced with an equal amount of DIG Easy Hyb containing 100 ng/mL of labelled and denatured *INMAP* or *POLR3B* probes and incubated at 68 °C overnight [38]. Chemiluminescent detection was performed according to the procedure of the DIG Northern Starter kit.

Cell culture, transient transfection and subcellular localisation analyses

HeLa cells used in these studies were maintained and passaged according to previously described standard methods [39] and were transfected with pEGFP-C3-truncated *INMAP* plasmid DNAs using Vigofect according to the manufacturer's instructions. 48 h after transfection, the localisation of the fusion protein was visualised with an

Olympus laser-scanning confocal microscope (Olympus Fluoview FV300, Japan) after labelling with DAPI for nuclear staining. HeLa cells transfected with the pEGFP-C3 vector were used as a control.

Reporter gene assays

HEK293T cells were cultured as described above and supplemented with 100 U/mL of penicillin and streptomycin. For luciferase assays, the recombinant vectors were transiently transfected into HEK293T cells using the high-efficiency transfection reagent Vigofect according to the manufacturer's instruction. 48 h later, the cells were harvested, and the luciferase activity assay was performed according to the protocols developed by Stratagene. Each experiment was performed in triplicate, and each assay was repeated at least three times. The means of the data from three individual transfected wells were calculated after normalisation for β -galactosidase.

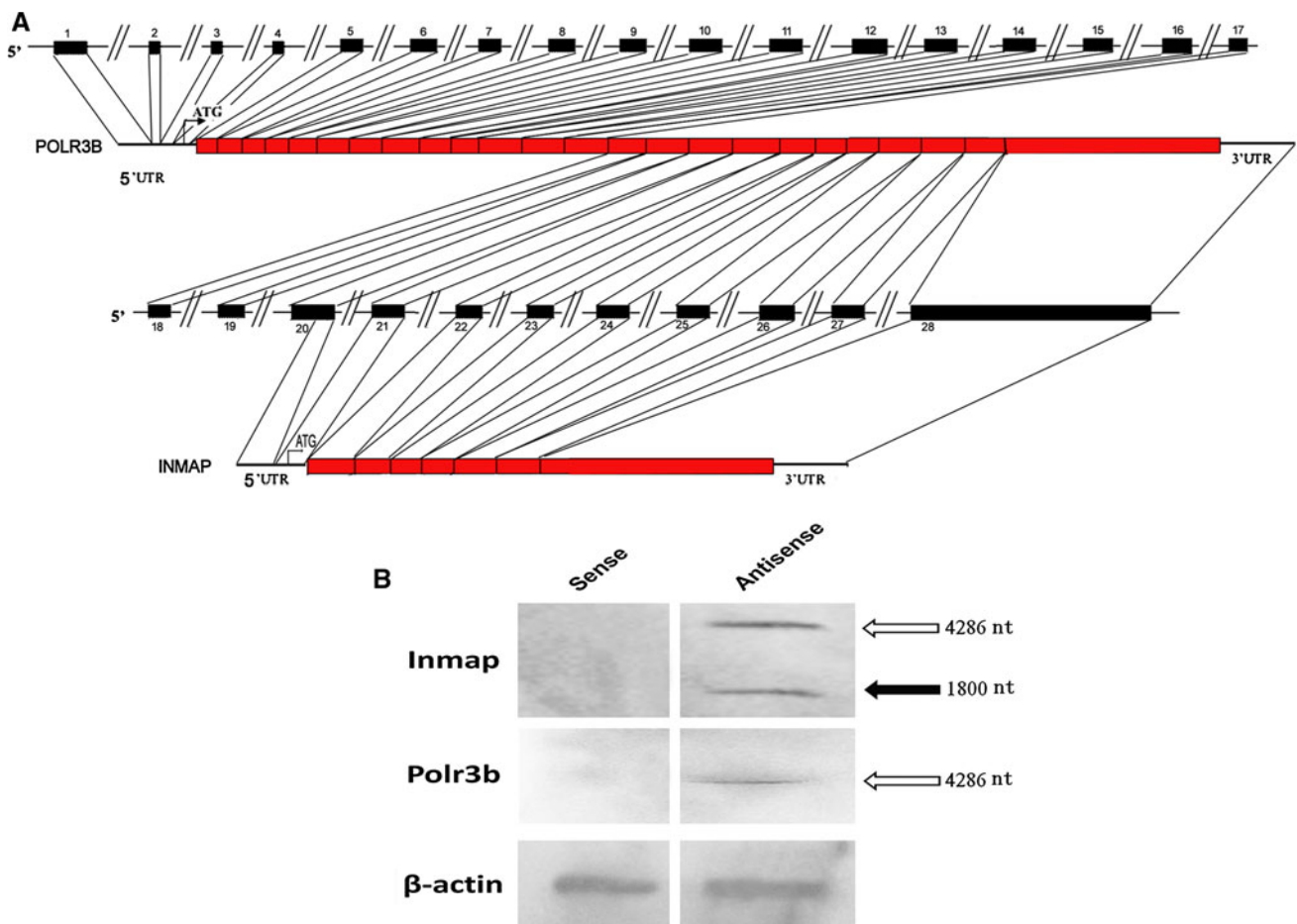
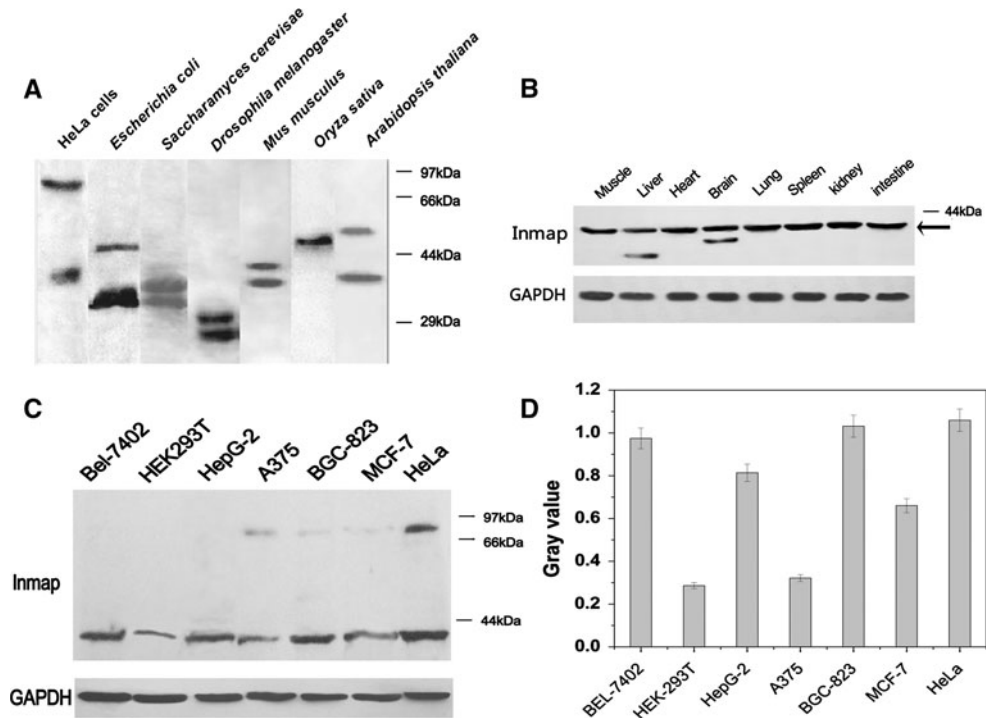


Fig. 1 Northern blot analysis of *POLR3B* and *INMAP* transcripts. **a** Genomic structure of *INMAP*. The exons are depicted by *black* boxes. The intron–exon organizations of *POLR3B* and *INMAP* are

shown. ATG indicates the translation start site. **b** Northern blot analysis of *INMAP* and *POLR3B* mRNAs in HeLa cells

Fig. 2 An INMAP-specific monoclonal antibody detects strong signals in a wide range of organisms. **a** Western blot analysis of total protein extracts from various organisms as indicated using an INMAP-specific monoclonal antibody. **b** Western blot analysis of INMAP protein expression in multiple mouse tissues. GAPDH was used as a loading control. **c** Western blot analysis of INMAP protein expression in several cell lines. GAPDH was used as a loading control. **d** Band quantification of Fig. 2c



Western blot analysis

Protein concentrations of cultured cells and mouse tissues were determined using the Bradford protein assay with bovine serum albumin (BSA) as the standard. Protein extracts were separated in 12 % SDS–polyacrylamide electrophoresis (SDS-PAGE) gels and electrotransferred onto nitrocellulose membranes. The membranes were blocked in 5 % fat-free milk and processed by incubation with primary and HRP-conjugated secondary antibodies. Protein bands were visualised using ECL and Kodak BioMix films.

Statistical analysis

The results are expressed as the mean \pm standard deviation (SD). Analysis of variance was performed using *t*-tests.

Results

INMAP, a novel truncated version of POLR3B

We previously reported that *INMAP* gene is highly homologous to the 3'-terminal sequence of *POLR3B* [37]. Figure 1a shows the proposed genomic organization of *INMAP* gene. Comparison of *POLR3B* genomic sequence and the cDNA sequence of *INMAP* revealed that the *INMAP* cDNA begins at a site within the 20th exon of *POLR3B*. All exon/intron boundaries followed the classical GT/AG rule (data not shown). Translation from an ATG

codon in the 21st exon of *INMAP* generates a protein of 343 amino acids corresponding to the region between amino acids 791 and 1,133 of *POLR3B*. Human genomic BLAST shows that *INMAP* is mapped to chromosome 12 (in the region of *POLR3B* at 12q23.3). As the use of an alternative promoter within the coding sequence of p53 has been shown to yield a truncated protein Δ 113p53 [40], we wanted to determine whether two transcripts exist that encode *POLR3B* and *INMAP*, respectively. To this end, we conducted Northern blot analysis of mRNAs prepared from HeLa cells using digoxigenin-labelled *INMAP* probe. As shown in Fig. 1b, we indeed detected two distinct bands with estimated sizes of 4,286 and 1,800 bases, sufficiently large to encode *POLR3B* and *INMAP*, respectively. In comparison, the digoxigenin-labelled *POLR3B* probe complementary to the 5'-terminal sequence of *POLR3B* detected only the upper band. Thus, the results suggest that the *INMAP* transcript is a novel truncated version of *POLR3B* probably transcribed from a promoter within the coding sequence of *POLR3B*.

Evolutionary conservation of INMAP

We have previously reported that *INMAP* is expressed in HeLa cells, murine erythroleukemia cells and the budding yeast [37]. Here, we performed Western blotting analysis of total proteins from HeLa cells, *Escherichia coli*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Mus musculus*, *Oryza sativa* and *Arabidopsis thaliana* with an *INMAP*-specific monoclonal antibody [37]. The result revealed the

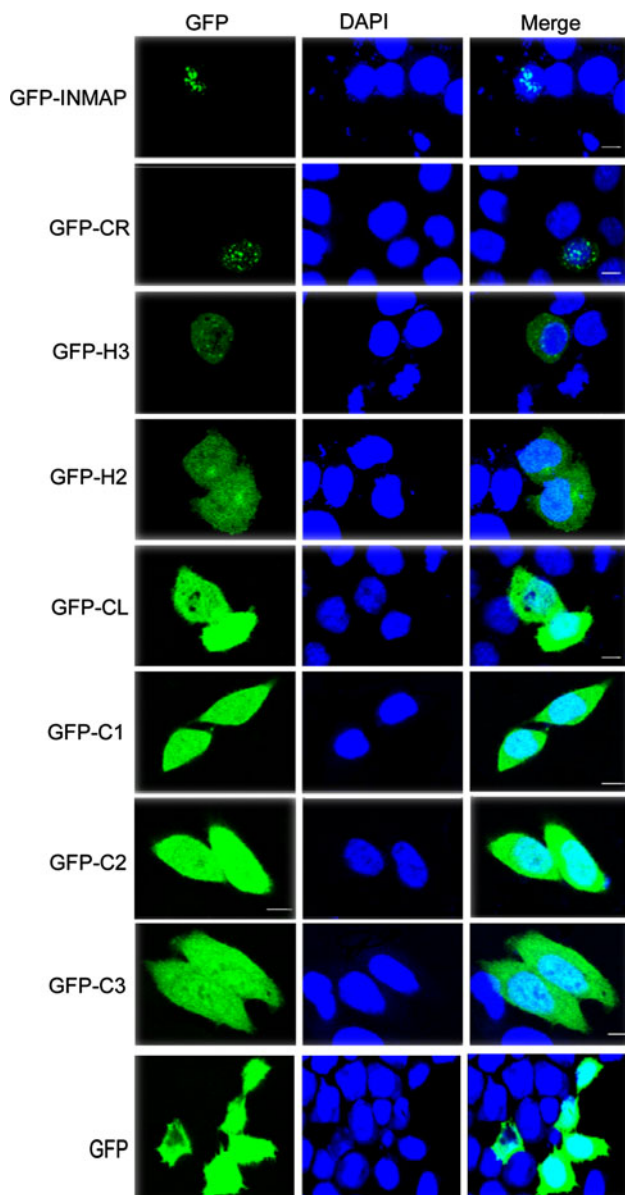


Fig. 3 Subcellular localisation of INMAP and various truncated versions of INMAP. Constructs expressing INMAP or various truncated versions of INMAP with a GFP tag were individually transfected into HeLa cells. The cells were counterstained with DAPI to label the DNA and examined by confocal microscopy

presence of immunoreactive polypeptides in all the seven species with molecular weights ranging from ~27 to 50 kDa (Fig. 2a). As shown, two bands were detected in all the species except *O. sativa*. In addition, the result suggests that INMAP might be evolutionarily conserved. Furthermore, Western blot analysis of eight tissues from adult mice detected a ~40 kDa band, matching the predicted molecular weight of INMAP, in skeletal muscle, heart, lung, spleen, kidney, intestine, liver and brain tissues, and an additional smaller band in the liver and brain (Fig. 2b). We also found that INMAP is expressed in the normal cell line HEK293T with a single ~40 kDa band as

well as in the liver cancer cell lines HepG-2 and Bel-7402 (Fig. 2c). As shown previously, the HeLa cell protein extract showed two distinct bands at ~40 and 80 kDa, respectively. The same two bands were also observed in A375, BGC-823 and MCF-7 cancer cell lines. The distinct appearance of the 80 kDa INMAP variant in cancer cells suggests a possible link to tumourigenesis. Furthermore, we found significantly elevated cellular levels of INMAP in HepG-2, Bel-7402, BGC-823, HeLa and MCF-7 cancer cell lines compared with normal cell lines after normalisation to GAPDH as a loading control (Fig. 2d), suggesting that up-regulation of INMAP may contribute to carcinogenesis.

Cellular localisation of INMAP in HeLa cells

By Western blot analysis of nuclear and cytoplasm fractions of cells, we have found that INMAP is primarily a nuclear protein during the interphase (Fig. S1). And our previous deletion analysis revealed that the C-terminal part of INMAP is responsible for its nuclear localisation [37]. Here, we constructed a series of C-terminally truncated versions of INMAP based on bioinformatics analysis of the domain organization (Fig. S2A). Using PCR with oligonucleotide primers (Table S1) and pEGFP-C3-INMAP template, we created plasmids to express seven fragments of INMAP (named H3, H2, C1, C2, C3, CR and CL) corresponding to peptides 209–294, 209–270, 101–200, 181–265, 244–343, 101–265 and 181–343 aa, respectively. Each of the fragments was ligated into the expression vector pEGFP-C3 for expression as a GFP fusion protein. The recombinant vectors were then transiently transfected into HeLa cells. As shown in Fig. 3, only the cells expressing either CR or H3 exhibited the typical dots in the interphase nucleus. By contrast, the other five fragments were expressed with diffuse GFP signals throughout both the nucleus and the cytoplasm similar to the localization pattern in the control cells expressing GFP alone. Together, the results indicate that the H3 domain, which is relatively shorter and contains three α -helices (Fig. S2), carries the signals required for the specific localisation of INMAP in the nucleus. It is necessary to be indicated that H2 in fact displays dotted signal but is concealed by diffuse signal, that is to say, inappropriate length may produce poor signal–noise ratio (Fig. S2B). Does it mean that a third α -helix, as in H3 or CR, enhances more specificity? Deeper investigation is required.

Over-expression of INMAP inhibits p53 and AP-1 transcriptional activities in HEK293T cells

As abnormal RNA Pol III activity is a feature of cancer cells in which p53 is inactive [41, 42], we wanted to determine whether over-expression of INMAP affects p53 regulated

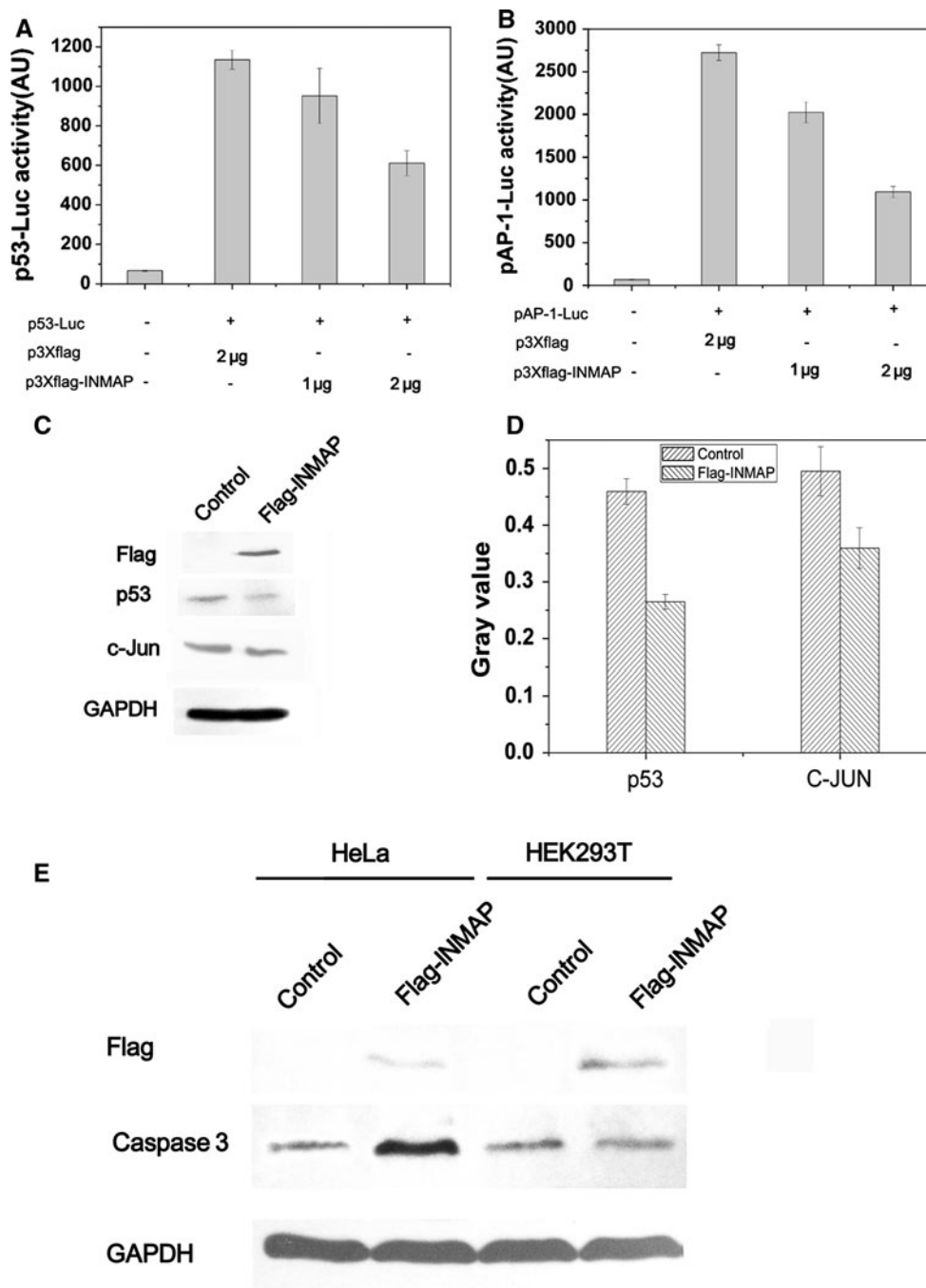


Fig. 4 Over-expression of INMAP suppressed the transcriptional activities of p53 and AP-1. (A, B) 48 h after transient transfection, the luciferase activity assay was performed according to the manufacturer's instruction. The data are the means of three repeats in a single transfection experiment after normalisation for β -galactosidase activity. **a** p53-Luc was co-transfected without or with 3XFLAG-INMAP fusion protein in varying doses (1, 2 μ g). **b** pAP-1-Luc was co-transfected without or with 3XFLAG-INMAP fusion protein in varying doses (1, 2 μ g). **c** Western blot of cell lysates of transfected

HEK293T cells. The membranes were probed with anti-FLAG antibody, anti-p53 antibody and anti-c-Jun antibody, respectively. GAPDH was used as a loading control. **d** Band quantification of Fig. 4c. **e** Western blot of cell lysates of transfected HeLa and HEK293T cells. The membranes were probed with anti-FLAG antibody and anti-caspase-3 antibody, respectively. GAPDH was used as a loading control. All experiments were performed in triplicate and repeated three times. The data presented are representative

genes. First, we performed pathway-specific reporter gene assays to measure the modulation of p53 by INMAP in HEK293T cells. A fixed amount of p53-Luc and varying

amounts of p3XFLAG-CMV-INMAP were co-transfected into HEK293T cells, and the total amount of DNA was equalised by adding the vector p3XFLAG-CMV. Figure 4a

and Fig. S3A show that INMAP suppressed the dose-dependent activation of the p53 reporter gene ($P < 0.05$). As the transcription factor AP-1 is known to regulate p53 expression [21–27], we next tested the effect of INMAP on the activity of AP-1 and found that the expression of INMAP significantly inhibited AP-1 activity in a dose-dependent manner (Fig. 4b and Fig. S3B). In addition, Western blot analysis showed that over-expression of INMAP caused down-regulation of p53 and c-Jun proteins in HEK293T cells (Fig. 4c, d). As shown in Fig. 4e, caspases-3, a preapoptotic signalling cascade protein, is up-regulated in HeLa cells with over-expression of INMAP. While, over-expression of INMAP in HEK293T cells does not influence on expression of caspase-3. Together, our data demonstrate that over-expression of INMAP inhibited the transcriptional activities of p53 and AP-1.

Discussion

AP-1 and p53 are tumour suppressors that may induce cell-cycle arrest or apoptosis in response to environmental duress [43–45]. Cancers generally evade this defence by inactivating p53 [46]. p53 can activate the c-Jun NH2-terminal kinase (JNK) pathway [47], and AP-1 has been linked to the modulation of p53 pathways to explain the role of AP-1 in cell survival [48]. Recently, it has been reported that MKK7, the upstream activator of JNK, functions as a major tumour suppressor in lung and mammary cancers in mouse through the kinases JNK1 and JNK2, and this signalling pathway directly combines oncogenic and genotoxic stress to the stability of p53 [49]. Besides, the growth arrest DNA damage-inducible gene 45 β (GADD45 β) which binds tightly to MKK7 forms a homodimeric complex and it does not disrupt the effect of MKK7 [50, 51]. Abnormal RNA Pol III activity is a feature of cancer cells in which p53 is inactive [41, 42]. Thus, functional interaction between p53 and RNA Pol III may play an important role in the control of cell growth and, perhaps, tumour formation. Our results suggest that INMAP, as a sort of ‘abnormal RNA Pol III subunit’, may participate in the transcriptional regulation of certain genes. Our unpublished finding indicated that transfection with the anti-sense INMAP resulted in cell death, which may be due to the inactivation of the mRNAs for both POLR3B and INMAP. Perhaps more work should be performed to distinguish and discern the actually functional role of both POLR3B and INMAP protein on cell-cycle or tumour formation. In addition, our observation of the up-regulation of INMAP in several cancer cell lines suggests a possible role for INMAP in cancer. This hypothesis is consistent with the dose-dependent suppression of the transcriptional activity of AP-1 and p53 by INMAP

over-expression in HEK293T cells (Fig. 4). Furthermore, we found that the cellular levels of p53 and c-Jun proteins were lower in cells that over-expressed INMAP than in control cells. Finally, we have found that the different function of INMAP on cell apoptosis may be related with dissimilar cells. Therefore, INMAP may play a role in the signal transduction through AP-1 and p53 which may ultimately affect the cell-cycle progression, leading to tumorigenesis or apoptosis.

We have previously reported the association of INMAP with α -tubulin, γ -tubulin and NuMA, suggesting a role for INMAP in the formation and stabilization of the mitotic spindle [37]. The inhibition of p53 by INMAP over-expression is consistent with the reports that abnormal POLR3B is a feature of cancer cells in which p53 is inactive [41, 42], conforming to the association of the AP-1-p53 pathway with carcinogenesis [52, 53].

Acknowledgments This study was supported by the National Natural Science Foundation of China (no. 30971470) to Q. J. Liang, Beijing Natural Science Foundation (no. 5122017) to Q. J. Liang, the open fund of Key Laboratory of Cell Proliferation and Regulation Biology, Ministry of Education to Y. B. Zheng and the open fund of Beijing Key Laboratory of Gene Resource and Molecular Development to Y. L. Zhou. We thank Dr. Yue Wang (A-STAR, Singapore) and Dr. Jian Kuang (University of Texas M.D., Anderson Cancer Center, USA) for critical reading.

References

1. Robert G, William J (1969) Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature* 224:235
2. Roeder RG (2003) The eukaryotic transcriptional machinery: complexities and mechanisms unforeseen. *Nat Med* 9:1239–1244
3. Geiduschek EP, Kassavetis GA (2001) The RNA polymerase III transcription apparatus. *J Mol Biol* 310:1–26
4. Paule MR, White RJ (2000) Survey and summary transcription by RNA polymerases I and III. *Nucleic Acids Res* 28:1283–1298
5. Cairns CA, White RJ (1998) p53 is a general repressor of RNA polymerase III transcription. *EMBO J* 17:3112–3123
6. Veras I, Rosen EM, Schramm L (2009) Inhibition of RNA polymerase III transcription by BRCA1. *J Mol Biol* 387:523–531
7. Goodfellow SJ, Graham EL, Kantidakis T, Marshall L, Coppins BA, Oficjalska-Pham D, Gérard M, Lefebvre O, White RJ (2008) Regulation of RNA polymerase III transcription by Maf1 in mammalian cells. *J Mol Biol* 378:481–491
8. Jacob J, Cabarcas S, Veras I, Zaveri N, Schramm L (2007) The green tea component EGCG inhibits RNA polymerase III transcription. *Biochem Biophys Res Commun* 360:778–783
9. Tétreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, Fribourg S, Schiffmann R, Brais B, Vanderver A, Bernard G (2011) Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. *Am J Hum Genet* 89:652–655
10. Yee NS, Gong W, Huang Y, Lorent K, Dolan AC, Maraia RJ, Pack M (2007) Mutation of RNA Pol III subunit *rpc2/polr3b* leads to deficiency of subunit Rpc11 and disrupts zebrafish digestive development. *PLoS Biol* 5:e312
11. Karin M, Liu Z, Zandi E (1997) AP-1 function and regulation. *Curr Opin Cell Biol* 9:240–246

12. Thépot D, Weitzman JB, Barra J, Segretain D, Stinnakre MG, Babinet C, Yaniv M (2000) Targeted disruption of the murine junD gene results in multiple defects in male reproductive function. *Development* 127:143–153
13. Jochum W, Passequé E, Wagner EF (2001) AP-1 in mouse development and tumorigenesis. *Oncogene* 20:2401–2412
14. Behrens A, Sibilila M, Wagner EF (1999) Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat Genet* 21:326–329
15. Kovary K, Bravo R (1991) The jun and fos protein families are both required for cell cycle progression in fibroblasts. *Mol Cell Biol* 11:4466–4472
16. Wang H, Xie Z, Scott RE (1997) Induction of AP-1 activity associated with c-Jun and JunB is required for mitogenesis induced by insulin and vanadate in SV40-transformed 3T3T cells. *Mol Cell Biochem* 168:21–30
17. Brown JR, Nigh E, Lee RJ, Ye H, Thompson MA, Saudou F, Pestell RG, Greenberg ME (1998) Fos family members induce cell cycle entry by activating cyclin D1. *Mol Cell Biol* 18:5609–5619
18. Schreiber M, Kolbus A, Piu F, Szabowski A, Möhle-Steinlein U, Tian J, Karin M, Angel P, Wagner EF (1999) Control of cell cycle progression by c-Jun is p53 dependent. *Genes Dev* 13:607–619
19. Hennigan RF, Stambrook PJ (2001) Dominant negative c-jun inhibits activation of the cyclin D1 and cyclin E kinase complexes. *Mol Biol Cell* 12:2352–2363
20. Xiong Y, Hannon G, Zhang H, Casso D, Kobayashi R, Beach D (1993) p21 is a universal inhibitor of cyclin kinases. *Nature* 366:701–704
21. Ameyar-Zazoua M, Wisniewska MB, Bakiri L, Wagner EF, Yaniv M, Weitzman JB (2005) AP-1 dimers regulate transcription of the p14/p19ARF tumor suppressor gene. *Oncogene* 24:2298–2306
22. Bakiri L, Lallemand D, Bossy-Wetzel E, Yaniv M (2000) Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1 expression. *EMBO J* 19:2056–2068
23. Passegue E, Wagner EF (2000) JunB suppresses cell proliferation by transcriptional activation of p16INK4a expression. *EMBO J* 19:2969–2979
24. Schmitt CA, Fridman JS, Yang M, Baranov E, Hoffman RM, Lowe SW (2002) Dissecting p53 tumor suppressor functions in vivo. *Cancer Cell* 1:289–298
25. Sherr CJ, McCormick F (2002) The RB and p53 pathways in cancer. *Cancer Cell* 2:103–112
26. Gasco M, Shami S, Crook T (2002) The p53 pathway in breast cancer. *Breast Cancer Res* 4:70–76
27. Eischen CM, Weber JD, Roussel MF, Sherr CJ, Cleveland JL (1999) Disruption of the ARF–Mdm2–p53 tumor suppressor pathway in Myc-induced lymphomagenesis. *Gene Dev* 13:2658–2669
28. Leach FS, Tokino T, Meltzer P, Burrell M, Oliner JD, Smith S, Hill DE, Sidransky D, Kinzler KW, Vogelstein B (1993) p53 Mutation and MDM2 amplification in human soft tissue sarcomas. *Cancer Res* 53:2231–2234
29. Bodner S, Minna J, Jensen S, D'amico D, Carbone D, Mitsudomi T, Fedorko J, Buchhagen D, Nau M, Gazdar A (1992) Expression of mutant p53 proteins in lung cancer correlates with the class of p53 gene mutation. *Oncogene* 7:743–749
30. Reifenberger J, Ring GU, Gies U, Cobbers L, Oberstrass J, An HX, Niederacher D, Wechsler W, Reifenberger G (1996) Analysis of p53 mutation and epidermal growth factor receptor amplification in recurrent gliomas with malignant progression. *J Neuropathol Exp Neurol* 55:822–831
31. Tornaletti S, Pfeifer GP (1994) Slow repair of pyrimidine dimers at p53 mutation hotspots in skin cancer. *Science* 263:1436–1438
32. Hamelin R, Laurent-Puig P, Olschwang S, Jego N, Asselain B, Remykov Y, Girodet J, Salmon R, Thomas G (1994) Association of p53 mutations with short survival in colorectal cancer. *Gastroenterology* 106:42–48
33. Tolbert D, Lu X, Yin C, Tantama M, Van Dyke T (2002) p19ARF is dispensable for oncogenic stress-induced p53-mediated apoptosis and tumor suppression in vivo. *Mol Cell Biol* 22:370–377
34. Lomazzi M, Moroni MC, Jensen MR, Frittoli E, Helin K (2002) Suppression of the p53- or pRB-mediated G1 checkpoint is required for E2F-induced S-phase entry. *Nat Genet* 31:190–194
35. Schlereth K, Beinoraviciute-Kellner R, Zeitlinger MK, Bretz AC, Sauer M, Charles JP, Vogiatzi F, Leich E, Samans B, Eilers M (2010) DNA binding cooperativity of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol Cell* 38:356–368
36. Goloudina A, Mazur S, Appella E, Garrido C, Demidov O (2012) Wip1 sensitizes p53-negative tumors to apoptosis by regulating the Bax/Bcl-xL ratio. *Cell Cycle* 11:1883–1887
37. Shen E, Lei Y, Liu Q, Zheng Y, Song C, Marc J, Wang Y, Sun L, Liang Q (2009) Identification and characterization of INMAP, a novel interphase nucleus and mitotic apparatus protein that is involved in spindle formation and cell cycle progression. *Exp Cell Res* 315:1100–1116
38. Peterhaensel C, Obermaier I, Rueger B (1998) Nonradioactive northern blot analysis of plant RNA and the application of different haptens for reprobing. *Anal Biochem* 264:279–283
39. Wei Y, Shen E, Zhao N, Liu Q, Fan J, Marc J, Wang Y, Sun L, Liang Q (2008) Identification of a novel centrosomal protein CrpF46 involved in cell cycle progression and mitosis. *Exp Cell Res* 314:1693–1707
40. Chen J, Ng SM, Chang C, Zhang Z, Bourdon J-C, Lane DP, Peng J (2009) p53 isoform $\Delta 113p53$ is a p53 target gene that antagonizes p53 apoptotic activity via BclxL activation in zebrafish. *Genes Dev* 23:278–290
41. White RJ (2004) RNA polymerase III transcription and cancer. *Oncogene* 23:3208–3216
42. Marshall L, White RJ (2008) Non-coding RNA production by RNA polymerase III is implicated in cancer. *Nat Rev Cancer* 8:911–914
43. Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. *Nature* 408:307–310
44. Lee W-T, Chang C-W (2010) Bax is upregulated by p53 signal pathway in the SPE B-induced apoptosis. *Mol Cell Biochem* 343:271–279
45. Jee Y-H, Jeong W-I, Kim T-H, Hwang I-S, Ahn M-J, Joo H-G, Hong S-H, Jeong K-S (2005) p53 and cell-cycle-regulated protein expression in small intestinal cells after fast-neutron irradiation in mice. *Mol Cell Biochem* 270:21–28
46. Vousden KH, Lu X (2002) Live or let die: the cell's response to p53. *Nat Rev Cancer* 2:594–604
47. Miled C, Pontoglio M, Garbay S, Yaniv M, Weitzman JB (2005) A genomic map of p53 binding sites identifies novel p53 targets involved in an apoptotic network. *Cancer Res* 65:5096–5104
48. Verde P, Casalino L, Talotta F, Yaniv M, Weitzman JB (2007) Deciphering AP-1 function in tumorigenesis. *Cell Cycle* 6:2632–2639
49. Schramek D, Kotsinas A, Meixner A, Wada T, Elling U, Pospisilik JA, Neely GG, Zwick RH, Sigl V, Forni G (2011) The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression. *Nat Genet* 43:212–219
50. Tornatore L, Marasco D, Dathan N, Vitale RM, Benedetti E, Papa S, Franzoso G, Ruvo M, Monti SM (2008) Gadd45 β forms a homodimeric complex that binds tightly to MKK7. *J Mol Biol* 378:97–111

51. Tornatore L, Monti SM, Marasco D, Dathan N, Vitale RM, Benedetti E, Pedone C, Papa S, Franzoso G, Ruvo M (2009) Gadd45 β dimerization does not affect MKK7 binding. *Peptides for Youth* 367–368
52. Mahata S, Bharti AC, Shukla S, Tyagi A, Husain SA, Das BC (2011) Berberine modulates AP-1 activity to suppress HPV transcription and downstream signaling to induce growth arrest and apoptosis in cervical cancer cells. *Mol Cancer* 10:39
53. Rajput S, Mandal M (2012) Antitumor promoting potential of selected phytochemicals derived from spices: a review. *Eur J Cancer Prev* 21:205–215