

Answer 1:

### Bibliographic Information

**Decitabine-induced demethylation of 5' CpG island in GADD45A leads to apoptosis in osteosarcoma cells.** Al-Romaih, Khaldoun; Sadikovic, Bekim; Yoshimoto, Masia; Wang, Yuzhuo; Zielenska, Maria; Squire, Jeremy A. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Can. Neoplasia (Ann Arbor, MI, United States) (2008), 10(5), 471-480. Publisher: Neoplasia Press Inc., CODEN: NEOPFL ISSN: 1522-8002.  
<http://www.neoplasia.com/pdf/manuscript/v10i05/neo08174.pdf> Journal; Online Computer File written in English. AN 2008:871363 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

GADD45 genes are epigenetically inactivated in various types of cancer and tumor cell lines. To date, defects of the GADD45 gene family have not been implicated in osteosarcoma (OS) oncogenesis, and the role of this pathway in regulating apoptosis in this tumor is unknown. The therapeutic potential of Gadd45 in OS emerged when our previous studies showed that GADD45A was reexpressed by treatment with the demethylation drug decitabine. In this study, we analyze the OS cell lines MG63 and U2OS and show that on treatment with decitabine, a significant loss of DNA methylation of GADD45A was assocd. with elevated expression and induction of apoptosis. In vivo affects of decitabine treatment in mice showed that untreated control xenografts exhibited low nuclear staining for Gadd45a protein, whereas the nuclei from xenografts in decitabine-treated mice exhibited increased amts. of protein and elevated apoptosis. To show the specificity of this gene for decitabine-induced apoptosis in OS, GADD45A mRNAs were disrupted using short interference RNA, and the ability of the drug to induce apoptosis was reduced. Understanding the role of demethylation of GADD45A in reexpression of this pathway and restoration of apoptotic control is important for understanding OS oncogenesis and for more targeted therapeutic approaches.

Answer 2:

### Bibliographic Information

**Inhibitory effect of 5-aza-2'-deoxycytidine on human gastric cancer xenografts in nude mice.** Tian, Xianglong; Zhong, Jie; Li, Biao; Huang, Wei; Zhang, Yifan; Wang, Jun; Gu, Yanyun. Department of Gastroenterology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, Peop. Rep. China. Shanghai Jiaotong Daxue Xuebao, Yixueban (2007), 27(5), 533-536. Publisher: Shanghai Jiaotong Daxue Xuebao Yixueban Bianjibu, CODEN: SJDXB8 Journal written in Chinese. CAN 148:346031 AN 2008:119882 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

The objective was to observe the effect of 5-aza-2'-deoxycytidine (5-aza-CdR) on the normal epithelial specific-1 gene (NES1) and the growth of human gastric cancer xenografts in nude mice, and to explore the possible anti-tumor mechanisms and search for new treatment for gastric cancer. Human gastric cancer xenograft model in nude mice was established and treated with 5-aza-CdR. The growth of xenografts in nude mice was obsd., and the status of methylation and protein expression of NES1 gene were detected by MSP and immunohistochem. resp. After treatment with 5-aza-CdR, the growth of the xenografts in nude mice was greatly inhibited ( $P < 0.01$ ). The inhibitory rate of tumor was 57.44%. The methylated exon3 of NES1 gene was found to be demethylated and the expression of NES1 protein also increased. 5-Zaz-CdR may reactivate antioncogene (NES1) silenced by denovomethylation, therefore, inhibit the growth and proliferation of gastric cancer cells in vivo.

Answer 3:

### Bibliographic Information

**Demethylation treatment of NES1 gene for breast carcinoma xenografted in nude mice.** Hu, Shengping; Li, Biao; Zhang, Yifan; Zhang, Min. Department of Nuclear Medicine, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai,

Peop. Rep. China. Shanghai Jiaotong Daxue Xuebao, Yixueban (2007), 27(1), 72-75. Publisher: Shanghai Jiaotong Daxue Xuebao Yixueban Bianjibu, CODEN: SJDXB8 Journal written in Chinese. CAN 148:345986 AN 2008:92019 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

The objective was to study the anti-tumor effect of 5-aza-2'-deoxycytidine (5-aza-dC) on breast carcinoma xenografted in nude mice. The model of breast carcinoma xenografted in nude mice was established. Ten mice were randomized into the treatment group (treated with 5-aza-dC) and control group (treated with PBS). The mass of the tumors before and after treatment were measured in the two groups, the inhibition rate of the tumor was calcd., and the growth curve was drawn. Immunohistochem. and Western blotting were employed to detect the expression of normal epithelial cell specific-1 (NES1) gene. The inhibition rate of the tumor in the treatment group was 57.44%, which was significantly different from the control group ( $P < 0.01$ ). Results from immunohistochem. and Western blotting indicated that the expression of NES1 gene was significantly increased after the treatment with 5-aza-dC. NES1 gene may serve as an anti-tumor gene and mol. marker, whose absence is closely related with the carcinogenesis and development of breast carcinoma. Demethylation treatment may increase the expression of NES1 gene and bring the anti-tumor effect, which paves a new way for the treatment of breast carcinoma.

Answer 4:

### Bibliographic Information

#### Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors.

Appleton, Kim; Mackay, Helen J.; Judson, Ian; Plumb, Jane A.; McCormick, Carol; Strathdee, Gordon; Lee, Chooi; Barrett, Sophie; Reade, Sarah; Jadayel, Dalal; Tang, Adrian; Bellenger, Katharine; Mackay, Lynsay; Setanoians, Albert; Schatzlein, Andreas; Twelves, Chris; Kaye, Stanley B.; Brown, Robert. Centre for Oncology and Applied Pharmacology, Cancer Research UK Beatson Laboratories, Glasgow University, Glasgow, UK. Journal of Clinical Oncology (2007), 25(29), 4603-4609. Publisher: American Society of Clinical Oncology, CODEN: JCONDN ISSN: 0732-183X. Journal written in English. CAN 148:159114 AN 2007:1282019 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

**Purpose:** The DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (decitabine) induces DNA demethylation and re-expression of epigenetically silenced genes, and increases carboplatin sensitivity of tumor xenograft models. We designed a clin. study to det. the feasibility of delivering a dose of decitabine, combined with carboplatin, that would be capable of producing equiv. biol. effects in patients with solid tumors. **Patients and Methods:** In a two-stage design, 33 patients received escalating doses of decitabine administered as a 6-h infusion on day 1 followed by carboplatin, area under the concn.-time curve (AUC) 5 (cohort 1) and AUC 6 (cohort 2), on day 8 of a 28-day cycle. Pharmacodynamic analyses included 5-methyl-2'-deoxycytidine levels, MAGE1A CpG island methylation, and fetal Hb (HbF) expression. **Results:** The major toxicity was myelosuppression. Dose limiting toxicities, prolonged grade 4 neutropenia (one patient), and sepsis and grade 3 anorexia/fatigue (one patient), were seen in two of four patients treated with decitabine 135 mg/m<sup>2</sup> and carboplatin AUC 5. Dose limiting toxicity comprising neutropenic sepsis (one patient) and grade 3 fatigue (one patient) was seen in two of 10 patients treated at decitabine 90 mg/m<sup>2</sup> and carboplatin AUC 6. Decitabine induced dose-dependent, reversible demethylation in peripheral-blood cells (PBCs) maximally at day 10. Furthermore, decitabine 90 mg/m<sup>2</sup> induced demethylation of the MAGE1A CpG island in PBCs, buccal cells, and tumor biopsies, as well as elevation of HbF expression. **Conclusion:** Decitabine can be combined safely with carboplatin at a dose and schedule that causes epigenetic changes equiv. to or greater than that obsd. in mice with carboplatin-sensitized xenografts. The recommended dose/schedule for phase II trials is decitabine 90 mg/m<sup>2</sup> (day 1) followed by carboplatin AUC 6 (day 8) every 28 days.

Answer 5:

### Bibliographic Information

**Combining epigenetic and cytotoxic therapy in the treatment of solid tumors.** Plimack, Elizabeth R.; Stewart, David J.; Issa,

Jean-Pierre J. The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA. *Journal of Clinical Oncology* (2007), 25(29), 4519-4521. Publisher: American Society of Clinical Oncology, CODEN: JCONDN ISSN: 0732-183X. Journal; General Review written in English. CAN 148:134704 AN 2007:1282001 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

A review. The research of Appleton et al. (2007) entitled "Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors" is reviewed with commentary and refs. Appleton et al. conducted trial combining decitabine and carboplatin in advanced solid tumors. This dose-finding trial uses a series of doses of decitabine that, per cycle, all fall within the range of low doses shown to induce hypomethylation in vitro and in vivo. Furthermore, decitabine was administered 8 days before initiation of cytotoxic therapy, in keeping with preclin. models. The investigators conducted two sep. dose escalations of decitabine, the first with carboplatin fixed at area under the concn. time curve (AUC) 5 and the second at AUC 6, concluding that the recommended phase II dosing for this combination is decitabine 90 mg/m<sup>2</sup> administered on day 1 followed by carboplatin AUC 6 on day 8 of a 28-day cycle. Of the 30 patients assessable for response, one patient with melanoma had a partial response and three other patients had stable disease. The majority of responses clustered at the recommended combination dose.

Answer 6:

### Bibliographic Information

#### **Modulation by decitabine of gene expression and growth of osteosarcoma U20S cells in vitro and in xenografts:**

**identification of apoptotic genes as targets for demethylation.** Al-Romaih, Khaldoun; Somers, Gino R.; Bayani, Jane; Hughes, Simon; Prasad, Mona; Cutz, Jean-Claude; Xue, Hui; Zielenska, Maria; Wang, Yuzhuo; Squire, Jeremy A. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Can. *Cancer Cell International* (2007), 7 No pp. given. Publisher: BioMed Central Ltd., CODEN: CCIACC ISSN: 1475-2867. <http://www.cancerci.com/content/pdf/1475-2867-7-14.pdf> Journal; Online Computer File written in English. CAN 148:92491 AN 2007:1259156 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Background: Methylation-mediated silencing of genes is one epigenetic mechanism implicated in cancer. Studies regarding the role of modulation of gene expression utilizing inhibitors of DNA methylation, such as decitabine, in osteosarcoma (OS) have been limited. A biol. understanding of the overall effects of decitabine in OS is important because this particular agent is currently undergoing clin. trials. The objective of this study was to measure the response of the OS cell line, U2OS, to decitabine treatment both in vitro and in vivo. Results: Microarray expression profiling was used to distinguish decitabine-dependent changes in gene expression in U2OS cells, and to identify responsive loci with demethylated CpG promoter regions. U2OS xenografts were established under the sub-renal capsule of immune-deficient mice to study the effect of decitabine in vivo on tumor growth and differentiation. Reduced nuclear methylation levels could be detected in xenografts derived from treated mice by immunohistochem. utilizing a 5-methylcytidine antibody. Decitabine treatment reduced tumor xenograft size significantly ( $p < 0.05$ ). Histol. anal. of treated U2OS xenograft sections revealed a lower mitotic activity ( $p < 0.0001$ ), increased bone matrix prodn. ( $p < 0.0001$ ), and a higher no. of apoptotic cells ( $p = 0.0329$ ). Microarray expression profiling of U2OS cultured cells showed that decitabine treatment caused a significant induction ( $p < 0.0025$ ) in the expression of 88 genes. Thirteen had a  $\geq 2$ -fold change, 11 of which had CpG-island-assocd. promoters. Interestingly, 6 of these 11 were pro-apoptotic genes and decitabine resulted in a significant induction of cell death in U2OS cells in vitro ( $p < 0.05$ ). The 6 pro-apoptotic genes (GADD45A, HSPA9B, PAWR, PDCD5, NFKBIA, and TNFAIP3) were also induced to  $\geq 2$ -fold in vivo. Quant. methylation pyrosequencing confirmed that the tested pro-apoptotic genes had CpG-island DNA demethylation as a result of U2OS decitabine treatment both in vitro and in xenografts.

Conclusion: These data provide new insights regarding the use of epigenetic modifiers in OS, and have important implications for therapeutic trials involving demethylation drugs. Collectively, these data have provided biol. evidence that one mode of action of decitabine may be the induction of apoptosis utilizing promoter-CpG demethylation of specific effectors in cell death pathways in OS.

Answer 7:

### Bibliographic Information

**Effects of 5-aza-2'-deoxycytidine on human laryngeal carcinoma xenograft in nude mice.** Zhang, Song; Kong, Weijia; Guo, Changkai; Wang, Yanjun; Han, Yuechen. Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Peop. Rep. China. Zhongguo Zhongliu Linchuang (2005), 32(21), 1212-1215. Publisher: Zhongguo Zhongliu Linchang Bianji Weiyuanhui, CODEN: ZZLIEP ISSN: 1000-8179. Journal written in Chinese. CAN 146:134767 AN 2006:1068456 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

The effects of 5-aza-2'-deoxycytidine on the growth of tumor and transcription regulation of DAPK (death-assocd. protein kinase) gene in the xenografted of nude mice were investigated to find new target of laryngeal cancer therapy. The model of human laryngeal carcinoma xenograft in nude mice was set up. The growth of those xenografts in nude mice was obsd. and a growth curve of the xenografted drawn, after treated by 5-aza-2'-deoxycytidine. RT-PCR and immunohistochem. were used to det. the expressions of DAPK mRNA and protein. No significant difference for body wt. of the nude mice was found between the medication group and the controls, after treatment with 5-aza-2'-deoxycytidine. The size of human laryngeal carcinoma xenografts in the nude mice of medication group was smaller than those in the controls. There was a statistically significant difference between the medication group and the controls, because no expression of DAPK was obsd. in the control group without treatment of 5-aza-2'-deoxycytidine. On the contrary, the expression of DAPK was obsd. in the treatment group. It indicated that 5-aza-2'-deoxycytidine might slow down the growth of tumor by reactivating tumor suppressor genes silenced by promoter methylation in vivo.

Answer 8:

#### Bibliographic Information

**Inhibitory effect of 5-aza-2'-deoxycytidine on human nasopharyngeal carcinoma xenograft in nude mice.** Zhang, Song; Kong, Weijia; Wang, Yanjun; Han, Yuechen; Zhang, Dan. Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, Peop. Rep. China. Aizheng (2005), 24(10), 1201-1205. Publisher: Sun Yat-sen Daxue, Aizheng Zhongxin, CODEN: AIZHE4 ISSN: 1000-467X. Journal written in Chinese. CAN 145:499715 AN 2006:744189 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

#### Abstract

This study was to observe the inhibitory effect of 5-Aza-2'-deoxycytidine (5Aza-CdR) on the growth of human nasopharyngeal carcinoma (NPC) cells and xenografts in nude mice, explore the possible mechanisms, and search for new treatment target of NPC. NPC cell line CNE cells were treated with 5-Aza-CdR; the methylation status of death-assocd. protein kinase (DAPK) gene was evaluated by methylation-specific polymerase chain reaction (PCR). The model of human NPC xenograft in nude mice was constructed and treated with 5-Aza-CdR; the xenograft growth in nude mice was obsd., and the mRNA and protein expression of DAPK were detected by reverse transcription-PCR (RT-PCR) and immunohistochem. No expression of DAPK mRNA was found in CNE cells and the xenografts in nude mice without treatment of 5-Aza-CdR. After treatment, the expression of DAPK mRNA in CNE cells and the xenografts was increased along with the increasing concn. of 5-Aza-CdR; the growth of CNE cells and the xenografts in nude mice were obviously inhibited, and the methylated DAPK gene was reactivated. Four weeks after treatment, no significant difference was found in body wt. of nude mice between 5-Aza-CdR group and control group [(22.35±2.02) g vs. (21.68±2.14) g, t=0.011, P>0.05]; the vol. of xenografts was significantly smaller in 5-Aza-CdR group than in control group [(195.32±27.57) mm<sup>3</sup> vs. (343.67±23.08) mm<sup>3</sup>, t=10.11, P<0.01]. 5-Aza-CdR may reactivate antioncogene silenced by de novo methylation, therefore, inhibit the growth of CNE cells in vivo and in vitro.

Answer 9:

#### Bibliographic Information

**Inhibition of DNA Methylation Sensitizes Glioblastoma for Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Mediated Destruction.** Eramo, Adriana; Pallini, Roberto; Lotti, Fiorenza; Sette, Giovanni; Patti, Mariella; Bartucci, Monica; Ricci-Vitiani, Lucia; Signore, Michele; Stassi, Giorgio; Larocca, Luigi M.; Crino, Lucio; Peschle, Cesare; De Maria, Ruggero.

Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanita, Rome, Italy. Cancer Research (2005), 65(24), 11469-11477. Publisher: American Association for Cancer Research, CODEN: CNREA8 ISSN: 0008-5472. Journal written in English. CAN 144:68418 AN 2005:1311927 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

Life expectancy of patients affected by glioblastoma multiforme is extremely low. The therapeutic use of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been proposed to treat this disease based on its ability to kill glioma cell lines in vitro and in vivo. Here, the authors show that, differently from glioma cell lines, glioblastoma multiforme tumors were resistant to TRAIL stimulation because they expressed low levels of caspase-8 and high levels of the death receptor inhibitor PED/PEA-15. Inhibition of methyltransferases by decitabine resulted in considerable up-regulation of TRAIL receptor-1 and caspase-8, down-regulation of PED/PEA-15, inhibition of cell growth, and sensitization of primary glioblastoma cells to TRAIL-induced apoptosis. Exogenous caspase-8 expression was the main event able to restore TRAIL sensitivity in primary glioblastoma cells. The antitumor activity of decitabine and TRAIL was confirmed in vivo in a mouse model of glioblastoma multiforme. Evaluation of tumor size, apoptosis, and caspase activation in nude mouse glioblastoma multiforme xenografts showed dramatic synergy of decitabine and TRAIL in the treatment of glioblastoma, whereas the single agents were scarcely effective in terms of redn. of tumor mass, apoptosis induction, and caspase activation. Thus, the combination of TRAIL and demethylating agents may provide a key tool to overcome glioblastoma resistance to therapeutic treatments.

Answer 10:

### Bibliographic Information

**Inhibition of human Kasumi-1 xenograft tumor growth in nude mice by combination of phenylbutyrate and 5-Aza-2'-deoxycytidine.** Hao, Changlai; Lin, Dong; Wang, Lihong; Xing, Haiyan; Wang, Min; Wang, Jianxiang. Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, Peop. Rep. China. Zhonghua Xueyexue Zazhi (2004), 25(11), 658-661. Publisher: Zhongguo Yixue Kexueyuan Xueyexue Yanjiuso, CODEN: CHTCD7 ISSN: 0253-2727. Journal written in Chinese. CAN 143:339146 AN 2005:968461 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

The tumor suppression efficacy of histone deacetylase inhibitor, phenylbutyrate (PB), in combination with DNA methylation inhibitor 5-Aza-2-deoxycytidine (5-Aza-CdR) in the treatment of Kasumi-1 xenograft tumor in nude mice and its mechanism were investigated. The nude mice model of Kasumi-1 xenograft tumor was established by s.c. inoculation. Latency of tumor formation, the ability of Kasumi-1 cells pre treated with PB to form the xenograft tumor, and the tumor suppression activity of PB and 5-Aza-CdR by i.p. injection in xenografted mice model were detected. Cell differentiation and cell cycle parameters of the tumor cells were analyzed by flow cytometry anal., apoptosis by TUNEL in situ hybridization, and tumor microvessel d. (MVD) by immunohistochem. study. The latency of tumor formation in mice with or without previous lienectomy was 17-23 and 40-50 days, resp. Tumor cells xenografted could not be found in other tissues than in inoculation area, and still harbored the specific t(8;21) and AML1-ETO fusion gene. When the xenografted mice models treated with PB, 5-Aza-CdR, or both, the tumor growth inhibition rates were 49.07%, 25.69% and 87.46% (P <0.05),. The apoptosis indexes (AI) of tumor cells were (2.25 ± 0.85)%, (1.32 ± 0.68)%, and (5.41 ± 1.56)% (P <0.05), and the microvessel densities (MVD) were 21.69 ± 6.25, 28.34 ± 4.24 and 9.48 ± 3.21 (P <0.01), resp. All the data above were significantly different from that in control (P <0.05). The expression of CD11b and CD13 antigen of the tumor cells was increased in xenografted mice model treated with PB when compared with the control [(12.08 ± 1.02)% and (54.91 ± 2.72)%], resp. (P <0.01), and tumor cells showed a cell cycle arrest with increased G0/G1-phase cells and decreased S-phase cells. PB inhibited the growth of Kasumi-1 xenograft tumor by inducing tumor cell apoptosis and differentiation, and suppressing its angiogenesis in vivo. 5-Aza-CdR could significantly enhance the antitumor activity of PB.

Answer 11:

### Bibliographic Information

**Methylation-Associated Silencing of Death-Associated Protein Kinase Gene in Laryngeal Squamous Cell Cancer.** Kong, Wei-Jia; Zhang, Song; Guo, Changkai; Zhang, Sulin; Wang, Yanjun; Zhang, Dan. Department of Otolaryngology, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, Peop. Rep. China. *Laryngoscope* (2005), 115(8), 1395-1401. Publisher: Lippincott Williams & Wilkins, CODEN: LARYA8 ISSN: 0023-852X. Journal written in English. CAN 144:67668 AN 2005:728817 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

### Abstract

**Objectives/Hypothesis:** Death-assocd. protein kinase (DAPK) is a Ca/calmodulin-regulated Ser/Thr kinase that functions as a pos. mediator of programmed cell death. It has been found that DAPK gene is frequently inactivated by its promoter hypermethylation in some cancers and tumor cell lines. However, it is not clear whether promoter hypermethylation of DAPK gene exists in laryngeal squamous cell cancer (LSCC). The aim of this study was to investigate the promoter methylation status of the DAPK gene in LSCC and the effect of 5-Aza-2'-deoxycytidine (5-Aza-CdR), a demethylating agent, on Hep-2 cells, a human laryngeal cancer cell line, and on xenografts of Hep-2. **Methods:** Methylation-specific polymerase chain reaction (PCR) and reverse-transcription PCR techniques were used to det. the promoter methylation status and mRNA expression of DAPK gene in LSCC. Furthermore, Hep-2 cells in vitro and in vivo were treated by 5-Aza-CdR to explore the effect of demethylating agents on DAPK mRNA expression and tumor growth. **Results:** Hypermethylation of DAPK gene promoter was found in 39 (67.2%) of 58 LSCC samples. There was no significant difference in the promoter hypermethylation rate among the samples of different histol. grades or samples from patients with different T stages. However, there was significant difference in methylation status of DAPK gene between the samples from patients in N0 stages and those from patients in N1 stages. No promoter hypermethylation of DAPK gene was found in any of the five normal laryngeal tissue samples. DAPK mRNA expression was not detected in tumor specimens with promoter hypermethylation. On the contrary, DAPK mRNA expression was obsd. in the unmethylated tumor specimens, specimens from tissues adjacent to the tumor, and normal laryngeal tissues samples. Promoter hypermethylation of DAPK gene was found, and no DAPK mRNA expression was detected in Hep-2 cells. DAPK mRNA expression in Hep-2 cells and xenografts could be restored by treating cells and xenografts with 5-Aza-CdR.

The tumors' xenografts, induced by way of Hep-2 cell injection in nude mice treated with 5-Aza-CdR, were obviously smaller than those in nude mice treated with phosphate-buffered saline. **Conclusions:** Abnormal loss of DAPK expression could be assocd. with aberrant promoter region methylation in the LSCC. 5-Aza-CdR may slow the growth of Hep-2 cells in vitro and in vivo by reactivating tumor suppressor gene DAPK silenced by de novo methylation.

Answer 12:

### Bibliographic Information

**Modulation by decitabine of gene expression and growth of osteosarcoma U2OS cells in vitro and in xenografts: Identification of apoptotic genes as targets for demethylation.** Al-Romaih Khaldoun; Somers Gino R; Bayani Jane; Hughes Simon; Prasad Mona; Cutz Jean-Claude; Xue Hui; Zielenska Maria; Wang Yuzhuo; Squire Jeremy A Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, M5G 1L5. jeremy.squire@utoronto.ca *Cancer cell international* (2007), 7 14. Journal code: 101139795. E-ISSN:1475-2867. Journal; Article; (JOURNAL ARTICLE) written in English. PubMed ID 17845729 AN 2007617293 In-process for MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

### Abstract

**ABSTRACT: BACKGROUND:** Methylation-mediated silencing of genes is one epigenetic mechanism implicated in cancer. Studies regarding the role of modulation of gene expression utilizing inhibitors of DNA methylation, such as decitabine, in osteosarcoma (OS) have been limited. A biological understanding of the overall effects of decitabine in OS is important because this particular agent is currently undergoing clinical trials. The objective of this study was to measure the response of the OS cell line, U2OS, to decitabine treatment both in vitro and in vivo. **RESULTS:** Microarray expression profiling was used to distinguish decitabine-dependent changes in gene expression in U2OS cells, and to identify responsive loci with demethylated CpG promoter regions. U2OS xenografts were established under the sub-renal capsule of immune-deficient mice to study the effect of decitabine in vivo on tumor growth and differentiation. Reduced nuclear methylation levels could be detected in xenografts derived from treated mice by immunohistochemistry utilizing a 5-methylcytidine antibody. Decitabine treatment reduced tumor xenograft size significantly ( $p < 0.05$ ). Histological

analysis of treated U2OS xenograft sections revealed a lower mitotic activity ( $p < 0.0001$ ), increased bone matrix production ( $p < 0.0001$ ), and a higher number of apoptotic cells ( $p = 0.0329$ ). Microarray expression profiling of U2OS cultured cells showed that decitabine treatment caused a significant induction ( $p < 0.0025$ ) in the expression of 88 genes. Thirteen had a  $\geq 2$ -fold change, 11 of which had CpG-island-associated promoters. Interestingly, 6 of these 11 were pro-apoptotic genes and decitabine resulted in a significant induction of cell death in U2OS cells in vitro ( $p < 0.05$ ). The 6 pro-apoptotic genes (GADD45A, HSPA9B, PAWR, PDCD5, NFKBIA, and TNFAIP3) were also induced to  $\geq 2$ -fold in vivo.

Quantitative methylation pyrosequencing confirmed that the tested pro-apoptotic genes had CpG-island DNA demethylation as a result of U2OS decitabine treatment both in vitro and in xenografts. **CONCLUSION:** These data provide new insights regarding the use of epigenetic modifiers in OS, and have important implications for therapeutic trials involving demethylation drugs. Collectively, these data have provided biological evidence that one mode of action of decitabine may be the induction of apoptosis utilizing promoter-CpG demethylation of specific effectors in cell death pathways in OS.