Exp Oncol 2007
29, 1, 71–73

SHORT COMMUNICATION

Experimental ONCOLOGY

SMYD3 TANDEM REPEATS POLYMORPHISM IS NOT ASSOCIATED WITH THE OCCURRENCE AND METASTASIS OF HEPATOCELLULAR CARCINOMA IN A CHINESE POPULATION

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A variable number of tandem repeats (VNTR) polymorphism in regulatory region of *SMYD3* coding for histone methyltransferase has been shown to be associated with colorectal cancer, hepatocellular carcinoma (HCC), and breast cancer in Japanese population. *Aim* of the study is to investigate the potential association between the functional *SMYD3* tandem repeats polymorphism and HCC in Chinese population. *Material and Methods:* The case-control study included 200 HCC patients and 261 healthy controls. The VNTR polymorphism in the promoter of *SMDY3* was genotyped by PCR and direct-sequencing analysis. Odds ratio and 95% confidence interval were used to estimate the association between the polymorphisms and risk of HCC. *Results:* The allele frequencies for *SMYD3* 2 and 3 repeats were 15.71% and 84.29% among controls; and 12.75%, and 87.25% among cases ($P = 0.22$). The odds ratio for 3/3 *versus* 2/2 and 2/3 genotypes was 1.30 ($P = 0.18$). The frequencies of 3 alleles were not increased with HCC stage increased (trend test, $P = 0.45$). *Conclusion: SMYD3* polymorphism is not associated with the occurrence and metastasis of HCC in Chinese population.
Key Words: **SMYD3, polymorphism, hepatocellular carcinoma.**

SMYD3, a histone methyltransferase, was first found upregulated in human colorectal cancer (CRC) and hepatocellular carcinoma (HCC) [1]. Its overexpression promotes cell proliferation particularly in tumors, indicating that *SMYD3* may play an important role in carcinogenesis [1]. Recently, Tsuge et al. [2] reported a variable number of the tandem repeats (VNTR) polymorphism CCGCC in the 5′ flanking region of *SMYD3*, which is a binding site of E2F-1. The E2F-1-binding affinity is higher to three repeats and results in an increased promoter activity compared with the two repeats. Furthermore, the three repeats have been identified as a high-risk allele associated with CRC, HCC, and breast cancer in a Japanese population [2]. However, the results from other study were not consistent. For example, the association of 3/3 polymorphism of *SMYD3* could not be found with both familial breast cancer and high-risk familial breast cancer cases [3]. Genetic disorders are complex to certain diseases, particularly for cancer, and genetic susceptibility to disease may vary among populations due to allele differences in both frequencies and composition as illustrated by the recent data generated by the HapMap project [4]. To investigate the potential association between the functional *SMYD3* tandem repeats polymorphism and HCC in the Chinese population, we conducted this case-control study among 200 HCCs and 261 healthy controls.

Subjects and samples. This study included 200 HCC patients and 261 healthy controls. All subjects were genetically unrelated ethnic Han Chinese. Patients with HCC were newly diagnosed based on pathological analyses and classified according to the tumor-node-metastasis classification [5] at Queen Mary Hospital, Hong Kong. Population controls were partially from healthy blood donors randomly collected from Red Cross of Hong Kong and partially from healthy individuals in the same period as the HCC cases were collected and most of them were enrolled in other previous studies of our department [6]. The study was approved by the local Institutional Review Board. Mean age (± SD) and gender proportion in each group were as follows: HCC, 54.44 ± 10.82, and 82.5% male; controls, 44.30 ± 17.22, and 53.3% male. Of the HCC patients, 85% were hepatitis B virus positive whereas 6% were hepatitis C virus positive. Among the 200 HCC patients, the distribution of tumor-node-metastasis stage I, II, II, and IV were 39%, 26%, 33.5%, and 1.5%, respectively. Genomic DNA was extracted from the peripheral blood leukocytes using the QIAamp blood kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

VNTR polymorphism of SMDY3. The VNTR polymorphism in the promoter of *SMDY3* was genotyped by PCR and direct-sequencing analysis. Primers used for PCR VNTR DNA fragments were the same as described previously [2]. The resulting PCR fragments were then subjected to DNA sequencing analysis by an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA) using the BigDye Terminator (v3.1).

Statistical analysis. The association between the polymorphisms and risk of HCC was estimated using odds ratio and its 95% confidence interval, which were calculated by unconditional logistic regression and adjusted for age and gender. All analyses were performed with Statistical Analysis System software (version 6.12; SAS Institute, Cary, NC).

The distribution of the VNTR polymorphism of *SMYD3* in this study is listed in Table 1. The allele frequencies for *SMYD3* 2 repeats and 3 repeats were 15.71% and 84.29% among the 261 controls; and 12.75%, and 87.25% among the 200 HCC cases. These differences were not statistically significant

Received: December 29, 2006.
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Abbreviations used: HCC – hepatocellular carcinoma; VNTR – variable number of tandem repeats.

(P = 0.22). The frequency of 3/3 genotype was 75.5% in HCC group and 69.3% in control group. The odds ratio of 3/3 genotype compared with 2/2 or 2/3 genotype was 1.30 (95% confidence interval, 0.81–2.06), which was not statistically significant (P = 0.18). From both allele and repeat frequencies of *SMYD3*, there was no significant difference between control and HCC group. Furthermore, we analyzed the correlation between the *SMYD3* genotype and metastasis of HCC (Table 2), but the distributions of *SMYD3* 3/3 genotype among different stages of HCC were similar (79.5%, 71.2%, and 74.3% in stages I, II, and III + IV, respectively; $P_{\text{for trend}}$ = 0.45).

Table 1. *SMYD3* polymorphism and HCC risk

SMYD3	Controls (n = 261) n (%)	Cases (n = 200) n (%)	Odds ratio (95% CI)	*P* value
2/2	2 (0.8)	2 (1.0)	1.00	
2/3	78 (29.9)	47 (23.5)		
3/3	181 (69.3)	151 (75.5)	1.30 (0.81–2.06)	0.180
2 allele	82 (15.71)	51 (12.75)	1	
3 allele	440 (84.29)	349 (87.25)	1.28 (0.86–1.89)	0.220

Table 2. *SMYD3* genotype and allele frequencies among patients with different tumor stages

Study group	Total subjects		2/2 or 2/3		3/3[a]	
	No	(%)	No	(%)	No	(%)
Tumor-node-metastasis-stage						
I	78	(39.0)	16	(20.5)	62	(79.5)
II	52	(26.0)	15	(28.8)	37	(71.2)
III + IV	70	(35.0)	18	(25.7)	52	(74.3)

[a]$P_{\text{for trend}}$ = 0.450.

VNTR polymorphism of *SMYD3* has been firstly reported to be a susceptibility factor in several human cancers including HCC in the Japanese population [2]. However, the results from other study were not consistent. Most recently, Frank et al. [3] did not found the association of 3/3 polymorphism of *SMYD3* with both familial breast cancer and high-risk familial breast cancer cases. The present study revealed that there were no statistically significant differences of *SMYD3* VNTR polymorphism in allele and genotype frequencies between HCC patients and healthy controls. In addition, we did not find any correlations between the *SMYD3* polymorphism and different stages of HCC disease. These results indicated that *SMYD3* VNTR polymorphism is not associated with HCC in terms of HCC risk and disease development.

We'd like to notice that our data are controversial to those of Japanese group [2]. One of the possibilities is that differences of a genetic polymorphism as a risk factor in two populations may be affected by various gene-environment interactions during cancer development. For example, geographical differences in HCC incidence partially reflect variations of viral infection [7]. In Japan, hepatitis C virus-associated HCC incidence is 70%, whereas hepatitis B virus (70%) is the major cause of liver chronic infection leading to HCC in South Asia including Hong Kong [7]. In our study, 85% of the HCC patients were hepatitis B virus positive and only 6% were hepatitis C virus positive. Therefore, it would be of interest to investigate whether the role of *SYMD3* polymorphism in carcinogenesis is dependent on the type of viral infection in HCC.

In conclusion, there was found no association of *SMYD3* tandem repeats polymorphism with HCC occurrence and metastasis in the Chinese population. This is the first null report in HCC and more independent studies might be needed to verify the association between this polymorphism and cancer in different populations.

ACKNOWLEDGEMENTS

This study was supported by Sun C.Y. Research Foundation for Hepatobiliary and Pancreatic Surgery of the University of Hong Kong.

REFERENCES

1. **Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP, Li M, Yagyu R, Nakamura Y.** *SMYD3* encodes a histone methyltransferase involved in the proliferation of cancer cells. Nat Cell Biol 2004; **6**: 731–40.

2. **Tsuge M, Hamamoto R, Silva FP, Ohnishi Y, Chayama K, Kamatani N, Furukawa Y, Nakamura Y.** A variable number of tandem repeats polymorphism in an E2F-1 binding element in the 5′ flanking region of SMYD3 is a risk factor for human cancers. Nat Genet 2005; **37**: 1104–7.

3. **Frank B, Hemminki K, Wappenschmidt B, Klaes R, Meindl A, Schmutzler RK, Bugert P, Untch M, Bartram CR, Burwinkel B.** Variable number of tandem repeats polymorphism in the SMYD3 promoter region and the risk of familial breast cancer. Int J Cancer 2005; **118**: 2917–8.

4. **Goldstein DB, Cavalleri GL.** Understanding human diversity. Nature 2005; **437**: 1241–2.

5. **Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M.** AJCC cancer staging handbook: TNM classification of malignant tumors, 6th ed. New York: Springer, 2002.

6. **Chan VS, Chan KY, Chen Y, Poon LL, Cheung AN, Zheng B, Chan KH, Mak W, Ngan HY, Xu X, Screaton G, Tam PK, Austyn JM, Chan LC, Yip SP, Peiris M, Khoo US, Lin CL.** Homozygous L-SIGN (CLEC4M) plays a protective role in SARS Coronavirus infection. Nat Genet 2006; **38**: 38–46.

7. **Llovet JM, Burroughs A, Bruix J.** Hepatocellular carcinoma: Lancet 2003; **362**: 1907–17.

ОТСУТСТВИЕ СВЯЗИ МЕЖДУ ПОЛИМОРФИЗМОМ ТАНДЕМНЫХ ПОВТОРОВ *SMYD3* И ВОЗНИКНОВЕНИЕМ И МЕТАСТАЗИРОВАНИЕМ КАРЦИНОМЫ ПЕЧЕНИ У НАСЕЛЕНИЯ КИТАЯ

Известно, что полиморфизм тандемных повторов (VNTR) в регуляторном участке гена *SMYD3,* кодирующем метилтрансферазу гистонов, ассоциирован с развитием рака прямой кишки, карциномы печени (HCC) и рака молочной железы и является популяционно-зависимой характеристикой. *Цель* работы состояла в исследовании возможной связи между VNTR геном *SMYD3* и развитием HCC у населения Китая. *Материалы и методы:* исследование типа "случай-контроль" проводили с участием 200 пациентов с HCC и 261 здорового донора. Полиморфизм VNTR в промоторной области гена *SMDY3* генотипировали методами PCR и прямого секвенирования. Для оценки связи между полиморфизмом и риском развития HCC использовали отношение шансов (OR) и 95% доверительные интервалы. *Результаты:* частота аллелей для повторов *SMYD3* 2 и 3 составила 15,71 и 84,29% в контрольной группе и 12,75 и 87,25% — в группе больных ($P = 0,22$). OR генотипов 3/3 *versus* 2/2 и 2/3 составило 1,30 ($P = 0.18$). Частота 3 аллелей не возрастала при повышении стадии заболевания ($P = 0,45$). *Выводы:* полиморфизм гена *SMYD3* не ассоциирован с развитием и метастазированием HCC у населения Китая.

Ключевые слова: **SMDY3, полиморфизм, карцинома печени.**