

## **AlkB homolog (ABH)ファミリー分子を標的とするがん治療創薬**

辻川 和文 (大阪大学大学院薬学研究科)

### **Evaluation of AlkB homolog family molecules as molecular targets for cancer therapy**

Kazutake TSUJIKAWA, Osaka University

1. Tasaki M, Shimada K, Kimura H, Tsujikawa K, Konishi N. (2011)

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

Br J Cancer, 104: 700-706.

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.

2. Shimada K, Nakamura M, Anai S, De Velasco M, Tanaka M, Tsujikawa K, Ouji Y, Konishi N. (2009)

#### **A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression.**

Cancer Res, 69: 3157-3164.

We recently identified a novel human AlkB homologue, ALKBH8, which is expressed in various types of human cancers including human urothelial carcinomas. In examining the role and function of ALKBH8 in human bladder cancer development in vitro, we found that silencing of ALKBH8 through small interfering RNA transfection reduced reactive oxygen species (ROS) production via down-regulation of NAD(P)H oxidase-1 (NOX-1) and induced apoptosis through subsequent activation of c-jun NH(2)-terminal kinase (JNK) and p38. However, we also found that JNK and p38 activation resulted in phosphorylation of H2AX (gammaH2AX), a variant of mammalian histone H2A, which contributes to the apoptosis induced by silencing ALKBH8 and NOX-1. Silencing of ALKBH8 significantly suppressed invasion, angiogenesis, and growth of bladder cancers in vivo as assessed both in the chorioallantoic membrane assay and in an orthotopic mouse model using green fluorescent protein-labeled KU7 human urothelial carcinoma cells. Immunohistochemical examination showed high expression of ALKBH8 and NOX-1 proteins in high-grade, superficially and deeply invasive carcinomas (pT(1) and >pT(2)) as well as in carcinoma in situ, but not in low-grade and noninvasive phenotypes (pT(a)). These findings indicate an essential role for ALKBH8 in urothelial carcinoma cell survival mediated by NOX-1-dependent ROS signals, further suggesting new therapeutic strategies in human bladder cancer by inducing JNK/p38/gammaH2AX-mediated cell death by silencing of ALKBH8.

3. Shimada K, Nakamura M, Higuchi T, Yamamoto H, Tsujikawa K, Konishi N. (2008)

#### **Prostate cancer antigen-1 contributes to cell survival and invasion through DDR1 in human prostate cancer.**

Cancer Sci, 99: 39-45.

A novel gene, prostate cancer antigen (PCA)-1, was recently reported to be expressed in the prostate; however, its biological roles remain unclear. Knockdown of the PCA-1 gene by small interfering RNA transfection induced apoptosis through reducing the expression of the anti-apoptotic molecule Bcl-xl and cytoplasmic release of cytochrome c in the androgen-independent prostate

cancer cell line PC3. Moreover, in vitro matrigel and in vivo chorioallantoic membrane assays showed that silencing of PCA-1 significantly downregulated discoidin receptor (DDR)-1 expression, resulting in suppression of cancer-cell invasion. Transfection with PCA-1 increased the levels of both Bcl-xl and DDR1, which made the cells more invasive through the upregulation of matrix metalloproteinase 9 in DU145. Interestingly, long-term culture using androgen-free medium increased the level of PCA-1 and the related expression of Bcl-xl and DDR-1 in the androgen-sensitive cancer cell line LNCaP, suggesting that PCA-1 signaling is associated with androgen independence. Immunohistochemical analysis in a series of 169 prostate carcinomas showed that PCA-1 and DDR1 were strongly expressed in prostate cancer cells, including preneoplastic lesions, but there was little or no expression in normal epithelium. Moreover, the expression of PCA-1 and DDR-1 was associated with a hormone-independent state of prostate cancer. Taken together, we propose that PCA-1-DDR-1 signaling is a new important axis involved in malignant potential prostate cancer associated with hormone-refractory status.

4. Tsujikawa K, Koike K, Kitae K, Shinkawa A, Arima H, Suzuki T, Tsuchiya M, Makino Y, Furukawa T, Konishi N, Yamamoto H. (2007)

**Expression and sub-cellular localization of human AlkB homolog family molecules.**

J Cell Mol Med, 11: 1105-1116.

AlkB is an Escherichia coli protein that catalyses the oxidative demethylation of 1-methyladenine and 3-methylcytosine in DNA and RNA. The enzyme activity of AlkB is dependent on a 2-oxoglutarate- and Fe(II)-dependent (2OG-Fe(II)) oxygenase domain. Human AlkB homologues (hABH), hABH1, hABH2 and hABH3, which also possess the 2OG-Fe(II) oxygenase domain, have previously been identified. Recent bioinformatics analysis suggests the existence of an additional five ABH genes in humans. In this study, we identified the hABH4-hABH7 mRNAs and determined their expression in human tissues. Moreover, an hABH2 splice variant lacking the 2OG-Fe(II) oxygenase domain and a new gene, hABH8, were cloned from testis cDNA. hABH8 possesses not only the 2OG-Fe(II) oxygenase domain but both an RNA-binding motif and a methyl-transferase domain. mRNA of the eight hABH molecules was detected in the 16 normal human tissues examined. The sub-cellular localization of EmGFP-hABH8 was restricted to the cytoplasm. EmGFP-hABH1, 3, 4, 6 and 7 were localized in both the cytoplasm and nuclei. Interestingly, the EmGFP-hABH2 splice variant localized in nucleoplasm with a dot-like pattern. In some HeLa cells transfected with EmGFP-hABH5, dot-like fluorescence was also detected in the cytoplasm. These observations provide important information for the future annotation of the hABH family of molecules.

5. Konishi N, Nakamura M, Shimada K, Mitsui E, Yoshikawa R, Yamamoto H, Tsujikawa K. (2005)

**High expression of a new marker PCA-1 in human prostate carcinoma.**

Clin Cancer Res, 11: 5090-5097.

**PURPOSE:** Identifying the genetic factors involved in prostate carcinogenesis is critical. Novel cancer-specific markers aid in early detection, in differentiating between cancer and nonmalignant disorders, and in monitoring clinical of prostate disease. We therefore examined differential gene displays in an attempt to identify genes that may be involved in prostate carcinogenesis.

**EXPERIMENTAL DESIGN:** Applying fluorescent differential display analysis to human prostate carcinomas, we have identified and cloned several cDNA transcripts. Antisera were raised against synthetic peptides and used in Western blot and immunohistochemical analyses. The mRNAs were also analyzed by real-time reverse transcription-PCR. For functional analysis, we assessed methylmethane sulfonate (MMS)-induced toxicity in COS-7 cells after cDNA transfection.

**RESULTS:** We identified a gene, designated prostate cancer antigen-1 (pca-1), which shows high mRNA expression in prostate carcinoma. Database analysis of the deduced amino acid sequence of PCA-1 indicated high similarity to Escherichia coli AlkB, a DNA alkylation damage repair enzyme. By immunohistochemical analysis, PCA-1 was expressed in a high number of both prostate carcinoma samples and in the atypical cells within high-grade prostatic intraepithelial neoplasias but not in benign prostatic hyperplasia or normal adjacent tissues. PCA-1-transfected COS-7 cells further showed resistance against MMS-induced cell death.

**CONCLUSIONS:** These findings suggest that PCA-1 could be a useful diagnostic marker. Furthermore, because this human counterpart of AlkB exhibits a protective function against alkylation damage in mammalian cells, PCA-1 may also serve as a therapeutic target molecule for prostate cancer.