Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2008 March 21; 14(11): 1741-1748
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Harry HX Xia, PhD, MD, *Series Editor*

DNA methylation in hepatocellular carcinoma

Iris Tischoff, Andrea Tannapfel

Iris Tischoff, Andrea Tannapfel, Institute of Pathology, Ruhr-University of Bochum, Bürkle-de-la-Camp-Platz 1, Bochum 44789, Germany
Correspondence to: Iris Tischoff, MD, Institute of Pathology, Ruhr-University of Bochum, Bürkle-de-la-Camp-Platz 1, Bochum 44789, Germany. iris.tischoff@rub.de
Telephone: +49-234-3024955 Fax: +49-234-3024809
Received: October 30, 2007 Revised: January 5, 2008

Abstract

As for many other tumors, development of hepatocellular carcinoma (HCC) must be understood as a multistep process with accumulation of genetic and epigenetic alterations in regulatory genes, leading to activation of oncogenes and inactivation or loss of tumor suppressor genes (TSG). In the last decades, in addition to genetic alterations, epigenetic inactivation of (tumor suppressor) genes by promoter hypermethylation has been recognized as an important and alternative mechanism in tumorigenesis. In HCC, aberrant methylation of promoter sequences occurs not only in advanced tumors, it has been also observed in premalignant conditions just as chronic viral hepatitis B or C and cirrhotic liver. This review discusses the epigenetic alterations in hepatocellular carcinoma focusing DNA methylation.

Key words: Hepatocellular carcinoma; DNA methylation; Histone modification; Tumor suppressor genes

Peer reviewer: Sharon Demorrow, Division of Research and Education, Scott and White Hospital and The Texas A&M University System, Health Science Center College of Medicine, Temple, Texas 76504, United States

Tischoff I, Tannapfel A. DNA methylation in hepatocellular carcinoma. *World J Gastroenterol* 2008; 14(11): 1741-1748 Available from: URL: http://www.wjgnet.com/1007-9327/14/1741.asp DOI: http://dx.doi.org/10.3748/wjg.14.1741

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancer worldwide. It shows a wide geographical variation with low incidence areas in North America and Europe and high incidence areas in Africa and Asia. 70%-80% of hepatocellular carcinoma occurs in cirrhotic liver. In high incidence areas, such as Asia and Africa, HCC is strongly associated with chronic viral hepatitis B and C and liver cirrhosis. Nutritional factors, toxins and metabolic diseases contribute also to hepatocarcinogenesis[1,2].

As for many other tumors, development of HCC is due to a multistep process with accumulation of genetic and epigenetic alterations in regulatory genes, leading to activation of oncogenes and inactivation or loss of tumor suppressor genes (TSG).

In the last three decades, cancer has been understood as a summary of altered genetic and epigenetic events. The epigenetic pathway is, in contrast to genetic events, a reversible alteration and characterized by three main mechanisms: (1) DNA hypermethylation leading to inactivation, (2) DNA hypomethylation causing genomic instability, (3) histone modifications affecting chromatin conformation.

These processes, especially aberrant DNA methylation and histone modifications, are closely linked with each other by a protein complex of transcript activators and repressors and alter mRNA transcript expression of affected genes[3].

Characteristically, DNA methylation does not change the genetic information. It just alters the readability of the DNA and results in inactivation of genes by subsequent mRNA transcript repression.

In humans and other mammals, CpG island methylation is an important physiological mechanism. The inactivated X-chromosome of females, silenced alleles of imprinted genes or inserted viral genes and repeat elements are inactivated through promoter methylation[4,5].

DNA HYPERMETHYLATION

Promoter hypermethylation

Hypermethylation of CpG islands in promoter sequences is associated with silencing of tumor suppressor genes and tumor-related genes by subsequent downregulation of mRNA transcript expression. Epigenetic silenced genes are involved in important molecular pathways of carcinogenesis e.g., cell cycle regulation, apoptosis, DNA repair or cell adhesion.

According to other types of malignant tumors, in hepatocellular carcinomas, aberrant methylation of several TSG and tumor-related genes such as *RASSF1A, hMLH1* or *SOCS1* was frequently observed[6].

CpG island hypermethylation is not only seen in HCC

Table 1 Methylation in hepatocellular carcinoma

Gene	Location	Function	Methylation frequency (%)	Ref.
$p16^{INK4a}$	9q21	CDK inhibitor	17-83	[10,11]
$p14^{ARF}$	9q21	MDM2 inhibitor	25-30	[7,12]
CASP8	2q33	Apoptosis	72	[17]
TMS1/ASC	16p11.2	Apoptosis	80	[23]
E-Cadherin	16q22.1	Cell adhesion	33-67	[28-30]
M-Cadherin	16q24.1	Cell adhesion	55	[34]
H-Cadherin	16q24.2-3	Cell adhesion	21	[17]
TIMP3	22q12	MMP inhibitor	13-19	[37-39]
hMLH1	3p21.3	Mismatch repair	18-44	[47-50]
hMSH2	2p21-22	Mismatch repair	68	[47,49,50]
hMSH3	5q11-12	Mismatch repair	75	[47,49,50]
MGMT	10q26	DNA repair	22-39	[53-55]
GSTP1	11q13	Detoxification	41-58	[53,58-61]
SOCS-1	16p13.13	Cytokine inhibitor	60	[67]
SOCS-3	17q25.3	Cytokine inhibitor	33	[68]
RASSF1A	3p21.3	Apoptosis	54-95	[71,75,76]
BLU	3p21.3	Unknown	20	[71]
SEMA3B	3p21.3	Apoptosis	80	[71]
FHIT	3p14.2	histidine triad protein	71	[86]

tumor tissue. Even in premalignant conditions such as dysplastic nodules or cirrhotic liver, promoter methylation of several kinds of TSG, e.g. E-cadherin, *GSTP1* or 6^{Ink4a} was demonstrated (Table 1 shows promoter methylation of different tumor-related genes in HCC).

Proliferation and apoptosis

One of the most important pathways affected in HCC are the Rb (Retinoblastoma) gene and INK4a-ARF pathway[7]. The INK4a-ARF locus is coding two cell-cycle regulatory proteins, $p16^{INK4a}$ and $p14^{ARF}$, acting through the Rb-CDK4 and p53 pathways. $p16^{INK4a}$ binds to cyclin-dependent protein kinase 4 (CDK4) and inhibits the ability of CDK4 to interact with cyclin D1. $p14^{ARF}$ prevents the *p53* degradation through its binding to MDM-2 and induces cell cycle arrest[8,9].

$p16^{INK4a}$ is one of the most altered tumor suppressor gene in human cancer. In HCC, loss of $p16^{INK4a}$ is mainly caused by aberrant promoter methylation, whereas deletions and mutations of this gene locus are infrequently seen. CpG island promoter methylation was reported from 55% to 73%, but aberrant methylation occurred also in non-cancerous liver tissue with cirrhosis in 29% or chronic hepatitis B and C up to 23%[10,11]. Compared to $p16^{INK4a}$ methylation, $p14^{ARF}$ promoter methylation was observed less frequently in 8% to 20% of HCC. It was demonstrated that inactivation of $p14^{ARF}$ is due to homozygous deletions. No correlation was found between *p53* mutations and promoter methylation of $p16^{INK4a}$ or $p14^{ARF}$[7,12].

Caspase 8 (CASP8) is a key apoptotic gene that is involved in death receptor and the mitochondrial pathways and acts as initiator CASP[13]. *CASP8* is silenced by aberrant hypermethylation of its promoter in childhood neuroblastomas[14,15]. In HCC, *CASP8* aberrant promoter methylation was reported by Yu *et al*[16] with a frequency up to 72%.

TMS1/ASC, another proapoptotic gene, functions as a negative regulator of nuclear factor kappaB (NF-κB) and blocks transcription of survival signals[17]. First, *TSM1* was identified as a target of methylation-induced silencing in cell lines overexpressing DNMT1. Epigenetic inactivation of *TMS1* was demonstrated in human glioblastomas, ovarian cancer, human melanoma, colorectal carcinomas or in lung cancer and breast cancer[18-22]. In HCC, *TMS1* promoter methylation was observed in 80%[23].

In hepatocellular carcinoma cell lines, restoration of *TMS1* transcript was induced by demethylating agent 5'-AZA and trichostatin, a histone deacetylase inhibitor. Furthermore, in these cell lines *TMS1* DNA methylation was associated with histone H3 lysine 9 hypoacetylation and trimethylation[24].

Cell adhesion and invasion

E-cadherin (CDH1): E-cadherin, a member of calcium-mediated membrane glycoproteins, is expressed in all epithelial cells acting as an adhesion molecule. Inactivation of E-cadherin induces loss of adherens junctions and impairment of cell adhesiveness and cell proliferation signalling pathways. In tumours, reduction of E-cadherin expression results in tumour progression, cell invasion and formation of metastasis[25,26].

Downregulation of E-cadherin, caused by genetic and epigenetic mechanism, is a frequent event in most type of epithelial carcinomas. In poorly-differentiated breast and gastric cancer, somatic mutations of E-cadherin are frequently found. Further, in all cases of familial gastric cancer, loss of E-cadherin is mainly caused by germline mutations[27]. In other types of tumors, mutations are infrequent events and repression of E-cadherin is mainly caused by aberrant promoter methylation.

In according to CC, mutations of E-cadherin are rare events in HCC. Reduced or loss of E-cadherin expression is mainly caused by aberrant CpG island methylation with a detectable frequency from 33% to 67%[28-30]. Wei *et al*[31] described, that loss of E-cadherin was closely associated with loss of heterozygosity (LOH) of E-cadherin and CpG hypermethylation. In precancerous conditions just as dysplastic nodules or liver tissue with chronic hepatitis or cirrhosis, aberrant E-cadherin methylation was detected in 8% and 46%, respectively[29,30].

Other factors, except epigenetic inactivation or mutations, leading to inactivation of E-cadherin include transcriptional repression by binding of transcriptional factors, e.g. the repressors Snail or Sip-1 to CDH1-E box elements[32,33].

But not only E-cadherin, as a member of cadherin genes, is epigenetically altered in HCC. Yamada *et al*[34] reported the highest methylation frequency of M-cadherin with a frequency to 55% among seven elucidated cadherin genes. Methylation-induced silencing of H-Cadherin (CDH13) was observed by Yu *et al*[17], reaching 21%.

TIMP-3: Tissue inhibitor of metalloproteinase-3 (*TIMP-3*) leads to inhibition of cell migration and angiogenesis. In human carcinoma cell lines, overexpression of *TIMP-3* suppresses cell growth and induces apoptosis by stabilization of TNF-alpha receptors on the cell surface[35,36]. A *TIMP-3* downregulation was demonstrated

in different kinds of tumors, mostly mediated by CpG island promoter methylation[6].

In HCC, *TIMP-3* methylation is an infrequent event reaching 13% to 19%. No methylation was found in normal liver tissue. Lü *et al*[37] demonstrated *TIMP-3* methylation in 25% of hepatocellular cancer emboli in portal veins. The aberrant promoter methylation is accompanied by loss or reduced *TIMP-3* mRNA and protein expression[37-39].

TFPI-2: *TFPI-2* is a Kunitz-type serine protease inhibitor that represses cellular invasion in several kinds of tumors, e.g. in lung cancer or pancreas carcinomas, by suppressing plasmin-mediated activation of MMP-1 and MMP-3 or inhibition of plasmin and trypsin activity[40-43].

Wong *et al*[44] observed *TFPI-2* downregulation with reduced or loss mRNA transcript expression in HCC with a frequency of 90%. In 47% of the observed HCC, aberrant CpG methylation was seen, but not in normal liver tissue. In HCC cell lines with epigenetically induced silencing, a *TFPI-2* mRNA transcript re-expression was induced by combined treatment with the demethylating agent 5'-AZA-DC and the histone deacetylase inhibitor TSA.

DNA repair

Mismatch repair system: Defects in DNA repair mechanisms may result in accumulation of mutations and genomic instability. The mismatch repair system (MMR) is one of the most important DNA repair mechanisms correcting errors in DNA replication. Defects of the MMR leading to microsatellite instabilities (MSI) have been observed in approximately 15% of sporadic colorectal and gastric carcinomas[45,46]. Promoter methylation of MMR genes in HCC occurred with a frequency of 5% to 13% for *hMLH1*, 68% for *hMSH2* and 75% for *hMSH3*. A high methylation frequency of *hMSH2* and *hMSH3* was observed in HCC corresponding non neoplastic liver tissue, especially in cirrhotic liver tissue, reaching 55 % and 70%, but not in normal liver tissue. No correlation was found neither to viral hepatitis nor to MSI status and DNA methylation of analyzed MMR genes[47-50].

MGMT (O6-methylguanine DNA methyltransferase): O6-methylguanine DNA methyltransferase (*MGMT*) is another important DNA repair gene with the highest activity in the liver[51]. *MGMT* protects cells from DNA damage caused by mutagenic and cytotoxic agents leading to alkylation at O6-guanine. Loss or reduced *MGMT* expression due to CpG islands methylation was detected in several kinds of human cancers[52]. In HCC, aberrant methylation occurred with a frequency of 22% to 39%, whereas the *MGMT* promoter shows higher methylation levels in chronic viral hepatitis associated HCC[53-55]. Interestingly, Su *et al*[53] reported that *MGMT* promoter methylation occurred to a similar extent in non neoplastic liver tissue compared to HCC.

GSTP1 (Glutathione S-transferase P1): The detoxificating glutathione S-transferase P1 (*GSTP1*) gene protects cells from cytotoxic and carcinogenic influences in due to inactivation of electrophilic carcinogens by conjugation with glutathione. Promoter methylation of *GSTP1* is best analyzed in prostate cancer. *GSTP1* methylation is an early event in prostatic carcinogenesis, because in high-grade prostatic intraepithelial neoplasia loss of *GSTP1* expression is caused by DNA methylation. Many other tumor types including breast cancer and cholangiocarcinoma showed a *GSTP1* hypermethylated promoter[56,57]. In HCC, methylation of the *GSTP1* gene occurred in 41% to 85%[53,58-61]. Zhang elucidated *GSTP1* methylation in HCC in presence of environmental chemical carcinogens. A significant correlation was observed with higher aflatoxin B_1 (AFB_1)-DNA adducts in tumor tissue in contrast to tumor tissue without or lower levels of AFB_1-DNA adducts. However, no association was found between *GSTP1* methylation and polycyclic aromatic hydrocarbon-DNA adducts[59]. So far, aberrant methylation of *GSTP1* is not only detectable in tumor tissue, Wang *et al*[62] observed a *GSTP1* hypermethylation in serum of HCC patients.

Suppressors of cytokine signaling (SOCS): Suppressors of cytokine signaling 1 and 3 (SOCS-1 and SOCS-3) are intracellular proteins that act as negative regulators of Janus kinase (JAK) and signal transducer and activators of signaling pathways (STAT). The JAK/STAT signaling pathway plays an important role in cell growth and differentiation or immune reaction mediated by cytokines. Cytokines activate JAK's by binding to membrane receptors that leads to phosphorylation of STAT's and activates target genes. *SOCS1* and *SOCS-3* bind direct and indirect to JAK's and inhibit the phosphorylation of STAT's and activation of target genes[63,64].

Aberrant methylation of *SOCS-1* and *SOCS-3* promoter sequence has been reported in several kinds of human cancer. *SOCS-1* and *SOCS-3* CpG island hypermethylation is an early event in human carcinogenesis. Recently, we have shown methylation-induced downregulation of *SOCS-1* and *SOCS-3* in precursor lesions of Barrett's adenocarcinomas and precursor lesions of squamous carcinomas of head and neck[65,66]. In HCC, aberrant promoter methylation of *SOCS-1* and *SOCS-3* occurred with a frequency of 60% and 33%, respectively[67,68]. Methylation of the *SOCS-1* gene was detected in HCV-induced chronic hepatitis and liver cirrhosis, reaching 45%, whereas the methylation frequency increased with fibrosis stage with the highest proportion in liver cirrhosis[69,70].

Methylation hot spot 3p

The short arm of human chromosome 3 belongs to regional methylation hot spots in addition to chromosomal locus 11p and 17p. Alterations of the genetic information on chromosome 3 are one of the most frequent and earliest steps in the carcinogenesis of several types of tumors. LOH of chromosome 3p occurred in about 30% of hepatocellular carcinomas[71].

In different kinds of human cancer, epigenetic inactivation *via* promoter methylation of several genes located on 3p, including *RASSF1A* on 3p21.3, *hMLH1* at 3p21.3, *RARβ 2* at 3p24.2, was shown.

One of the most frequent observed and most

epigenetically inactivated genes of 3p is *RASSF1A,* a multifunctional tumor suppressor gene that protects cells from genomic instability and transformation by stabilizing the microtubules[72,73]. An aberrant promoter methylation was detected in about 50% of malignant tumors. In renal cell carcinoma and small cell lung cancer, the highest prevalence was observed, reaching about 91%[74]. In HCC, hypermethylation occurred in approximately 54% to 95%, whereas HBV-associated HCC showed higher levels of *RASSF1A* methylation compared to HCC without risk factors. *RASSF1A* methylation occurs not only in HCC, methylation is even observed in non-neoplastic precancerous conditions like cirrhotic liver and chronic hepatitis[71].

Semaphorin 3B (*SEMA3B)* and *BLU* are two other putative tumour suppressor genes located on 3p21.3, whereas the function of *BLU* still remains unclear. In lung cancer, *BLU* overexpression inhibits tumor colony formation efficiency. Qiu *et al*[77] reported that *BLU* might function as an environmental stress-responsive gene, regulated by E2F, at least in nasopharyngeal carcinomas. However, *BLU* methylation is a rare event in human cancer. We detected *BLU* promoter methylation in about 20% of our examined HCC[71,78].

SEMA3B, a member of the Semaphorin family, suppresses tumor formation in lung cancer and induces apoptosis. It has been demonstrated that *SEMA3B* induced apoptosis is antagonized by $VEGF^{165}$ in due to an interaction with NP-1 receptor[79,80].

Aberrant methylation of *SEMA3B* was detected in lung cancer and gliomas[81,82]. We reported a high prevalence of *SEMA3B* methylation in HCC, reaching 80%. In contrast, the tumor surrounding non-neoplastic liver exhibited an unmethylated *SEMA3B* promoter. Further, *RASSF1A* and *SEMA3B* expression was restored by treatment with the demethylating drug 5-AZA-C in HCC cell lines, suggesting that promoter hypermethylation is responsible for silencing transcript expression[71,72,81].

The fragile histidine triad *(FHIT)* gene, located to 3p14.2, embraces FRA3b, the most actively fragile site in humans[83,84]. Functional and structural alterations of *FHIT* were identified in several kinds of human cancer. Methylation induced silencing was described in lung and breast cancers[85]. In HCC, promoter methylation of *FHIT* is a frequent and early event. Sun *et al*[86] observed *FHIT* hypermethylation with a frequency of 71% in HCC, 64% in non neoplastic liver tissue and 14 % in normal liver.

CpG island methylator phenotype (CIMP)

Carcinomas with high rates of accumulated aberrant promoter methylation of tumor-related genes are characterized as $CIMP^+$ (CpG island methylator phenotype). $CIMP^+$ was first described for colorectal and gastric cancer by Toyota and Issa in 1999[87,88]. Shen *et al*[89] reported that $CIMP^+$ is associated with environmental exposures in HCC. HCC from patients without precancerous conditions or risk factors, respectively, showed significantly lower levels of methylation than HCC arising from patients with chronic hepatitis B and C or patients with cirrhosis.

CIMP positive HCC (tumours with five genes that are concordantly methylated) showed a significantly association with methylation of the TSG *p14, p15, p16 ER, RASSF1A* or *WT1* and elevated serum alpha-fetoprotein (AFP) levels. Further, $CIMP^+$ was commonly seen in HCC with increased serum AFP levels[90,91].

DNA-methyltransferases (DNMT)

DNA hypermethylation is catalyzed by the family of DNA methyltransferases (DNMT) including DNMT1, DNMT3a and DNMT3b. DNMT1 is required for maintenance of DNA methylation whereas DNMT3a and DNMT3b function as de novo DNA methyltransferases[92-94]. DNMT2 was former described as DNMT because of its strong similarity with m5C methyltransferases of pro- and eukaryotes. But it was recently shown that DNMT2 does not methylate DNA. It's the first described RNA cytosine methyltransferase that methylate position 38 in Aspartic acid transfer RNA[95].

In human cancer just as in HCC, an upregulation of DNMT activity is seen in contrast to global hypomethylation. Park *et al*[96] described a significantly overexpression of DNMT1 and DNMT3b in HCC compared to non-neoplastic liver tissue. DNMT3a showed similar or higher expression levels. Saito *et al*[97] observed higher expression levels of all three DNMTs in HCC and cirrhotic liver than in normal liver. Increased DNMT1 and DNMT3a expression was also reported in dysplastic nodules[98].

According to other tumors, no correlation was seen between DNMT upregulation and promoter hypermethylation-induced inactivation of tumor-related genes. The certain mechanisms of DNMT upregulation remains still unclear, but it is suggested that aberrant DNMT activity, especially of DNMT1, is due to rapid proliferation of cancer cells because DNMT1 binds to proliferating cell nuclear antigen (PCNA)[99-101].

DNA hypomethylation on pericentromeric satellite regions results in chromosomal instability. During hepatocarcinogenesis, DNA hypomethylation of these regions was reported in HCC and precancerous conditions. The splice variant of DNMT3b, DNMT3b4 that may lack DNA methyltransferase activity is associated with DNA hypomethylation on pericentromeric satellite regions. Saito *et al*[102] reported that overexpresssion of DNMT3b4 was seen in cirrhotic liver, chronic hepatitis and HCC whereas increased DNMT3b4 levels correlated with DNA hypomethylation on pericentromeric satellite regions.

DNA HYPOMETHYLATION

In human cancer, global DNA hypomethylation leads to genomic instability, affects repeated DNA sequences, tissue-specific genes and proto-oncogenes or causes loss of imprinting with a biallelic expression, just as in case of *IGF2*. Further, the level of DNA hypomethylation increases with tumor progression[103,104]. In recent years, DNA hypomethylation was shown in several human cancer and some premalignant alterations, i.e. colorectal adenomas and carcinomas, adenocarcinoma of prostate, breast cancer or intestinal type of gastric carcinoma and hepatocellular carcinoma, respectively[105-108].

Lin *et al*[109] observed 5-methylcytosine (m5C) content in hepatocellular carcinogenesis by comparing hepatocellular carcinoma with non neoplastic liver, including cirrhotic livers. In all cancer tissues, 5-methylcytosine was significantly reduced. No difference of the m5C content was detected in cirrhotic and non cirrhotic liver tissue. The reduced 5mC level was associated with large tumor size and poorly histopathological grade.

It is suggested that (re-)activation of retroposons might be associated with global hypomethylation because approximately 90% of all m5C lies in these elements. An association between hypomethylation and transposon activation, especially of LINE-1 transposons, has been observed in human testicular carcinoma cell lines, urothelial carcinoma cell lines and teratocarcinoma cell lines[110-112]. But in HCC, an activation of transposable elements just as LINE-1 retrotransposons *via* hypomethylation could not be detected yet[109].

HISTONE MODIFICATION

Histone modifications are strongly associated with formation of the nucleosome structure and are closely linked to CpG island methylation by interacting with Methyl-CpG-binding proteins (MBD's) and DNA methyltransferases (DNMT's). Modifications including methylation, acetylation or phosphorylation of certain position of the histone tails. Whereas histone methylation is associated either to activation or to repression, histone hypoacetylation mediated by histone deacetylases leads mostly to DNA relaxation and subsequent accessibility for transcriptional factors with repression of transcription.

Lee *et al*[113] reported that HCC with low survival expressed higher levels of genes involved in histone modifications just as PTMA and SET, two proteins that are members of inhibitors of histoneacetyltransferases complex.

P73, a member of the TP53 family represses AFP expression during normal hepatic development by chromatin structure alterations. In hepatoma cells, transactivated p73 suppresses endogenous AFP transcription via reducing of acetylated histone H3 lysine 9 and increasing dimethylated histone H3 lysine 9[114].

CONCLUSION

In hepatocarcinogenesis, aberrant methylation of tumor related genes occurs not only in advanced tumour stages, it's a frequent and early event. Promoter methylation of different kinds of tumor suppressor genes including *p16*, *SOCS1* and *SOCS3* or *RASSF1A*, has been demonstrated in premalignant conditions just as chronic hepatitis or liver cirrhosis. Moreover, the frequency of aberrant promoter methylation increases during the progression from precancerous lesion to HCC. In HBV or HCV-associated chronic hepatitis, methylation frequency of detected genes is significantly higher than in non-neoplastic non-viral liver tissue. Therefore, epigenetic changes in preneoplastic or early neoplastic stages may serve as indicator or "biomarker" for screening of patients with an increased risk for HCC.

Further, HCC is one of the most common causes of cancer death worldwide with a poor prognosis. Only few therapeutic interventions exist. It was demonstrated that re-expression of tumor suppressor genes that are epigenetically silenced is possible by using demethylating and histone modifying agents. In the next years, this might be a possible therapeutic approach analogue to other malignant diseases, e.g. myelodysplastic syndrome, but the used therapeutic agents that influence DNA hypermethylation are toxic and lead to genome wide alteration of the methylation pattern with possible activating of oncogenes or imprinted genes. Another possible aspect of chemotherapy might be to modulate the epigenetically involved pathways by using small molecules that are more specific. But further investigations in clinical trails are needed to prove and integrate epigenetic pathway modulating agents.

REFERENCES

1 **Beasley RP**, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; **2**: 1129-1133
2 **Parkin DM**, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94**: 153-156
3 **Baylin SB**, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. *Trends Genet* 2000; **16**: 168-174
4 **Riggs AD**, Pfeifer GP. X-chromosome inactivation and cell memory. *Trends Genet* 1992; **8**: 169-174
5 **Razin A**, Cedar H. DNA methylation and genomic imprinting. *Cell* 1994; **77**: 473-476
6 **Esteller M**, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. *Cancer Res* 2001; **61**: 3225-3229
7 **Tannapfel A**, Busse C, Weinans L, Benicke M, Katalinic A, Geissler F, Hauss J, Wittekind C. INK4a-ARF alterations and p53 mutations in hepatocellular carcinomas. *Oncogene* 2001; **20**: 7104-7109
8 **Liggett WH Jr**, Sidransky D. Role of the p16 tumor suppressor gene in cancer. *J Clin Oncol* 1998; **16**: 1197-1206
9 **Sharpless NE**, DePinho RA. The INK4A/ARF locus and its two gene products. *Curr Opin Genet Dev* 1999; **9**: 22-30
10 **Tannapfel A**, Wittekind C. Genes involved in hepatocellular carcinoma: deregulation in cell cycling and apoptosis. *Virchows Arch* 2002; **440**: 345-352
11 **Kaneto H**, Sasaki S, Yamamoto H, Itoh F, Toyota M, Suzuki H, Ozeki I, Iwata N, Ohmura T, Satoh T, Karino Y, Satoh T, Toyota J, Satoh M, Endo T, Omata M, Imai K. Detection of hypermethylation of the p16(INK4A) gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus. *Gut* 2001; **48**: 372-377
12 **Peng CY**, Chen TC, Hung SP, Chen MF, Yeh CT, Tsai SL, Chu CM, Liaw YF. Genetic alterations of INK4alpha/ARF locus and p53 in human hepatocellular carcinoma. *Anticancer Res* 2002; **22**: 1265-1271
13 **Medema JP**, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J* 1997; **16**: 2794-2804
14 **Teitz T**, Wei T, Valentine MB, Vanin EF, Grenet J, Valentine VA, Behm FG, Look AT, Lahti JM, Kidd VJ. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med* 2000; **6**: 529-535
15 **Banelli B**, Casciano I, Croce M, Di Vinci A, Gelvi I, Pagnan G, Brignole C, Allemanni G, Ferrini S, Ponzoni M, Romani M. Expression and methylation of CASP8 in neuroblastoma: identification of a promoter region. *Nat Med* 2002; **8**: 1333-1335; author reply 1335

16 **Yu J**, Ni M, Xu J, Zhang H, Gao B, Gu J, Chen J, Zhang L, Wu M, Zhen S, Zhu J. Methylation profiling of twenty promoter-CpG islands of genes which may contribute to hepatocellular carcinogenesis. *BMC Cancer* 2002; **2**: 29

17 **McConnell BB**, Vertino PM. TMS1/ASC: the cancer connection. *Apoptosis* 2004; **9**: 5-18

18 **Stone AR**, Bobo W, Brat DJ, Devi NS, Van Meir EG, Vertino PM. Aberrant methylation and down-regulation of TMS1/ASC in human glioblastoma. *Am J Pathol* 2004; **165**: 1151-1161

19 **Terasawa K**, Sagae S, Toyota M, Tsukada K, Ogi K, Satoh A, Mita H, Imai K, Tokino T, Kudo R. Epigenetic inactivation of TMS1/ASC in ovarian cancer. *Clin Cancer Res* 2004; **10**: 2000-2006

20 **Guan X**, Sagara J, Yokoyama T, Koganehira Y, Oguchi M, Saida T, Taniguchi S. ASC/TMS1, a caspase-1 activating adaptor, is downregulated by aberrant methylation in human melanoma. *Int J Cancer* 2003; **107**: 202-208

21 **Yokoyama T**, Sagara J, Guan X, Masumoto J, Takeoka M, Komiyama Y, Miyata K, Higuchi K, Taniguchi S. Methylation of ASC/TMS1, a proapoptotic gene responsible for activating procaspase-1, in human colorectal cancer. *Cancer Lett* 2003; **202**: 101-108

22 **Virmani A**, Rathi A, Sugio K, Sathyanarayana UG, Toyooka S, Kischel FC, Tonk V, Padar A, Takahashi T, Roth JA, Euhus DM, Minna JD, Gazdar AF. Aberrant methylation of TMS1 in small cell, non small cell lung cancer and breast cancer. *Int J Cancer* 2003; **106**: 198-204

23 **Kubo T**, Yamamoto J, Shikauchi Y, Niwa Y, Matsubara K, Yoshikawa H. Apoptotic speck protein-like, a highly homologous protein to apoptotic speck protein in the pyrin domain, is silenced by DNA methylation and induces apoptosis in human hepatocellular carcinoma. *Cancer Res* 2004; **64**: 5172-5177

24 **Zhang C**, Li H, Zhou G, Zhang Q, Zhang T, Li J, Zhang J, Hou J, Liew CT, Yin D. Transcriptional silencing of the TMS1/ASC tumour suppressor gene by an epigenetic mechanism in hepatocellular carcinoma cells. *J Pathol* 2007; **212**: 134-142

25 **Hashimoto M**, Niwa O, Nitta Y, Takeichi M, Yokoro K. Unstable expression of E-cadherin adhesion molecules in metastatic ovarian tumor cells. *Jpn J Cancer Res* 1989; **80**: 459-463

26 **Bussemakers MJ**, van Moorselaar RJ, Giroldi LA, Ichikawa T, Isaacs JT, Takeichi M, Debruyne FM, Schalken JA. Decreased expression of E-cadherin in the progression of rat prostatic cancer. *Cancer Res* 1992; **52**: 2916-2922

27 **Guilford P**, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. *Nature* 1998; **392**: 402-405

28 **Matsumura** T, Makino R, Mitamura K. Frequent down-regulation of E-cadherin by genetic and epigenetic changes in the malignant progression of hepatocellular carcinomas. *Clin Cancer Res* 2001; **7**: 594-599

29 **Kwon GY**, Yoo BC, Koh KC, Cho JW, Park WS, Park CK. Promoter methylation of E-cadherin in hepatocellular carcinomas and dysplastic nodules. *J Korean Med Sci* 2005; **20**: 242-247

30 **Kanai Y**, Ushijima S, Hui AM, Ochiai A, Tsuda H, Sakamoto M, Hirohashi S. The E-cadherin gene is silenced by CpG methylation in human hepatocellular carcinomas. *Int J Cancer* 1997; **71**: 355-359

31 **Wei Y**, Van Nhieu JT, Prigent S, Srivatanakul P, Tiollais P, Buendia MA. Altered expression of E-cadherin in hepatocellular carcinoma: correlations with genetic alterations, beta-catenin expression, and clinical features. *Hepatology* 2002; **36**: 692-701

32 **Batlle E**, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, Garcia De Herreros A. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* 2000; **2**: 84-89

33 **Cano A**, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2000; **2**: 76-83

34 **Yamada S**, Nomoto S, Fujii T, Takeda S, Kanazumi N, Sugimoto H, Nakao A. Frequent promoter methylation of M-cadherin in hepatocellular carcinoma is associated with poor prognosis. *Anticancer Res* 2007; **27**: 2269-2274

35 **Bian J**, Wang Y, Smith MR, Kim H, Jacobs C, Jackman J, Kung HF, Colburn NH, Sun Y. Suppression of in vivo tumor growth and induction of suspension cell death by tissue inhibitor of metalloproteinases (TIMP)-3. *Carcinogenesis* 1996; **17**: 1805-1811

36 **Mannello F**, Gazzanelli G. Tissue inhibitors of metalloproteinases and programmed cell death: conundrums, controversies and potential implications. *Apoptosis* 2001; **6**: 479-482

37 **Lu GL**, Wen JM, Xu JM, Zhang M, Xu RB, Tian BL. Relationship between TIMP-3 expression and promoter methylation of TIMP-3 gene in hepatocellular carcinoma. *Zhonghua Binglixue Zazhi* 2003; **32**: 230-233

38 **Lee S**, Lee HJ, Kim JH, Lee HS, Jang JJ, Kang GH. Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. *Am J Pathol* 2003; **163**: 1371-1378

39 **Lai HJ**, Lo SJ. Epigenetic methylation of TIMP-3 may play a role in HBV-associated hepatocellular carcinoma. *Chang Gung Med J* 2005; **28**: 453-455

40 **Rao CN**, Liu YY, Peavey CL, Woodley DT. Novel extracellular matrix-associated serine proteinase inhibitors from human skin fibroblasts. *Arch Biochem Biophys* 1995; **317**: 311-314

41 **Sprecher CA**, Kisiel W, Mathewes S, Foster DC. Molecular cloning, expression, and partial characterization of a second human tissue-factor-pathway inhibitor. *Proc Natl Acad Sci USA* 1994; **91**: 3353-3357

42 **Rao CN**, Cook B, Liu Y, Chilukuri K, Stack MS, Foster DC, Kisiel W, Woodley DT. HT-1080 fibrosarcoma cell matrix degradation and invasion are inhibited by the matrix-associated serine protease inhibitor TFPI-2/33 kDa MSPI. *Int J Cancer* 1998; **76**: 749-756

43 **Rao CN**, Mohanam S, Puppala A, Rao JS. Regulation of ProMMP-1 and ProMMP-3 activation by tissue factor pathway inhibitor-2/matrix-associated serine protease inhibitor. *Biochem Biophys Res Commun* 1999; **255**: 94-98

44 **Wong CM**, Ng YL, Lee JM, Wong CC, Cheung OF, Chan CY, Tung EK, Ching YP, Ng IO. Tissue factor pathway inhibitor-2 as a frequently silenced tumor suppressor gene in hepatocellular carcinoma. *Hepatology* 2007; **45**: 1129-1138

45 **Kim H**, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994; **145**: 148-156

46 **Oliveira C**, Seruca R, Seixas M, Sobrinho-Simoes M. The clinicopathological features of gastric carcinomas with microsatellite instability may be mediated by mutations of different "target genes": a study of the TGFbeta RII, IGFII R, and BAX genes. *Am J Pathol* 1998; **153**: 1211-1219

47 **Park JH**, Cho SB, Lee WS, Park CH, Joo YE, Kim HS, Choi SK, Rew JS, Lee JH, Kim SJ. Methylation pattern of DNA repair genes and microsatellite instability in hepatocelluar carcinoma. *Korean J Gastroenterol* 2006; **48**: 327-336

48 **Matsukura S**, Soejima H, Nakagawachi T, Yakushiji H, Ogawa A, Fukuhara M, Miyazaki K, Nakabeppu Y, Sekiguchi M, Mukai T. CpG methylation of MGMT and hMLH1 promoter in hepatocellular carcinoma associated with hepatitis viral infection. *Br J Cancer* 2003; **88**: 521-529

49 **Wang L**, Bani-Hani A, Montoya DP, Roche PC, Thibodeau SN, Burgart LJ, Roberts LR. hMLH1 and hMSH2 expression in human hepatocellular carcinoma. *Int J Oncol* 2001; **19**: 567-570

50 **Zhang CJ**, Li HM, Yau LM, Suen KW, Zhou GY, Yu F, Liew CT. Methylation of mismatch repair gene (MMR) in primary hepatocellular carcinoma. *Zhonghua Binglixue Zazhi* 2004; **33**: 433-436

51 **Gerson SL**, Trey JE, Miller K, Berger NA. Comparison of O6-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissues. *Carcinogenesis* 1986; **7**: 745-749

52 **Esteller M**, Hamilton SR, Burger PC, Baylin SB, Herman

JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* 1999; **59**: 793-797

53 **Su PF**, Lee TC, Lin PJ, Lee PH, Jeng YM, Chen CH, Liang JD, Chiou LL, Huang GT, Lee HS. Differential DNA methylation associated with hepatitis B virus infection in hepatocellular carcinoma. *Int J Cancer* 2007; **121**: 1257-1264

54 **Matsukura S**, Soejima H, Nakagawachi T, Yakushiji H, Ogawa A, Fukuhara M, Miyazaki K, Nakabeppu Y, Sekiguchi M, Mukai T. CpG methylation of MGMT and hMLH1 promoter in hepatocellular carcinoma associated with hepatitis viral infection. *Br J Cancer* 2003; **88**: 521-529

55 **Zhang YJ**, Chen Y, Ahsan H, Lunn RM, Lee PH, Chen CJ, Santella RM. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation and its relationship to aflatoxin B1-DNA adducts and p53 mutation in hepatocellular carcinoma. *Int J Cancer* 2003; **103**: 440-444

56 **Brooks JD**, Weinstein M, Lin X, Sun Y, Pin SS, Bova GS, Epstein JI, Isaacs WB, Nelson WG. CG island methylation changes near the GSTP1 gene in prostatic intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 531-536

57 **Esteller M**, Corn PG, Urena JM, Gabrielson E, Baylin SB, Herman JG. Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. *Cancer Res* 1998; **58**: 4515-4518

58 **Lee S**, Lee HJ, Kim JH, Lee HS, Jang JJ, Kang GH. Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. *Am J Pathol* 2003; **163**: 1371-1378

59 **Zhang YJ**, Chen Y, Ahsan H, Lunn RM, Chen SY, Lee PH, Chen CJ, Santella RM. Silencing of glutathione S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. *Cancer Lett* 2005; **221**: 135-143

60 **Zhong S**, Tang MW, Yeo W, Liu C, Lo YM, Johnson PJ. Silencing of GSTP1 gene by CpG island DNA hypermethylation in HBV-associated hepatocellular carcinomas. *Clin Cancer Res* 2002; **8**: 1087-1092

61 **Anzola M**, Cuevas N, Lopez-Martinez M, Saiz A, Burgos JJ, de Pancorbo MM. No association between GSTP1 gene aberrant promoter methylation and prognosis in surgically resected hepatocellular carcinoma patients from the Basque Country (Northern Spain). *Liver Int* 2003; **23**: 249-254

62 **Wang J**, Qin Y, Li B, Sun Z, Yang B. Detection of aberrant promoter methylation of GSTP1 in the tumor and serum of Chinese human primary hepatocellular carcinoma patients. *Clin Biochem* 2006; **39**: 344-348

63 **Starr R**, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ. A family of cytokine-inducible inhibitors of signalling. *Nature* 1997; **387**: 917-921

64 **Endo TA**, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S, Yoshimura A. A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* 1997; **387**: 921-924

65 **Weber A**, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, Dietz A, Wittekind C, Tannapfel A. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. *Oncogene* 2005; **24**: 6699-6708

66 **Tischoff I**, Hengge UR, Vieth M, Ell C, Stolte M, Weber A, Schmidt WE, Tannapfel A. Methylation of SOCS-3 and SOCS-1 in the carcinogenesis of Barrett's adenocarcinoma. *Gut* 2007; **56**: 1047-1053

67 **Okochi O**, Hibi K, Sakai M, Inoue S, Takeda S, Kaneko T, Nakao A. Methylation-mediated silencing of SOCS-1 gene in hepatocellular carcinoma derived from cirrhosis. *Clin Cancer Res* 2003; **9**: 5295-5298

68 **Niwa Y**, Kanda H, Shikauchi Y, Saiura A, Matsubara K, Kitagawa T, Yamamoto J, Kubo T, Yoshikawa H. Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. *Oncogene* 2005; **24**: 6406-6417

69 **Miyoshi H**, Fujie H, Moriya K, Shintani Y, Tsutsumi T, Makuuchi M, Kimura S, Koike K. Methylation status of suppressor of cytokine signaling-1 gene in hepatocellular carcinoma. *J Gastroenterol* 2004; **39**: 563-569

70 **Yoshida T**, Ogata H, Kamio M, Joo A, Shiraishi H, Tokunaga Y, Sata M, Nagai H, Yoshimura A. SOCS1 is a suppressor of liver fibrosis and hepatitis-induced carcinogenesis. *J Exp Med* 2004; **199**: 1701-1707

71 **Tischoff I**, Markwarth A, Witzigmann H, Uhlmann D, Hauss J, Mirmohammadsadegh A, Wittekind C, Hengge UR, Tannapfel A. Allele loss and epigenetic inactivation of 3p21.3 in malignant liver tumors. *Int J Cancer* 2005; **115**: 684-689

72 **Dammann R**, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nat Genet* 2000; **25**: 315-319

73 **Liu L**, Tommasi S, Lee DH, Dammann R, Pfeifer GP. Control of microtubule stability by the RASSF1A tumor suppressor. *Oncogene* 2003; **22**: 8125-8136

74 **Dreijerink K**, Braga E, Kuzmin I, Geil L, Duh FM, Angeloni D, Zbar B, Lerman MI, Stanbridge EJ, Minna JD, Protopopov A, Li J, Kashuba V, Klein G, Zabarovsky ER. The candidate tumor suppressor gene, RASSF1A, from human chromosome 3p21.3 is involved in kidney tumorigenesis. *Proc Natl Acad Sci USA* 2001; **98**: 7504-7509

75 **Schagdarsurengin U**, Wilkens L, Steinemann D, Flemming P, Kreipe HH, Pfeifer GP, Schlegelberger B, Dammann R. Frequent epigenetic inactivation of the RASSF1A gene in hepatocellular carcinoma. *Oncogene* 2003; **22**: 1866-1871

76 **Zhong S**, Yeo W, Tang MW, Wong N, Lai PB, Johnson PJ. Intensive hypermethylation of the CpG island of Ras association domain family 1A in hepatitis B virus-associated hepatocellular carcinomas. *Clin Cancer Res* 2003; **9**: 3376-3382

77 **Qiu GH**, Tan LK, Loh KS, Lim CY, Srivastava G, Tsai ST, Tsao SW, Tao Q. The candidate tumor suppressor gene BLU, located at the commonly deleted region 3p21.3, is an E2F-regulated, stress-responsive gene and inactivated by both epigenetic and genetic mechanisms in nasopharyngeal carcinoma. *Oncogene* 2004; **23**: 4793-4806

78 **Agathanggelou A**, Dallol A, Zochbauer-Muller S, Morrissey C, Honorio S, Hesson L, Martinsson T, Fong KM, Kuo MJ, Yuen PW, Maher ER, Minna JD, Latif F. Epigenetic inactivation of the candidate 3p21.3 suppressor gene BLU in human cancers. *Oncogene* 2003; **22**: 1580-1588

79 **Tomizawa Y**, Sekido Y, Kondo M, Gao B, Yokota J, Roche J, Drabkin H, Lerman MI, Gazdar AF, Minna JD. Inhibition of lung cancer cell growth and induction of apoptosis after reexpression of 3p21.3 candidate tumor suppressor gene SEMA3B. *Proc Natl Acad Sci USA* 2001; **98**: 13954-13959

80 **Castro-Rivera E**, Ran S, Thorpe P, Minna JD. Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect. *Proc Natl Acad Sci USA* 2004; **101**: 11432-11437

81 **Kuroki T**, Trapasso F, Yendamuri S, Matsuyama A, Alder H, Williams NN, Kaiser LR, Croce CM. Allelic loss on chromosome 3p21.3 and promoter hypermethylation of semaphorin 3B in non-small cell lung cancer. *Cancer Res* 2003; **63**: 3352-3355

82 **Hesson L**, Bieche I, Krex D, Criniere E, Hoang-Xuan K, Maher ER, Latif F. Frequent epigenetic inactivation of RASSF1A and BLU genes located within the critical 3p21.3 region in gliomas. *Oncogene* 2004; **23**: 2408-2419

83 **Mitelman F**, Mertens F, Johansson B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat Genet* 1997; **15**: 417-474

84 **Smeets DF**, Scheres JM, Hustinx TW. The most common fragile site in man is 3p14. *Hum Genet* 1986; **72**: 215-220

85 **Zochbauer-Muller S**, Fong KM, Maitra A, Lam S, Geradts J, Ashfaq R, Virmani AK, Milchgrub S, Gazdar AF, Minna JD. 5' CpG island methylation of the FHIT gene is correlated with loss of gene expression in lung and breast cancer. *Cancer Res*

2001; **61**: 3581-3585
86 **Sun Y**, Geng XP, Zhu LX, Xiong QR, Qian YB, Dong GY, Li XM. Clinicopathological significance of aberrant methylation of the fragile histidine triad gene in patients with hepatocellular carcinoma. *Zhonghua Waike Zazhi* 2006; **44**: 609-612
87 **Toyota M**, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999; **96**: 8681-8686
88 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; **59**: 5438-5442
89 **Shen L**, Ahuja N, Shen Y, Habib NA, Toyota M, Rashid A, Issa JP. DNA methylation and environmental exposures in human hepatocellular carcinoma. *J Natl Cancer Inst* 2002; **94**: 755-761
90 **Liu WJ**, Wang L, Wang JP, Li JQ, Zhang CQ, Zheng L, Yuan YF. Correlations of CpG island methylator phenotype and OPCML gene methylation to carcinogenesis of hepatocellular carcinoma. *Ai Zheng* 2006; **25**: 696-700
91 **Zhang C**, Li Z, Cheng Y, Jia F, Li R, Wu M, Li K, Wei L. CpG island methylator phenotype association with elevated serum alpha-fetoprotein level in hepatocellular carcinoma. *Clin Cancer Res* 2007; **13**: 944-952
92 **Bestor TH**. Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. *EMBO J* 1992; **11**: 2611-2617
93 **Saito Y**, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Expression of mRNA for DNA methyltransferases and methyl-CpG-binding proteins and DNA methylation status on CpG islands and pericentromeric satellite regions during human hepatocarcinogenesis. *Hepatology* 2001; **33**: 561-568
94 **Nagai M**, Nakamura A, Makino R, Mitamura K. Expression of DNA (5-cytosin)-methyltransferases (DNMTs) in hepatocellular carcinomas. *Hepatol Res* 2003; **26**: 186-191
95 **Goll MG**, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* 2006; **311**: 395-398
96 **Park HJ**, Yu E, Shim YH. DNA methyltransferase expression and DNA hypermethylation in human hepatocellular carcinoma. *Cancer Lett* 2006; **233**: 271-278
97 **Saito Y**, Kanai Y, Nakagawa T, Sakamoto M, Saito H, Ishii H, Hirohashi S. Increased protein expression of DNA methyltransferase (DNMT) 1 is significantly correlated with the malignant potential and poor prognosis of human hepatocellular carcinomas. *Int J Cancer* 2003; **105**: 527-532
98 **Choi MS**, Shim YH, Hwa JY, Lee SK, Ro JY, Kim JS, Yu E. Expression of DNA methyltransferases in multistep hepatocarcinogenesis. *Hum Pathol* 2003; **34**: 11-17
99 **Eads CA**, Danenberg KD, Kawakami K, Saltz LB, Danenberg PV, Laird PW. CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. *Cancer Res* 1999; **59**: 2302-2306
100 **Feo F**, Pascale RM, Simile MM, De Miglio MR, Muroni MR, Calvisi D. Genetic alterations in liver carcinogenesis: implications for new preventive and therapeutic strategies. *Crit Rev Oncog* 2000; **11**: 19-62
101 **Chuang LS**, Ian HI, Koh TW, Ng HH, Xu G, Li BF. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* 1997; **277**: 1996-2000
102 **Saito Y**, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Overexpression of a splice variant of DNA methyltransferase 3b, DNMT3b4, associated with DNA hypomethylation on pericentromeric satellite regions during human hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2002; **99**: 10060-10065
103 **Gama-Sosa MA**, Slagel VA, Trewyn RW, Oxenhandler R, Kuo KC, Gehrke CW, Ehrlich M. The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Res* 1983; **11**: 6883-6894
104 **Kim YI**, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, Selhub J, Mason JB. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. *Cancer* 1994; **74**: 893-899
105 **Bedford MT**, van Helden PD. Hypomethylation of DNA in pathological conditions of the human prostate. *Cancer Res* 1987; **47**: 5274-5276
106 **Feinberg AP**, Gehrke CW, Kuo KC, Ehrlich M. Reduced genomic 5-methylcytosine content in human colonic neoplasia. *Cancer Res* 1988; **48**: 1159-1161
107 **Cravo M**, Pinto R, Fidalgo P, Chaves P, Gloria L, Nobre-Leitao C, Costa Mira F. Global DNA hypomethylation occurs in the early stages of intestinal type gastric carcinoma. *Gut* 1996; **39**: 434-438
108 **Shen L**, Fang J, Qiu D, Zhang T, Yang J, Chen S, Xiao S. Correlation between DNA methylation and pathological changes in human hepatocellular carcinoma. *Hepatogastroenterology* 1998; **45**: 1753-1759
109 **Lin CH**, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, Liaw YF. Genome-wide hypomethylation in hepatocellular carcinogenesis. *Cancer Res* 2001; **61**: 4238-4243
110 **Bratthauer GL**, Fanning TG. Active LINE-1 retrotransposons in human testicular cancer. *Oncogene* 1992; **7**: 507-510
111 **Jurgens B**, Schmitz-Drager BJ, Schulz WA. Hypomethylation of L1 LINE sequences prevailing in human urothelial carcinoma. *Cancer Res* 1996; **56**: 5698-5703
112 **Florl AR**, Lower R, Schmitz-Drager BJ, Schulz WA. DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. *Br J Cancer* 1999; **80**: 1312-1321
113 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676
114 **Cui R**, Nguyen TT, Taube JH, Stratton SA, Feuerman MH, Barton MC. Family members p53 and p73 act together in chromatin modification and direct repression of alpha-fetoprotein transcription. *J Biol Chem* 2005; **280**: 39152-39160

S- Editor Liu JN **E- Editor** Yin DH