

***PTEN* Mutation Studies In Malaysian Colorectal Cancer Patients**

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Abstract. Tumour suppressor gene, *PTEN* has been found to be altered in various types of tumours such as breast, glioblastoma, prostate, endometrial cancers and colorectal carcinoma (CRC). Alterations have been found to be common in sporadic colorectal tumours. Our objective was to identify *PTEN* mutations in colorectal tumours and blood samples from Malaysian patients. We analysed genomic DNA from 27 paired normal and tumour tissue samples and 10 blood samples. Nineteen of these samples were from a previous study that had failed to identify *APC* and β -*catenin* gene mutations. Both these genes are involved in early events related to colorectal carcinoma tumorigenesis. The entire coding region and the flanking sites of the 9 exons of *PTEN* gene was amplified and conformation sensitive gel electrophoresis (CSGE) and sequencing was done to identify alterations. We found no *PTEN* alterations among the colorectal cancer samples we had examined and thereby conclude that there is a lack of *PTEN* involvement in the carcinogenesis of colorectal cancer in these patients.

Keywords. *PTEN*, mutations, colorectal carcinoma, CSGE

INTRODUCTION

Colorectal cancer is currently one of the most common cancers in Malaysia. The 2nd report of the National Cancer Registry reported that colon cancer is ranked the 3rd most frequent cancer with rectal cancer being ranked as the 5th most common cancer for both genders. The incidence of colon cancer in Chinese was 2 times higher compared to other ethnic groups. Colorectal cancer incidence among Malaysians increased over age but peaked after 50 years in both males and females.

Colorectal carcinogenesis is a multistep process with tumours presenting various genetic and epigenetic alterations inactivating tumour suppressor genes and and/or activating oncogenes (Ilyas *et al.*, 1999; Chung, 2000). The human tumour suppressor gene, *PTEN* (phosphatase and tensin homolog) (also known as *MMAC1/TEP1*), has been identified on chromosome 10q23 (Li *et al.*, 1997; Steck *et al.*, 1997). *PTEN* acts by inhibiting the activation of Akt/protein kinase B and is therefore involved in a major pathway controlling cell proliferation and survival (Maehama *et al.*, 1999; Besson *et al.*, 1999). The COOH-terminal region of *PTEN* protein has been found to be important for its activity as a tumour suppressor (Georgescu *et al.*, 1999). Mutations detected in exons 7, 8 and 9 of the *PTEN* gene, produced truncated proteins lacking the COOH-terminal region, which leads to

the inhibition of the tumour suppressor activity of *PTEN*.

PTEN mutations have been previously described in various cancers such as the thyroid, breast, prostate, bladder, brain and of endometrial origin. Germline mutations have been closely linked to patients with Cowden disease, Bannayan-Zonana syndrome and juvenile polyposis (Liau *et al.*, 1997; Marsh *et al.*, 1997; Di Cristofano *et al.*, 2000). Early studies found that *PTEN* mutations were rare in colorectal cancers (Okami *et al.*, 1998; Chang *et al.*, 1999). However, mutations at the poly(A)₆ tract of exons 7 and 8 of the *PTEN* gene, have been identified in approximately 18% of patients with colorectal tumours showing microsatellite instability (Guanti *et al.*, 2000; Nassif *et al.*, 2004). *PTEN* mutations also play an active role in promoting abnormal growth of colorectal cells (Guanti *et al.*, 2000). *APC* (adenomatous polyposis coli) gene and β -*catenin* (*CTTNB1*) gene, have both been linked closely to the development of colorectal cancer, however, the role of *PTEN* mutations in colorectal cancer is still being investigated.

In this study, we carried out a detailed analysis of the involvement of *PTEN* gene in colorectal cancer cases in

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Malaysians. Nineteen of the samples studied were previously screened for *APC* and β -*catenin* genes mutations, to elucidate their involvement in carcinogenesis of colorectal cancer in Malaysian patients, but no mutations were identified in both these genes. Therefore, this preliminary study seeks to verify the role of *PTEN* gene in colorectal cancer in Malaysian patients.

Twenty seven fresh tissues (normal and tumour) and 10 blood samples were collected from consenting colorectal cancer patients from the Hospital University Kebangsaan Malaysia. Patients were from all age groups and were histologically confirmed with colorectal carcinoma. Polymerase chain reaction was carried out with primers (Liaw *et al.*, 1997; Marsh *et al.*, 1998) for the 9 exons of *PTEN* gene. Mutation analysis was carried out using conformation sensitive gel electrophoresis (CSGE) as described by Ganguly *et al.*, 1993. After electrophoresis, gel was stained with Syber gold (Molecular Probes, USA) for 5 min, viewed under an UV illuminator. Samples suspected with heteroduplex bands were sequenced with the ABI PRISM BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 3.1 on the ABI PRISM 310 Genetic Analyser. Sequences obtained were compared with the published sequence.

Clinical data analysis was done for the 35 patients studied. Of these 14% (5/35) of the patients were between the age group of 25-49 and the remaining 86% (30/35) of the patients were above 50 years of age. Majority of the patients were female (68% - 24/35). All of the patients were diagnosed with tumours in stage Duke's B (51% - 18/35) and Duke's C (49% - 17/35) and were well differentiated (60% - 21/35) and presented in the rectum (65% - 23/35) (Table 1). Three patients had at least one family member with colorectal carcinoma (Table 2).

No germline *PTEN* mutations were found in the 4 patients with family history of colorectal cancer or the remaining sporadic colorectal cancer patients. As no *PTEN* mutations were found even in the 19 samples that did not carry *APC* gene and β -*catenin* gene mutations, we can safely say that these 3 genes are not involved in the carcinogenesis of colorectal cancer in the 19 patients.

Our findings are similar to those reported by other related studies (Okami *et al.*, 1998; Chang *et al.*, 1999; Liu *et al.*, 2004). One similar study reported that 84% of the colorectal cancer patients studied did not carry *PTEN* mutations. These patients (50%) of whom had cancer localized in the rectum, and had also been classified with Duke's A and B tumours (Dicuonzo *et al.*, 2001). 65% (23/35) of the patients in our study had cancer of the rectum and 51% (18/35) of them had Duke's B stage tumour, and no *PTEN* mutations were identified in this patients.

One study estimated that the frequency of *PTEN* alterations were approximately 2.7% in unselected cases of colorectal cancers in comparison to 18% in sporadic colorectal cancers with microsatellite instability (Guanti *et al.*, 2000). Recently, another study with 41 unselected sporadic

Table 1. Tumour presentation in colorectal cancer patients.

Site	Differentiation status			Stage	
	Number	Well	Moderate	Poor	Number
Caecum	5.7	Well	19		Duke's A -
Ascending colon	4	Moderate	12		Duke's B 18
Sigmoid colon	6	Poor	4		Duke's C 17
Rectum	23				Duke's D -

Note: None of the patients had tumours presented in the transverse and descending colon.

Table 2. Age and family history of 4 patients with a history of cancer

Patient no	Age	Family history
1	30	uncle (colorectal carcinoma)
2	47	sister (colorectal carcinoma) father (prostate cancer)
3	57	son and brother (colorectal carcinoma)
4	58	brother (nasopharyngeal carcinoma)

colorectal cancers identified 9 *PTEN* mutations, of which 8 were from microsatellite instable tumours (Nassif *et al.*, 2004), indicating that microsatellite instability in Malaysian sporadic cases should be analysed prior to mutation screening. Nassif also proposed that the higher mutation rate seen in their study may have been contributed by a more sensitive mutation screening method, dideoxy fingerprinting (ddF) compared to methods like single stranded conformational polymorphism (SSCP) which depends wholly on the nature of the mutation. As CSGE is totally dependent on the differential migration of DNA heteroduplexes and homoduplexes, it could have been a contributing factor to our findings even though CSGE has been found to be a sensitive and practical method for the screening and could be utilized in routine molecular diagnostic laboratories. (Zhang *et al.*, 2005).

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REFERENCES

- Besson, A., Robbins, S.M. and Yong, V.W. 1999. PTEN/MMAC1/TEP1 in signal transduction and tumorigenesis. *European Journal of Biochemistry* 263: 605-611.
- Chang J.G., Chen, Y.J., Perng, L.I., Wang, N.M., Kao, M.C., Yang, T.Y., Chang, C.P., Tsai, C.H. 1999. Mutational analysis of the PTEN/MMAC1 gene in cancer of the digestive tract. *European Journal of Cancer* 35: 647-651.
- Chung D.C. 2000. The genetic basis of colorectal cancer: insights into critical pathways of tumorigenesis. *Gastroenterology* 119: 854-865
- Depowski, P.L., Rosenthal, S.I. and Ross, J.S. 2001. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Modern Pathology* 14: 672-676.
- Dicuonzo, G., Angeletti, S., Farcia-Foncillas, J., Brugaralas, A., Okrouzhnou, Y., Santini, D., Tonini, G., Lorino, G., Cesaris, M.D., Baldi, A. 2001. Colorectal carcinomas and PTEN/MMAC1 gene mutations. *Clinical Cancer Research* 7: 4049-4053.
- Di Cristofano, A. and Pandolfi, P. 2000. The multiple roles of PTEN in tumour suppression. *Cell* 100:387-390.
- Ganguly, A., Rock, M.J., and Prockop D.J. 1993. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proceedings of the National Academy of Sciences of the United States of America*. 21: 10325-10329.
- Georgescu, M.M., Kirsch, K.H., Akagi, T., Shishido, T., Hanafusa, H. 1999. The tumour suppressor activity of PTEN is regulated by its carboxyl-terminal region. *Proceedings of National Academy of Sciences USA* 96: 10182-10187.
- Guanti, G., Resta, N., Simone, C., Cariola, F., Demma, I., Fiorente, P., Gentile, M. 2000. Involvement of PTEN mutations in the genetic pathways of colorectal carcinogenesis. *Human Molecular Genetics* 9: 283-287.
- Ilyas, M., Starub, J., Tomlinson, I.P. and Bodmer, W.R. 1999. Genetic pathways in colorectal and other cancers. *European Journal of Cancer* 35: 335-351
- Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliareis, C., Rodgers, L., McCombie, R., Bigner, S.H., et al. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275: 1943-1947.
- Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., et al. 1997. Germline mutations of PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nature Genetics* 16: 64-67.
- Liu, W.H., Kaur, M., Wang, G., Zhu, P., Zhang, Y., Makrigiorgos, G.M. 2004. Inverse PCR-based RFLP scanning identifies low-level mutation signatures in colon cells and tumours. *Cancer Research* 64: 2544-2551
- Machama, T. and Dixon, J.E. 1999. PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biology* 9: 125-8
- Marsh, D.J., Dahia, P.L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R.J., Eng, C. 1997. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nature Genetics* 16: 333-334
- Nassif, N.T., Lobo, G.P., Wu, X., Henderson, C.J.A., Morrison, C.D., Eng, C., Jalaludin, B., Segelov, E. 2004. PTEN mutations are common in sporadic microsatellite stable colorectal cancer. *Oncogene* 23:617-628.
- Okami, K., Wu, L., Riggins, G., Cairns, P., Goggins, M., Evron, E., Halachmi, N., Ahrendt, S.A., Reed, A.L., Hilgers, W., et al. 1998. Analysis of PTEN/MMAC1 alterations in aerodigestive tracts tumours. *Cancer Research* 58: 509-511
- Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., Frye, C., et al. 1997. Identification of a candidate tumor suppressor gene MMAC1 at chromosome 10q23.3 that is mutated in advanced cancer. *Nature Genetics* 15: 356-362.
- Zhang, X.N., He, X.H., and Li, J.C. 2005. PCR products with heterozygous mutations containing two types of heteroduplexes. *Chinese Medical Journal* 34: 471-420