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ORIGINAL ARTICLE

Prospective Study

Blood DNA methylation markers in prospectively identified hepatocellular carcinoma cases and controls from Taiwan

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Abstract

AIM: To determine if gene-specific DNA methylation in prospectively collected blood samples is associated with later development of hepatocellular carcinoma (HCC).

METHODS: Comparing genome-wide DNA methylation profiles using Illumina Human methylation 450K arrays, we previously identified a list of loci that were differentially methylated between tumor and adjacent nontumor tissues. To examine if dysregulation of DNA

methylation patterns observed in tumor tissues can be detected in white blood cell (WBC) DNA, we conducted a prospective case-control study nested within a community-based cancer screening cohort in Taiwan with 16 years of follow up. We measured methylation levels in ninety-six loci that were aberrant in DNA methylation in HCC tumor tissues compared to adjacent tissues. Baseline WBC DNA from 159 HCC cases and 312 matched controls were bisulfite treated and assayed by Illumina BeadArray. We used the χ^2 test for categorical variables and student's *t*-test for continuous variables to assess the difference in selected characteristics between cases and controls. To estimate associations with HCC risk, we used conditional logistic regression models stratified on the matching factors to calculate odds ratios (OR) and 95%CI.

RESULTS: We found that high methylation level in cg10272601 in *WNK2* was associated with increased risk of HCC, with an OR of 1.91 (95%CI: 1.27-2.86). High methylation levels in both cg12680131 in *TPO* and cg22511877 in *MYT1L*, however, were associated with decreased risk. The ORs (95%CI) were 0.59 (0.39-0.87) and 0.50 (0.33-0.77), respectively, for those with methylation levels of cg12680131 and cg22511877 above the median compared with those with levels below the median. These associations were still statistically significant in multivariable conditional logistic regression models after adjusting for hepatitis B virus infection and alcohol consumption.

CONCLUSION: These findings support the measurement of methylation markers in WBC DNA as biomarkers of HCC susceptibility but should be replicated in additional prospective studies.

Key words: DNA methylation; Epigenetics; Hepatitis B virus; Hepatocellular carcinoma; White blood cell DNA

Core tip: Hepatocellular carcinoma (HCC) is a highly fatal disease thus, the identification of biomarkers that could predict risk for development could enhance screening/early detection and prognosis. DNA methylation alterations are well established in HCC but whether changes in DNA methylation in white blood cells (WBC) are associated with increased risk of developing HCC is unknown. Taking advantage of a cancer screening program in Taiwan, we measured baseline WBC DNA methylation in prospectively collected blood samples at 96 CpG sites that were identified as differentially methylated in HCC tumors compared to adjacent tissues. Three were significantly associated with later development of HCC suggesting potential utility as a marker of risk.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most common cancers around the word[1]. Hepatitis B and C virus infection are the most important risk factors of HCC[2-4]. More recent studies have also identified the importance of exposure to alcohol, dietary aflatoxins and cigarette smoke[5-7].

The mechanisms of liver cancer induction are now known to include mutations in specific genes and epigenetic alterations such as changes in DNA methylation and microRNA expression. These changes lead to changes in expression of oncogenes and tumor suppressor genes[8-10]. DNA hypermethylation can silence tumor suppressor genes while hypomethylation can activate oncogenes[11,12]. Using Illumina Humanmethylation 27K and 450K BeadChips, we previously reported a distinct DNA methylation pattern between HCC tumor and paired adjacent nontumor tissues (NCBI's GEO database accession numbers GSE54503 and GSE37988)[13,14]. In one of the studies, we found 28017 CpG sites hypermethylated and 102495 hypomethylated in tumor tissues compared with paired adjacent tissues[14], suggesting their role in HCC tumorigenesis.

Using data on baseline white blood cell (WBC) DNA banked up to 16 years before diagnosis, we recently reported that global hypomethylation of Sat2, a repetitive element, was associated with increased HCC risk[15] and was also associated with high AFB_1 exposure[16]. These results suggest that decreased overall DNA methylation in WBC DNA can be used as a biomarker for HCC risk.

The main aim of this study was to examine whether the dysregulation of DNA methylation markers observed in tumor tissues can be detected in WBC DNA. We measured methylation levels in ninety-six loci in WBC DNA from 159 HCCs who developed cancer after enrollment in a community-based cancer screening program in Taiwan[5,6,15] and compared them with 312 controls who remained cancer free in the same cohort.

MATERIALS AND METHODS

Study population

This study included individuals who participated in a Cancer Screening Program cohort in Taiwan. This study was approved by both the Institutional Review Board of Columbia University and the Research Ethics Committee of the College of Public Health at National Taiwan University. We obtained written informed consent from all study subjects in this study.

Detail information regarding the cohort description and screening procedure and follow-up was provided in previous publications[5,6,15,16]. Between July 1990 and June 1992, 12020 males and 11924 females aged from

Table 1 Sociodemographic characteristics of hepatocellular carcinoma cases and matched controls

Variable	Cases *n* = 159	%	Controls *n* = 312	%	*P*
Age (yr, mean, SD)	52.8 (8.0)		53.1 (7.8)		0.72
BMI (mean, SD)	24.3 (3.6)		24.8 (3.7)		0.13
Gender					
Female	77	48	148	47	0.92
Male	82	52	164	53	
HBsAg					
Negative	65	41	238	76	< 0.0001
Positive	93	59	72	23	
Missing	1	< 1	2	< 1	
Anti-HCV					
Negative	109	69	243	78	< 0.0001
Positive	29	18	15	5	
Missing	21	13	54	17	
Smoking					
Never	97	61	184	59	0.67
Ever	62	39	128	41	
Alcohol					
Never	130	82	276	89	0.046
Ever	29	18	36	12	

HBsAg: Hepatitis B virus surface antigen; BMI: Body mass index; HCV: Hepatitis C virus.

30 to 65 years old and who lived in seven towns in Taiwan were enrolled in this study. Each participant filled out a structured questionnaire to collect information including demographic characteristics, history of alcohol intake and cigarette smoking, history of chronic disease and family history of cancers, including HCC. Each participant also donated a fasting blood sample during the time of recruitment.

In this study, we used blood collected from 159 participants who were diagnosed with HCC during the interval between their blood draw and June 2008. We also used blood from 312 controls who remained cancer free in the same cohort. Controls were selected by matching to each case by age (within 5 years), sex, residential area and time of recruitment (within 3 mo). Baseline WBCs were shipped to Columbia University on dry ice for DNA isolation and DNA methylation measurement.

DNA bisulfite conversion

We extracted genomic DNA from WBC using a salting out procedure. We bisulfite-treated an aliquot of DNA (500 ng) with EZ DNA methylation kits (Zymo Research, Orange, CA). The bisulfite DNA was resuspended in 20 μL of distilled water and stored at -20 ℃ until use.

Loci selection and methylation measurement

We selected 96 CpG sites that previously had shown either hyper- or hypomethylation in HCC tumor compared to paired adjacent nontumor tissues in our 450k array data[14]. We selected our target CpG sites from among the top 250 most hyper or hypomethylated sites. Our selection of targets was based on the following criteria: (1) the largest methylation differences between tumor and adjacent tissues; (2) half of the CpG sites showing hypomethylation and half hypermethylation; and (3) one site per gene. Due to the inability to design primers for some sites, we have 65 CpG sites with hypermethylation and 31 CpG sites with hypomethylation. DNA methylation analysis was measured using an Illumina GoldenGate assay with BeadArray technology. The arrays were customized to measure methylation covering the CpG sites identified in the 450k array. DNA methylation values were scored as β-values which ranges between 0 and 1.

Statistical analysis

We used the χ^2 test and/or student's *t*-test to assess the difference in selected variables between cases and controls. To estimate associations between methylation markers and HCC risk, we used a conditional logistic regression model using PROC PHREG procedure. Subjects were divided into different methylation groups: Those with methylation levels above the median value for all controls sample *vs* those below the median. In the multivariable model, we modeled the associations of methylation in cg10272601 in *WNK2*, cg12680131 in *TPO* and cg22511877 in *MYT1L* adjusting for, hepatitis B virus surface antigen (HBsAg) (Yes *vs* No), and history of alcohol intake (Ever *vs* Never) in the model. All analyses were performed with SAS software 9.2 (SAS Institute, Cary, NC).

RESULTS

The distributions of subjects' characteristics at baseline for cases and matched controls is given in Table 1. The distributions of matching factors including age, sex were similar between cases and controls. There were 51.7% and 52.5% males in cases and controls, respectively. The distribution of smoking was also similar, while the percentage of ever alcohol consumption was slightly lower in controls (11.5%) than in cases (18.2%). The percents positive for HBsAg and anti-HCV were higher in cases than in matched controls [58.5% *vs* 23.1% for HBsAg (+) and 18.2% *vs* 4.8% for anti-HCV (+)].

Table 2 presents the distributions of the 96 methylation markers by HCC status. The mean values of methylation vary by methylation markers. Fifty DNA methylation markers had mean methylation values below 10% in cases and controls. Nineteen DNA methylation markers had mean methylation values above 90%. About 27 DNA methylation markers had mean methylation levels between 10% and 90%. The mean levels of three DNA methylation markers were statistically significantly different between cases and controls, including cg10272601, cg12680131, and cg22511877. The mean methylation beta values for cg1027261 were 0.30 ± 0.07 for cases and 0.28 ± 0.08 for controls (P = 0.04). Values for cg12680131 were 0.80 ± 0.09 and 0.82 ± 0.11 for cases and controls, respectively (P = 0.02) and for cg22511877, 0.56 ± 0.17 for cases and 0.60 ± 0.16 for controls (P = 0.01).

Table 2 Distribution of DNA methylation by hepatocellular carcinoma status

Locus	Gene	HCC cases		Controls		P^1
		Mean	SD	Mean	SD	
cg00028598	*GABRA5*	0.92	0.04	0.92	0.07	0.81
cg00108164	*ACP1*	0.01	0.02	0.00	0.01	0.55
cg00249511	*SCT*	0.01	0.04	0.01	0.04	0.80
cg00753478	*LDHB*	0.09	0.08	0.08	0.06	0.12
cg00817367	*GRASP*	0.01	0.04	0.01	0.01	0.23
cg00939495	*DRD5*	0.22	0.10	0.22	0.12	0.95
cg01530024	*STK32B*	0.97	0.08	0.97	0.07	0.79
cg01566592	*RIMS2*	0.10	0.09	0.09	0.08	0.32
cg01860297	*BASP1*	0.96	0.03	0.95	0.08	0.49
cg02527669	*OBSL1*	0.02	0.02	0.03	0.05	0.53
cg02553663	*SECTM1*	0.03	0.04	0.03	0.03	0.65
cg02710296	*C1orf14*	0.33	0.11	0.33	0.11	0.92
cg02736548	*FAM109B*	0.08	0.09	0.08	0.09	0.46
cg03306486	*APC2*	0.02	0.02	0.01	0.02	0.44
cg03396005	*APCDD1*	0.92	0.04	0.92	0.06	0.99
cg03621881	*BRUNOL6*	0.04	0.05	0.03	0.04	0.70
cg04920951	*GSTP1*	0.01	0.07	0.00	0.02	0.22
cg05328339	*PTPRN2*	0.89	0.09	0.88	0.10	0.55
cg05661282	*ZNF154*	0.03	0.05	0.03	0.08	0.75
cg05699035	*KCNK2*	0.86	0.07	0.86	0.08	0.99
cg05833351	*CUGBP2*	0.95	0.07	0.95	0.08	0.70
cg05970721	*HS3ST2*	0.90	0.10	0.91	0.10	0.49
cg06382344	*TBR1*	0.02	0.03	0.03	0.05	0.13
cg06445348	*ILDR2*	0.02	0.06	0.01	0.01	0.24
cg06641285	*TIMP2*	0.02	0.02	0.02	0.05	0.74
cg07061738	*SMOC2*	0.94	0.08	0.94	0.11	0.75
cg07689503	*MTHFD2*	0.00	0.00	0.00	0.01	0.27
cg07759394	*GLB1L2*	0.01	0.03	0.01	0.02	0.44
cg07765706	*KCNQ3*	0.95	0.03	0.95	0.08	0.12
cg08328777	*DUOX1*	0.07	0.05	0.07	0.06	0.30
cg08714590	*FZD1*	0.86	0.12	0.86	0.12	0.43
cg08738570	*C1orf70*	0.09	0.10	0.09	0.08	0.69
cg09210956	*SNTG2*	0.67	0.09	0.67	0.12	0.93
cg09433131	*KCNB2*	0.94	0.06	0.93	0.10	0.43
cg09489445	*ZNF788*	0.01	0.03	0.01	0.04	0.92
cg09901035	*PLEKHG4B*	0.87	0.06	0.87	0.08	0.66
cg10272601	*WNK2*	0.30	0.07	0.28	0.08	0.04
cg10342963	*IGF1R*	0.81	0.13	0.79	0.15	0.07
cg11349423	*OPCML*	0.48	0.15	0.48	0.16	0.93
cg11377136	*PKDREJ*	0.03	0.03	0.03	0.03	0.69
cg11686528	*ABR*	0.01	0.07	0.01	0.06	0.60
cg12296772	*MTMR7*	0.07	0.06	0.07	0.07	0.78
cg12610564	*SLC39A12*	0.98	0.01	0.97	0.07	0.09
cg12680131	*TPO*	0.80	0.09	0.82	0.11	0.02
cg12852139	*MYO10*	0.96	0.02	0.95	0.06	0.70
cg13204512	*RNF135*	0.01	0.06	0.01	0.02	0.23
cg13517866	*SMOC2*	0.89	0.11	0.89	0.10	0.58
cg13564825	*PPP1R14A*	0.01	0.05	0.01	0.02	0.98
cg13604246	*ANKMY1*	0.11	0.08	0.11	0.09	0.68
cg13611121	*COL5A1*	0.80	0.08	0.80	0.10	0.73
cg13782274	*KCNQ2*	0.94	0.08	0.93	0.11	0.39
cg13791254	*FOXE1*	0.02	0.02	0.01	0.03	0.62
cg13879483	*USP44*	0.08	0.06	0.07	0.07	0.34
cg13895235	*PRKAR1B*	0.01	0.01	0.01	0.03	0.39
cg14183206	*HLA-L*	0.24	0.10	0.23	0.09	0.61
cg14486338	*KCNS2*	0.12	0.07	0.12	0.07	0.53
cg14644001	*PRRT1*	0.04	0.03	0.04	0.05	0.63
cg14645545	*SLC11A1*	0.20	0.12	0.19	0.12	0.83
cg14715697	*HRNBP3*	0.70	0.08	0.71	0.08	0.20
cg14866200	*SHISA3*	0.02	0.07	0.02	0.06	0.74
cg14988503	*CDKL2*	0.02	0.03	0.02	0.03	0.85
cg15092343	*MSX1*	0.07	0.05	0.07	0.04	0.48
cg15167871	*TCERG1L*	0.92	0.10	0.92	0.11	0.98
cg15549700	*AJAP1*	0.96	0.05	0.96	0.08	0.53
cg15760257	*SARM1*	0.01	0.01	0.01	0.05	0.35
cg17264670	*RGS17*	0.08	0.06	0.08	0.08	0.94
cg17497608	*FZD1*	0.83	0.11	0.84	0.12	0.43
cg17725364	*COL6A3*	0.96	0.10	0.96	0.09	0.86
cg18537730	*IZUMO1*	0.16	0.07	0.16	0.08	0.63
cg19429281	*ZNF702P*	0.02	0.01	0.02	0.03	0.40
cg19464917	*ISL2*	0.06	0.04	0.05	0.03	0.17
cg20129213	*RIMS2*	0.01	0.05	0.01	0.05	0.47
cg20399616	*BCAT1*	0.05	0.08	0.04	0.08	0.40
cg21385746	*LOC150568*	0.96	0.10	0.95	0.11	0.80
cg21472506	*OTX1*	0.01	0.04	0.01	0.04	0.98
cg21790626	*ZNF154*	0.04	0.04	0.05	0.05	0.32
cg22403469	*RIMBP2*	0.83	0.05	0.83	0.08	0.63
cg22511877	*MYT1L*	0.56	0.17	0.60	0.16	0.01
cg22524061	*OSR2*	0.23	0.09	0.22	0.09	0.48
cg22655988	*CRMP1*	0.96	0.08	0.96	0.10	0.77
cg22789900	*MIXL1*	0.00	0.01	0.01	0.04	0.55
cg23004031	*MGMT*	0.55	0.31	0.58	0.32	0.41
cg23391785	*DNM3*	0.02	0.06	0.01	0.04	0.28
cg23498518	*POM121L12*	0.79	0.07	0.80	0.10	0.36
cg23864180	*ADARB2*	0.90	0.06	0.91	0.07	0.26
cg24274117	*C20orf195*	0.03	0.07	0.04	0.07	0.52
cg24425838	*C2CD4D*	0.05	0.08	0.05	0.07	0.98
cg24432073	*CDKL2*	0.02	0.03	0.02	0.04	0.84
cg24563094	*FAM59B*	0.10	0.04	0.10	0.05	0.55
cg24602704	*ATP10A*	0.97	0.02	0.97	0.07	0.46
cg24816460	*CDYL*	0.03	0.07	0.03	0.07	0.51
cg25480336	*ZFP64*	0.01	0.02	0.01	0.01	0.16
cg25577023	*AMN*	0.09	0.09	0.09	0.09	0.82
cg25622366	*OTX1*	0.02	0.07	0.02	0.05	0.66
cg26010734	*EPHX3*	0.05	0.05	0.05	0.04	0.43
cg26841013	*WNT3A*	0.03	0.02	0.03	0.03	0.45

[1]*P* value for student's *t*-test.

Table 3 White blood cell DNA methylation and hepatocellular carcinoma risk

Locus		Cases/ controls	OR (95%CI)
WNK2 cg10272601	Below median (< 0.279)	56/157	1.0
	Above median (≥ 0.279)	103/155	1.91 (1.27-2.86)
TPO cg12680131	Below median (< 0.836)	102/157	1.0
	Above median (≥ 0.836)	57/155	0.59 (0.39-0.87)
MYT1L cg22511877	Below median (< 0.636)	105/159	1.0
	Above median (≥ 0.636)	54/153	0.50 (0.33-0.77)

The association between DNA methylation of cg10272601, cg12680131, and cg22511877 and HCC are given in Table 3. The OR for those with cg10272601 methylation above the median was 1.91 (95%CI: 1.27-2.86). Individuals with a cg12680131 methylation level above the median had lower risk of HCC, with an OR of 0.59 (95%CI: 0.39-0.87). The OR was 0.50 (95%CI: 0.33-0.77) for those with cg22511877 methylation above median.

Table 4 shows the multiple variables conditional logistic regression model. Overall, HBsAg (+) was associated with increased HCC risk (OR = 5.50, 95%CI: 3.34-9.03) compared with HBsAg(-). Ever smokers had a 2.1-fold increased risk of developing HCC (OR = 2.10, 95%CI: 1.08-4.07). The ORs (95%CI) were 2.26 (1.42-3.61), 0.55 (0.34-0.87), and 0.53 (0.32-0.88) for cg10272601, cg12680131, and cg22511877 hypermethylation.

Table 4 Multiple variables model for DNA methylation and hepatocellular carcinoma risk

Variable	OR (95%CI)	*P*
WNK2 cg10272601[1]	2.26 (1.42-3.61)	0.0006
TPO cg12680131[2]	0.55 (0.34-0.87)	0.01
MYT1L cg22511877[3]	0.53 (0.32-0.88)	0.01
HBsAg (positive *vs* negative)	5.50 (3.34-9.03)	< 0.0001
Alcohol (yes *vs* no)	2.10 (1.08-4.07)	0.03

[1]Above or below the median of 0.279; [2]Above or below the median of 0.836; [3]Above or below the median of 0.636. HBsAg: Hepatitis B virus surface antigen.

DISCUSSION

Alterations in methylation of cg10272601, cg12680131, and cg22511877 were associated with risk for later HCC development. Consistent with our tissue data, we found that a high methylation level in cg10272601 was associated with increased risk of HCC, while high methylation levels in both cg12680131 and cg22511877 were associated with decreased risk. In the 450k data, the mean beta values were 0.52 ± 0.22 for cg10272601, 0.28 ± 0.21 for cg12680131, and 0.34 ± 0.26 for cg22511877 in HCC tumors[14]. The corresponding beta values were 0.10 ± 0.06, 0.79 ± 0.08, 0.87±0.05, respectively, in adjacent nontumor tissues.

cg10272601 is located at transcription start site (TSS) 200 of *WNK2*, a gene encoding a serine-threonine kinase on chromosome 9q22.31[17]. *WNK2* acts as a tumor suppressor gene by suppressing the ERK/MAPK-pathway and downstream cell cycle progression[18] and *WNK2* expression inhibited colony formation[19], suggesting a role in cell growth suppression. Dense high methylation at the CpG island was associated with decreased WNK2 expression[19]. Hypermethylation of *WNK2* was reported in many cancers, including pancreatic ductal adenocarcinoma[20], HCC[14,21], and gliomas[22].

cg12680131 is located on chromosome 2p25 at TSS 200 of thyroid peroxidase (*TPO*), a key enzyme in thyroid hormone synthesis. Mutations in *TPO* are associated with several disorders of thyroid hormonogenesis[23]. The association of methylation and expression of TPO has not been studied and the role of TPO in carcinogenesis has not been reported. cg22511877 is located at a shore region of myelin transcription factor 1-like (*MYT1L*) also on chromosome 2p25. MYT1L is a main member of the MYT/NZF family of transcription factors[24,25]. Limited data suggests a polymorphism in *MYT1L* is associated with gastric cancer outcome in a Chinese population[26]. Future studies are needed to understand the mechanisms of hypomethylation of both *TPO* and *MYT1L* in hepatocarcinogenesis.

The main limitation of this study is that we did not adjust for multiple comparisons due to the limited sample size. However, in further data analysis, we also observed significant associations of methylation in these 3 CpG sites with HCC risk after adjusting for HBV infection and alcohol consumption, suggesting an independent effect in HCC risk.

This study, using prospective study design, allowed us to produce causal evidence on DNA methylation in WBC and cancer susceptibility[27]. Using information from HCC tumor tissues, our study investigated the associations of HCC-specific differentially methylated loci observed in tumor tissues in WBC DNA with HCC risk.

In summary, we provide new evidence that specific loci methylation in WBC DNA is associated with increased HCC susceptibility. These finding could lead to development of a simple non-invasive blood measure of DNA methylation to identify people at high risk of HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is a highly devastating disease with a poor prognosis. Thus, methods that allow the identification of individuals at elevated risk of HCC should greatly enhance screening for early diagnosis and improve prognosis. While several risk factors are well known such as infection with hepatitis B or C virus, not all viral-infected individuals develop cancer. Additional biomarkers of risk are therefore needed.

Research frontiers

It is known that tumors release DNA into the blood stream and that this DNA contains the same DNA alterations both mutations and changes in DNA methylation that are found in the tumor. Thus, researchers have been able to develop assays for tumor DNA in plasma/serum for early diagnosis. There is also limited data in some cancers, not HCC, that DNA methylation changes in blood cells differs between cases and controls.

Innovations and breakthroughs

This study is the first to investigate whether DNA methylation in specific genes in white blood cells is predictive of later HCC development.

Applications

While the study needs confirmation in another population, it suggests that it may be possible to develop risk prediction models that include white blood cell DNA methylation markers.

Peer-review

This is a very interesting paper. The authors found the correlation between DNA methylation and HCC occurring. The results provide sufficient experimental evidence or data to draw firm scientific conclusions.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Takano S**, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995; **21**: 650-655 [PMID: 7875662 DOI: 10.1002/hep.1840210308]

3 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405 DOI: 10.1056/NEJMoa013215]

4 **Thein HH**, Walter SR, Gidding HF, Amin J, Law MG, George J, Dore GJ. Trends in incidence of hepatocellular carcinoma after diagnosis of hepatitis B or C infection: a population-based cohort study, 1992-2007. *J Viral Hepat* 2011; **18**: e232-e241 [PMID: 21692938 DOI: 10.1111/j.1365-2893.2011.01440.x]

5 **Wang LY**, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang GT, Yang PM, Lee HS, Santella RM. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. *Int J Cancer* 1996; **67**: 620-625 [PMID: 8782648]

6 **Wu HC**, Wang Q, Yang HI, Ahsan H, Tsai WY, Wang LY, Chen SY, Chen CJ, Santella RM. Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 846-853 [PMID: 19273485 DOI: 10.1158/1055-9965.EPI-08-0697]

7 **Chen CJ**, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF, Chang WY, Sheen MC, Lin TM. Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. *Hepatology* 1991; **13**: 398-406 [PMID: 1847891 DOI: 10.1002/hep.1840130303]

8 **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 [PMID: 14507645 DOI: 10.1016/S0002-9440(10)63495-5]

9 **Herath NI**, Leggett BA, MacDonald GA. Review of genetic and epigenetic alterations in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2006; **21**: 15-21 [PMID: 16706806 DOI: 10.1111/j.1440-1746.2005.04043.x]

10 **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 [PMID: 12011226 DOI: 10.1093/jnci/94.10.755]

11 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769]

12 **Tycko B**. Genetic and epigenetic mosaicism in cancer precursor tissues. *Ann N Y Acad Sci* 2003; **983**: 43-54 [PMID: 12724211 DOI: 10.1111/j.1749-6632.2003.tb05961.x]

13 **Shen J**, Wang S, Zhang YJ, Kappil M, Wu HC, Kibriya MG, Wang Q, Jasmine F, Ahsan H, Lee PH, Yu MW, Chen CJ, Santella RM. Genome-wide DNA methylation profiles in hepatocellular carcinoma. *Hepatology* 2012; **55**: 1799-1808 [PMID: 22234943 DOI: 10.1002/hep.25569]

14 **Shen J**, Wang S, Zhang YJ, Wu HC, Kibriya MG, Jasmine F, Ahsan H, Wu DP, Siegel AB, Remotti H, Santella RM. Exploring genome-wide DNA methylation profiles altered in hepatocellular carcinoma using Infinium HumanMethylation 450 BeadChips. *Epigenetics* 2013; **8**: 34-43 [PMID: 23208076 DOI: 10.4161/epi.23062]

15 **Wu HC**, Wang Q, Yang HI, Tsai WY, Chen CJ, Santella RM. Global DNA methylation levels in white blood cells as a biomarker for hepatocellular carcinoma risk: a nested case-control study. *Carcinogenesis* 2012; **33**: 1340-1345 [PMID: 22581841 DOI: 10.1093/carcin/bgs160]

16 **Wu HC**, Wang Q, Yang HI, Tsai WY, Chen CJ, Santella RM. Global DNA methylation in a population with aflatoxin B1 exposure. *Epigenetics* 2013; **8**: 962-969 [PMID: 23867725 DOI: 10.4161/epi.25696]

17 **Hong C**, Moorefield KS, Jun P, Aldape KD, Kharbanda S, Phillips HS, Costello JF. Epigenome scans and cancer genome sequencing converge on WNK2, a kinase-independent suppressor of cell growth. *Proc Natl Acad Sci USA* 2007; **104**: 10974-10979 [PMID: 17578925 DOI: 10.1073/pnas.0700683104]

18 **Moniz S**, Veríssimo F, Matos P, Brazão R, Silva E, Kotelevets L, Chastre E, Gespach C, Jordan P. Protein kinase WNK2 inhibits cell proliferation by negatively modulating the activation of MEK1/ERK1/2. *Oncogene* 2007; **26**: 6071-6081 [PMID: 17667937 DOI: 10.1038/sj.onc.1210706]

19 **Jun P**, Hong C, Lal A, Wong JM, McDermott MW, Bollen AW, Plass C, Held WA, Smiraglia DJ, Costello JF. Epigenetic silencing of the kinase tumor suppressor WNK2 is tumor-type and tumor-grade specific. *Neuro Oncol* 2009; **11**: 414-422 [PMID: 19001526 DOI: 10.1215/15228517-2008-096]

20 **Dutruel C**, Bergmann F, Rooman I, Zucknick M, Weichenhan D, Geiselhart L, Kaffenberger T, Rachakonda PS, Bauer A, Giese N, Hong C, Xie H, Costello JF, Hoheisel J, Kumar R, Rehli M, Schirmacher P, Werner J, Plass C, Popanda O, Schmezer P. Early epigenetic downregulation of WNK2 kinase during pancreatic ductal adenocarcinoma development. *Oncogene* 2014; **33**: 3401-3410 [PMID: 23912455 DOI: 10.1038/onc.2013.312]

21 **Tao R**, Li J, Xin J, Wu J, Guo J, Zhang L, Jiang L, Zhang W, Yang Z, Li L. Methylation profile of single hepatocytes derived from hepatitis B virus-related hepatocellular carcinoma. *PLoS One* 2011; **6**: e19862 [PMID: 21625442 DOI: 10.1371/journal.pone.0019862]

22 **Moniz S**, Martinho O, Pinto F, Sousa B, Loureiro C, Oliveira MJ, Moita LF, Honavar M, Pinheiro C, Pires M, Lopes JM, Jones C, Costello JF, Paredes J, Reis RM, Jordan P. Loss of WNK2 expression by promoter gene methylation occurs in adult gliomas and triggers Rac1-mediated tumour cell invasiveness. *Hum Mol Genet* 2013; **22**: 84-95 [PMID: 23035050]

23 **Cangul H**, Aycan Z, Olivera-Nappa A, Saglam H, Schoenmakers NA, Boelaert K, Cetinkaya S, Tarim O, Bober E, Darendeliler F, Bas V, Demir K, Aydin BK, Kendall M, Cole T, Högler W, Chatterjee VK, Barrett TG, Maher ER. Thyroid dyshormonogenesis is mainly caused by TPO mutations in consanguineous community. *Clin Endocrinol* (Oxf) 2013; **79**: 275-281 [PMID: 23236987 DOI: 10.1111/cen.12127]

24 **Stevens SJ**, van Ravenswaaij-Arts CM, Janssen JW, Klein Wassink-Ruiter JS, van Essen AJ, Dijkhuizen T, van Rheenen J, Heuts-Vijgen R, Stegmann AP, Smeets EE, Engelen JJ. MYT1L is a candidate gene for intellectual disability in patients with 2p25.3 (2pter) deletions. *Am J Med Genet A* 2011; **155A**: 2739-2745 [PMID: 21990140 DOI: 10.1002/ajmg.a.34274]

25 **Kim JG**, Armstrong RC, v Agoston D, Robinsky A, Wiese C, Nagle J, Hudson LD. Myelin transcription factor 1 (Myt1) of the oligodendrocyte lineage, along with a closely related CCHC zinc finger, is expressed in developing neurons in the mammalian central nervous system. *J Neurosci Res* 1997; **50**: 272-290 [PMID: 9373037]

26 **Zhang Y**, Zhu H, Zhang X, Gu D, Zhou X, Wang M, Cao C, Zhang X, Wu X, Gong W, Tang Y, Zhou J, Tang C, Zhang Z, Chen J. Clinical significance of MYT1L gene polymorphisms in Chinese patients with gastric cancer. *PLoS One* 2013; **8**: e71979 [PMID: 24015200 DOI: 10.1371/journal.pone.0071979]

27 **Terry MB**, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM. DNA methylation in white blood cells: association with risk factors in epidemiologic studies. *Epigenetics* 2011; **6**: 828-837 [PMID: 21636973 DOI: 10.4161/epi.6.7.16500]

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