ALKBH3, a human AlkB homologue, contributes to cell survival in human non-small-cell lung cancer

M Tasaki*1, K Shimada2, H Kimura1, K Tsujikawa3 and N Konishi2

1Second Department of Internal Medicine, Nara Medical University School of Medicine, Shijo-cho, Kashihara, Nara 634-8521, Japan; 2Department of Pathology, Nara Medical University School of Medicine, Shijo-cho, Kashihara, Nara, Japan; 3Department of Immunology, Osaka University, Graduate School and School of Pharmaceutical Sciences, Yamadaoka, Suita, Osaka, Japan

BACKGROUND: We have demonstrated for the first time that a novel human AlkB homologue, ALKBH3, contributes to prostate cancer development, but its clinical and biological roles in lung cancer remain unclear.

METHODS: Expression of both mRNA and protein of PCA-1 was examined by RT–PCR and western blotting. We also assessed association with senescence and in vivo ALKBH3 treatment on orthotopic tumour cell inoculation, and analysed it clinicopathologically.

RESULTS: We have since found novel biological roles for ALKBH3 in human lung cancers, particularly in adenocarcinoma. Our immunohistochemical analysis of human adenocarcinomas and squamous cell carcinomas of the lung not only showed overexpression of ALKBH3 in these tumours but the percentage of cells positive for ALKBH3 also correlated statistically to recurrence-free survival in adenocarcinoma. Knockdown of ALKBH3 by siRNA transfection induced expression of p21WAF1/Cip1 and p27Kip1 in the human lung adenocarcinoma cell line A549, resulting in cell cycle arrest, senescence and strong suppression of cell growth in vitro. In vivo, peritoneal tumour growth and dissemination was inhibited in nude mice, previously inoculated with the A549 cell line, by intraperitoneal injection of ALKBH3 siRNA + atelocollagen, as demonstrated by the reduction in both number and diameter of tumours developing in the peritoneum.

CONCLUSION: We suggest that ALKBH3 contributes significantly to cancer cell survival and may be a therapeutic target for human adenocarcinoma of the lung.

Keywords: lung cancer; adenocarcinoma; ALKBH3; senescence

A number of investigators are engaged in examinations of the clinical, pathological and biological characteristics of both small-cell and non-small-cell lung cancers (NSCLC); yet more than 60,000 people die of lung cancer every year and the 5-year survival rate for patients with the disease remains about only 15% (Alberg et al, 2007; Toyota et al, 2008). Adenocarcinoma accounts for approximately 70% of NSCLC, and the recent increases in the number of cases indicate an urgent need to develop not only better treatment but also improve methods of early diagnosis. The epidermal growth factor receptor (EGFR) has recently been scrutinised as a potential target in lung adenocarcinoma therapy because of its overexpression in and association with poor prognosis of many solid tumours (Meert et al, 2002; Hirsch et al, 2003). The development of EGFR inhibitors gefitinib and erlotinib initially showed dramatic effects in the treatment of lung adenocarcinoma; however, tumours frequently acquire resistance to the drugs, resulting in treatment failure (Sharma et al, 2007; Linardou et al, 2002; Hirsch et al, 2005).

DNA alkylation damage repair mechanisms are known to be controlled by six genes (tag, ogt, ada, alka, aidB and alkB). Among them, ada, alka, aidB and alkB are induced on exposure to a sublethal dose of alkylating agents, called the adaptive response (Sedgwick and Lindahl, 2002). In Escherichia coli, the alkB gene product was identified as protein to carry out DNA repair by oxidative demethylation (Kataoka et al, 1983; Dinglay et al, 2000; Falnes et al, 2002; Trewick et al, 2002; Aas et al, 2003; Sedgwick et al, 2007) and repairs both DNA and RNA methylation (Falnes and Rognes, 2003; Falnes et al, 2007). Among eight AlkB homologues (ALKBH), designated hABH1 to hABH8, have since been identified in human tissues (Tsujikawa et al, 2007). In previous studies on prostate cancer conducted in our lab, we isolated a highly expressed protein that we originally designated as prostate cancer antigen-1 (PCA-1) (Konishi et al, 2005). We further characterised this protein in terms of its effects on prostate cancer cell survival and invasion through modulation of the discoidin domain receptor 1 (DDR1) (Di Marco et al, 1993; Vogel, 1999; Curat and Vogel, 2002; Ongusaha et al, 2003; Shimada et al, 2008). AlkB homologue-3 thus seems to participate in a wide range of biological functions involving survival and invasion of cancer cells.

In this study, we found not only overexpression of ALKBH3 in lung adenocarcinoma cells but also a correlation between expression profile and recurrence-free survival (RFS). In addition, ALKBH3 silencing through siRNA transfection effectively induced cellular senescence and growth suppression of lung adenocarcinoma cells both in vivo and in vitro. AlkB homologue-3 may thus join EGFR as both a new molecular target in cancer therapeutics.
and as another useful clinicopathological marker in the management of human non-small-cell lung cancer.

MATERIALS AND METHODS

Cell culture
The non-small-cell lung cancer cell line A549, originating from a human lung adenocarcinoma, and RERF-LC-AI, originating from a human squamous cell carcinoma, were purchased from RIKEN Bio Resource Center (Tsukuba, Ibaraki, Japan) and cultured in RPMI supplemented with 10% fetal bovine serum.

Antibodies and preparation of antisera
Antibodies to caspase-3 were supplied by PharMingen (San Diego, CA, USA), those to caspase-8 by Medical and Biological Laboratories Co., Ltd. (Nagoya, Japan), to caspase-9 by Cell Signaling Technology (Cambridge, MA, USA) and to actin by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-ALKBH3 antisera were raised in rabbits against the synthetic peptide of ALKBH3 (peptide sequence NKQKSKYLRGNRNS) as an antigen. Aliquots of 0.5 mg peptide were emulsified in equal volumes of Freund’s Complete Adjuvant and injected s.c. at several sites into each rabbit. Antiserum was prepared and the relative reactivity of the antisera evaluated against the synthetic peptide by ELISA; those antisera showing high titres were affinity-purified using SulfoLink (Pierce Biotech, Rockford, IL, USA).

Preparation of cell lysates and western blotting analysis
We resolved the cell lysates from A549 in SDS polyacrylamide gels and transferred them onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA), which were blocked in 5% skimmed milk at room temperature for 1 h. The membranes were then incubated with each of the antibodies described in the previous section for 1 h, followed by incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (Amersham Pharmacia Biotech, Piscataway, NJ, USA). We detected peroxidase activity on X-ray films using an enhanced chemiluminescence detection system.

siRNA transfection of ALKBH3
Transfections were carried out using the Lipofectamine system (Invitrogen, Tokyo, Japan) in accordance with the manufacturer’s protocol. We seeded 2 x 10^6 cells from each lung cancer cell line in 60 mm dish plates and transfected them with either 100 nmol of control RNA (Santa Cruz Biotechnology) or ALKBH3 siRNA. AlkB homologue-3 siRNA duplexes, generated with 3′-dTdT overhangs and prepared by Qiagen (Tokyo, Japan), were chosen against the following DNA target sequences for ALKBH3: 5′-TACCAGTGCTAAGAGCCATCTCC-3′ and 5′-ACCTGCTGAGGTCTTGTGAACAC-3′.

Tissue samples and immunohistochemistry
We obtained 86 specimens of human lung adenocarcinoma and 46 specimens of lung squamous cell carcinoma from patients at Nara Medical University Hospital. All patients provided informed consent before collection of specimens. Some patients received post-operative chemotherapy; however, no alkylating reagents such as cyclophosphamide, ifosfamide, melphanal and busulfan were administered.

The sections were incubated with the primary antibodies to ALKBH3 at 1:100 dilution for 16 h at 4 °C and the reactions were visualised using a Histofine kit (Nichirei, Tokyo, Japan) with aHistofine kit (Nichirei, Tokyo, Japan). We performed Western blotting analysis to further investigate the expression of ALKBH3.

Reverse transcription – PCR
Using the OneStep RT – PCR kit (Qiagen), we extracted total RNA from the homogenised A549 cell line using Trizol reagent and subjected it to reverse transcription – PCR (RT – PCR). PCR conditions were as follows: 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min, for a total of 35 cycles.

Table 1: Clinicopathologic characteristics in lung adenocarcinoma and squamous cell carcinoma

| Gender       | Adenocarcina | Squamous cell carcinoma |
|--------------|--------------|-------------------------|
| Male         | 45           | 39                      |
| Female       | 41           | 7                       |

| Age (years) | Adenocarcina | Squamous cell carcinoma |
|-------------|--------------|-------------------------|
| <70         | 41           | 12                      |
| 70 ≤        | 45           | 34                      |

| Stage | Adenocarcina | Squamous cell carcinoma |
|-------|--------------|-------------------------|
| I     | 46           | 22                      |
| II    | 21           | 12                      |
| III   | 5            | 6                       |
| IV    | 3            | 5                       |
| V     | 11           | 1                       |
| VI    | 0            | 0                       |
| Total | 86           | 46                      |

| Vascular invasion | Adenocarcina | Squamous cell carcinoma |
|-------------------|--------------|-------------------------|
| (+)               | 26           | 8                       |
| (−)               | 60           | 38                      |

| P53 factor | Adenocarcina | Squamous cell carcinoma |
|------------|--------------|-------------------------|
| (+)        | 21           | 14                      |
| (−)        | 65           | 32                      |

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were 95°C for 30 s, 55–60°C for 30 s and 72°C for 1 min through a total of 30 cycles. The PCR primer sequences for ALKBH3 were 5'-AGATGTACTGGTTCCCTGGC-3' (sense) and 5'-CCTCACCGGAACACATGGTAG-3' (antisense). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the primers used were 5'-ACCACAGTCCATGCCATCAC-3' (sense) and 5'-TCCACCACCTGTTGCTGTA-3' (antisense). The PCR products were analysed on 1.5% agarose gel and visualised by ethidium bromide staining.

Statistical analysis
Data were statistically analysed using the Student t-test or, for non-parametric analysis, the Kruskal–Wallis test. Survival analyses for biochemical recurrence were evaluated using the Kaplan–Meier method and the log-rank test. Results were considered significant at P < 0.05.

RESULTS
Expression of ALKBH3 in human NSCLC
Before we began the larger study, we initially performed a limited survey of the expression profile of ALKBH3 using four samples each of small-cell lung cancer, adenocarcinoma and squamous cell carcinoma (Figure 1). Immunohistochemical results showed that ALKBH3 was highly expressed in 75% of both adenocarcinoma and squamous cell carcinoma samples, but expressed to a lesser degree in only 25% of small-cell carcinomas. On the basis of these initial results, we examined the relationship between ALKBH3 expression and selected clinicopathological parameters in 132 surgical specimens of NSCLC, comprised of 86 human lung adenocarcinomas and 46 squamous cell carcinomas, in more detail (Table 1). Of those specimens, 50% of adenocarcinomas and 56.5% of squamous cell tumours demonstrated >30% of cells immunopositive for ALKBH3 (Figures 2A and B). In lung adenocarcinoma only, ALKBH3 positivity was also statistically associated with recurrence-free survival and with factors such as gender, tumour stage and degree of pleural invasion (P-factor) (Figure 2C); these associations did not hold for squamous cell carcinoma (data not shown).

ALKBH gene silencing and cell survival
At the beginning of the experiment, we evaluated RNA expression of ALKBH3 by real-time RT–PCR analysis as demonstrated in the following figures, but the results were not completely consistent with immunohistochemical data. AlkB homologue-3 protein in human lung-cancer cells may be stabilised by a posttranscriptional and/or post-translational mechanism including ubiquitin–proteasome signals. To confirm, we checked whether ALKBH3 was downregulated by siRNA transfection by both RT–PCR and western blotting in this study.

In the human lung adenocarcinoma cell line A549 RT–PCR and western blotting data showed that ALKBH3 gene expression was significantly reduced by transfection with 100 nM siRNA (Qiagen) for 72 h (Figure 3A). As demonstrated in Figure 3B and C, ALKBH3 gene silencing induced cell cycle arrest at the G1 phase, resulting in inhibition of cell growth.

Gene silencing through siRNA transfection: senescence and apoptosis
Cell cycle arrest is known to induce cytotoxicity, including cellular senescence and apoptosis. After silencing ALKBH3 by siRNA transfection, A549 cells were found to be senescent using the cellular senescence marker, SA-β-gal (Figure 4A), and we detected induction of p27 and p21 in a time-dependent manner (Figure 4B); however, apoptosis was not induced as evidenced by propidium iodide staining, and cleavage in caspases 3, 8 and 9 were not observed after ALKBH3 gene silencing (data not shown). It therefore appears that, in human lung adenocarcinoma cells, ALKBH3 knockdown inhibits cell survival, presumably through p21/p27-mediated cell cycle arrest at G1, followed by cellular senescence.

In vivo effects of ALKBH3 gene silencing on tumour growth
To study the effects of ALKBH3 gene silencing in vivo, we constructed an animal model of intraperitoneal inoculation of A549 and RERF-LC-AI cells using nude mice. At 7 and 14 days after intraperitoneal injection of cancer cells, control siRNA or ALKBH3 siRNA was intraperitoneally injected in the presence of...
atelocollagen as described in Materials and Methods. After 28 days, all mice were killed and tumour masses on/in the peritoneum and liver were removed and measured (Figures 5A and B). As shown in Figure 5C, the numbers of tumours formed in the peritoneum in our nude mouse model were significantly decreased in mice receiving ALKBH3 siRNA compared with mice receiving control siRNA. To validate our findings, we examined whether ALKBH3 gene silencing in vivo affects squamous cell carcinoma using the human lung squamous cell carcinoma cell line RERF-LC-A1, but no significant differences in tumour formation were observed between the two groups injected with either ALKBH3 siRNA or control RNA (data not shown). The results were thus in-line with clinicopathological data, both in adenocarcinoma and squamous cell carcinoma.

DISCUSSION

We originally detected ALKBH3 expression in the prostate, and were able to demonstrate that a number of molecules associated with ALKBH3 were involved in cancer metastasis or resistance to anticancer drugs. In this study, we show that ALKBH3 has important roles in the survival and progression of human NSCLC cells, both in vitro and in vivo. We also tried to clarify whether ALKBH3 influences cell cycle progression and survival in human lung carcinoma.

Eight mammalian AlkB homologues (ALKBH1-8) are currently identified (Kurowski et al, 2003). Furthermore, in 2007, the FTO (fat mass and obesity associated) gene was found to encode a functional homologue of AlkB (Gerken et al, 2007; Sanchez-Pulido

Figure 2  Immunohistochemical detection of ALKBH3 and Kaplan–Meier plots in adenocarcinomas. ALKBH3 is localised mainly in the cytoplasm of cancer cells. (−), < 30% of immunopositive cells A; (+), ≥ 30% of immunopositive cells B. (C) Kaplan–Meier plots of recurrence-free survival in patients with lung adenocarcinoma. There was significant difference between the two groups in terms of gender, pathological stage, ALKBH3 and pleural invasion factor (R-factor).

Figure 3  Downregulation of ALKBH3 induced cell cycle arrest in human adenocarcinoma cells. (A) A549 was transfected with 100 nM of ALKBH3 siRNA or control RNA. Expression levels of both mRNA and protein of ALKBH3 were examined by RT–PCR and western blotting, respectively. (B) Cell proliferation was quantitatively assessed by MTS assay. Columns indicate mean ± s.e. (C) Cell cycle analysis was performed by flow cytometry using propidium iodide, as described in Materials and Methods. Columns indicate mean ± s.e. **P<0.05.
and Andrade-Navarro, 2007), and two more genes, TET1 and TET2 (Tahiliani et al., 2009; Ito et al., 2010), suggested possibility as a similar mechanism.

ALKBH2 and ALKBH3 share the ability of E.coli AlkB to directly reverse nucleic acid damage in vitro (Falnes et al., 2002; Trewick et al., 2002), and we reported in a recent study that ALKBH8 has important roles in the survival and progression of human urothelial carcinoma both in vitro and in vivo (Shimada et al., 2009). The AlkB family of genes is one of several that control repair of the cytotoxic damage generated in both ssDNA and RNA by S_{32}-alkylating agents; the in vivo function of ALKBH3 is still unclear, but it has also been shown to repair DNA and RNA basepair lesions (Dinglay et al., 2000; Aas et al., 2003). Alkylation of DNA, RNA and proteins results in induction of cytotoxic and mutagenic DNA damage, most of which is subject to excision and postreplication repair. It is well known that, in response to DNA damage, activation of either p16/Rb, p19/p53/p21 or PTEN/p27 can initiate or enhance cellular senescence (Chu et al., 2008), resulting in growth reduction and inhibition. DNA damage elicited in response to extracellular stresses, including exposure to

Figure 4 Downregulation of ALKBH3 induced senescence in human adenocarcinoma cells. (A) Cells were fixed and stained with SA-β-gal and senescence-like phenotype was visualised under a microscope (×400). (B) After 48 h or 72 h cultivation, p21 and p27 induction was examined by western blotting. They were induced in a time-dependent manner: **P<0.05.

Figure 5 Silencing ALKBH3 reduced the peritoneal metastases of lung adenocarcinoma cells, whereas it did not reduce liver metastases. A and B, mice were given either control RNA or ALKBH3 siRNA + atelocollagen mixture at 7 and 14 days after peritoneal injection of A549 cells as described in materials and methods. The mice were killed on day 28. (C) Tumour size and the number of tumours in the peritoneum, as well as the number of liver metastases, were analysed. Columns, mean of 11 mice/group; bars, s.e.
chemotherapeutic drugs, can exhibit significant antitumour effects by inducing senescence, often termed premature senescence (Robles and Adami, 1998; Schmitt et al, 2002; Ricci and Zong, 2006); current chemotherapeutic drugs such as irinotecan, daunorubicin, hydroxyurea, retinoic acid and the previously described gefitinib are closely associated with cellular senescence through their cytotoxic effects (te Poel et al, 2002; Hotta et al, 2007). Cyclin-dependent kinase inhibitors and certain genes such as p16(INK4A), p21(CIP1/WAF1), p27(KIP1) and p53 have important roles in induction or maintenance of senescence by inhibiting the cell cycle progression at G1 arrest (Sherr and Roberts, 1999; Adams, 2009).

Cisplatin induces cell cycle arrest through the p16/p53-dependent induction or maintenance of senescence by inhibiting the cell cycle rather than apoptosis. We examined whether cell death induced by ALKBH3 silencing could also be due to apoptosis, but cleavages of PARP and caspases 3, 8 and 9 were not observed, leaving us to infer that senescence was the underlying cause of growth inhibition and death. Senescence has been shown to be involved in antitumour effect by various anticancer agents and by ionising radiation (Wainwright et al, 2001; Han et al, 2002; Mansilla et al, 2003). In this study, we evaluated the role of ALKBH3 in cancer cell survival, but not in the sensitivity to anticancer drugs including alkylating reagents. Knockdown experiments using siRNA revealed that ALKBH3 contributes to lung adenocarcinoma cell growth through accelerating G1/S transition. As generally accepted, cancer cells arrested at G1 phase are much more sensitive to DNA damaging reagents; therefore, ALKBH3 may be one of the key molecules that determine chemotherapeutic efficacy in lung adenocarcinomas. We further examine whether ALKBH3 gene overexpression or downregulation affects chemosensitivity. However, the surgical specimens were not exposed to chemotherapeutic drugs.

We found that silencing ALKBH3 through siRNA transfection significantly inhibited cell growth in human NSCLC in vitro and in vivo and that, in culture, p21 and p27 were upregulated following ALKBH3 knockdown. We know that p21 binds to CDK2, inhibiting kinase activity in various types of cancer cells and inducing cell cycle arrest at G1 with subsequent cellular senescence (Sherr and Roberts, 1995, 1999; Chang et al, 2000). p21 protein or mRNA is regulated at both the transcriptional and posttranscriptional levels. Among the transcription factors that increase p21 mRNA levels are Sp1, Sp3, E2Fs, STATs and AP2; in addition, p21 transcription is upregulated in response to DNA damage and to p53-mediated tumour suppressor signals (Gartel and Tyner, 1999). p27, on the other hand, inhibits the catalytic activity of CDK4, also resulting in p27(KIP1) gene silencing (Cher and Tyner, 1999). p27, on the other hand, inhibits the catalytic activity of CDK4, also resulting in p27(KIP1) gene silencing (Cher and Tyner, 1999). p27(KIP1) gene silencing inhibits cancer cell survival, and targeted downregulation, as was carried out in our in vivo study by injection of a siRNA and atelocollagen cocktail, could be a novel clinical tool for lung cancer therapy. Our immunohistochemical analysis further suggests that the ALKBH3 expression profile of tumours may be a predictive factor for tumour recurrence of adenocarcinoma, in particular, and may also join EGFR mutational analysis as a marker for sensitivity to chemoradiation.

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REFERENCES

Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, Akbari M, Sundheim O, Bjoras M, Slupphaug G, Seeberg E, Krokan HE (2003) Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. Nucleic Acids Res 31: 4291–4296

Adams PD (2009) Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 36: 2–14

Alberg AJ, Ford JG, Samet JM (2007) Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 132: 295–55S

Alexander K, Hinds PW (2001) Requirement for p27(KIP1) in retinoblastoma protein-mediated senescence. Mol Cell Biol 21: 3616–3631

Chang BD, Watanabe K, Broude EV, Fang J, Poole JC, Kalinichenko TV, Roninson IB (2000) Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. Proc Natl Acad Sci USA 97: 4291–4296

Chu IM, Hengst L, Slingerland JM (2008) The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev 8: 253–267

Curat CA, Vogel WF (2002) Discoidin domain receptor 1 controls growth and adhesion of mesangial cells. J Am Soc Nephrol 13: 2648–2656
Di Marco E, Cutuli N, Herrera L, Cancedda R, De Luca M (1993) Molecular cloning of trkE, a novel trk-related putative tyrosine kinase receptor isolated from normal human keratinocytes and widely expressed by normal human tissues. *J Biol Chem* 268: 24290–24295

Dinglay S, Trewick SC, Lindahl T, Sedgwick B (2000) Defective processing of methylated single-stranded DNA by *E. coli* AlkB mutants. *Genes Dev* 14: 2097–2107

Falnes PO, Johansen RE, Seeberg E (2002) AlkB-mediated oxidative demethylation reverses DNA damage in Escherichia coli. *Nature* 419: 178–182

Falnes PO, Kungland A, Alseth I (2007) Repair of methyl lesions in DNA and RNA by oxidative demethylation. *Neuroscience* 145: 1222–1232

Falnes PO, Rognes T (2003) DNA repair by bacterial AlkB proteins. *Res Microbiol* 154: 531–536

Gartel AL, Tyner AL (1999) Transcriptional regulation of the p21((WAF1/CIP1)) gene. *Exp Cell Res* 246: 280–289

Gerken T, Girard CA, Tung YC, Webbey C, Saudak V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins F, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O’Rahilly S, Schofield CJ (2007) The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 318: 1469–1472

Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, Hendrickson EA, Balan KV, Pantazis P, Wyche JH (2005) Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. *J Biol Chem* 277: 17154–17160

Hirsch FR, Varella-Garcia M, Bunn Jr PA, D’Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y (2010) Role of PCA-1 in human prostate carcinoma. *Oncologist* 15: 313–317

Hotta K, Tabata M, Kiura K, Kozuki T, Mascaux C, Meert AP, Martin B, Delmotte P, Berghmans T, Lafitte JJ, Mascaux C, Paesmans M, Steels E, Verdebout JM, Sculier JP (2002) The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J* 20: 975–981

Honugasa PP, Kim JJ, Farg L, Wong TW, Yancopoulos GD, Aaronsen SA, Lee SW (2003) p53 induction and activation of DDR1 kinase counteract p53-mediated apoptosis and influence p53 regulation through a positive feedback loop. *EMBO J* 22: 1289–1301

Ricci MS, Zong WX (2006) Chemotherapeutic approaches for targeting cell death pathways. *Oncologist* 11: 342–357

Robles SJ, Adami GR (1998) Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. *Oncogene* 16: 1113–1123

Sanchez-Pulido L, Andrade-Navarro MA (2007) The FTO (fat mass and obesity associated) gene codes for a novel member of the non-heme dioxygenase superfamily. *BMC Biochem* 8: 23

Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW (2002) A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. *Cell* 109: 335–346

Sedgwick B, Bates PA, Paik J, Jacobs SC, Lindahl T (2007) Repair of alkylated DNA: recent advances. *DNA Repair* 6: 429–442

Sedgwick B, Lindahl T (2002) Recent progress on the Ada response for inducible repair of DNA alkylation damage. *Oncogene* 21: 8886–8894

Sharma SV, Bell DW, Settlement J, Haber DA (2007) Epidermal growth factor receptor factor mutations in lung cancer. *Nat Rev 7*: 169–181

Sherr CJ, Roberts JM (1995) Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 9: 1149–1163

Sherr CJ, Roberts JM (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13: 1501–1512

Shimada K, Nakamura M, Anai S, De Velasco M, Tanaka M, Tsujikawa K, Ojji Y, Konishi N (2009) A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. *Cancer Res* 69: 3157–3164

Shimada K, Nakamura M, Ishida E, Higuchi T, Yamamoto H, Tsujikawa K, Konishi N (2008) Prostate cancer antigen-1 contributes to cell survival and invasion through discoidin receptor 1 in human prostate cancer. *Cancer Sci* 99: 39–48

Shimada K, Nakamura M, Ishida E, Kishi M, Yonehara S, Konishi N (2003) c-Jun NH2-terminal kinase-dependent Fas activation contributes to etoposide-induced apoptosis in p53-mutated prostate cancer cells. *Prostate* 55: 265–280

Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324: 930–935

to Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP (2002) DNA methylation and RNA by oxidative demethylation. *EMBO J* 21: 3798–3807

Tewrick SC, Henschaw TF, Hausinger RP, Lindahl T, Sedgwick B (2002) Oxidative demethylation by Escherichia coli AlkB directly reverts DNA base damage. *Nature* 419: 174–178

Toyoda Y, Nakayama T, Ioka A, Tsukuma H (2008) Trends in lung cancer incidence by histological type in Osaka, Japan. *Jpn J Clin Oncol* 38: 534–539

Trewick SC, Henschaw TF, Hausinger RP, Lindahl T, Sedgwick B (2002) Oxidative demethylation by Escherichia coli AlkB directly reverts DNA base damage. *Nature* 419: 174–178

Tsujikawa K, Koike K, Itakura A, Hanada H, Suzuki T, Tsuichiya M, Makino Y, Furukawa T, Konishi N, Yamamoto H (2007) Expression and sub-cellular localization of human ABH family molecules. *J Cell Mol Med* 11: 1105–1116

Vogel W (1999) Discoidin domain receptors: structural relations and functional implications. *FASEB J* 13(Suppl): S77–S82

Wainwright LJ, Lasorella A, Lavarone A (2001) Distinct mechanisms of cell cycle arrest control the decision between differentiation and senescence in human neuroblastoma cells. *Proc Natl Acad Sci USA* 98: 9396–9400