Genetic pattern of CYP1A1*2A (T>C) gene in patients with colorectal cancer in South Jakarta – Indonesia

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Abstract— The information of relationship between genetics and cancer in Indonesia, especially colon cancer, is still limited. This study aimed to determine the genetic pattern of colon cancer allele in Fatmawati Hospital, Jakarta -Indonesia. We conducted a case-control study of 40 colorectal cancer patients and 133 volunteers as controls. We observed CYP1A1*2A alleles using RFLP-PCR method. Three milliliters of blood was used to isolate DNA. Our observation showed of the genetic patterns CYP1A1*2A with the incidence of colorectal cancer showed 17 patients were variant-type homozygote and 12 patients were heterozygote type. In this research shown the common of genotyping type CYP1A1*2A of colorectal cancer from the patients are a homozygous variant type.

Keywords: CYP1A1*2A; colorectal cancer; Indonesia

I. INTRODUCTION

The information of the relationship between genetics and cancer had been carried out. Cytochrome P450 (CYP450s) is an enzyme that plays a pivotal role in the xenobiotics and drug metabolism. It has been widely reported that mutations in one of the nucleotides in this enzyme gene causing individual to be susceptible to some cancers. The CYP1A1 gene is one of the most widely studied genes, which associated with cancer. Several CYP1A1 polymorphisms had been reported on Human CYP Allele Nomenclature website showing evidences of the link between genetics and cancer incidences. The involvement of this enzyme activity in the activation or de-activation shows the individual’s susceptibility to cancer. The CYP1A1 polymorphism especially CYP1A1*2A (T>C) will produce a cutting site for MspI enzyme; it has been linked to individual sensitivity to colon cancer [1-5].

The study about genetics and cancer in Indonesia, especially colon cancer is still limited. Colorectal cancer is one of the three main types of cancers that commonly found in Indonesia. Epidemiological studies in Europe, Asia, and America have shown this disease is more prevalent in adults aged 45 to 50 years. Furthermore, there is still lack of information about colorectal cancer in Indonesia [6-9].

We screened for colorectal cancer patients from Fatmawati Hospital Jakarta-Indonesia, starting May until the end of 2014. There were 40 colorectal cancer patients who were new cases or old cases patients who routinely control to the hospital.

II. MATERIALS AND METHODS

A. Subjects

A total of 40 patients and 133 volunteers were recruited for this study. The inclusion criteria for cases were patients who were positively diagnosed as colon cancer and have been getting medical treatment (surgery, chemotherapy, etc.). All volunteers as controls should show a negative result for FOB test (Faecal Occult Blood test). Three milliliters of blood used for genetic screening. All patients and volunteers shall agree to participate in this study.

B. Methods: PCR-RFLP

The DNA from patients and controls was extracted from peripheral blood. PCR-based restriction fragment length polymorphism (RFLP) methods were used to analyze CYP1A1*2A.

The genetic examination commenced by isolating genome using a Genome Isolation Kit from Genaid® with catalog numbers: #GB300. The testing was continued by target gene amplification using PCR Kit Kapa2G Fast PCR Kits with catalog number #KK5609. The primers used were 5'-TAGGA GTCTT GTCTC ATGCC T-3' (sense) and 5'-CAGTG AAGAG GTGTA GCCGC T-3' (anti-sense), following experiments by Nisa et al (2012). The PCR steps are: denaturation I 95°C for 3 minutes; thirty-five cycles of PCR: denaturation II 95°C for three minutes, annealing 60°C for 15 seconds and elongation 72°C for two seconds; and the final extension 72°C for five seconds, with large PCR product is 340 base-pair (bp). The results were observed using electrophoresis techniques in order to 1.5% of agarose for 60 minutes. Electrophoresis results were observed using UV light. The next stage is cutting by enzyme MspI fragment using RFLP technique.

C. Data Processing and Statistical Analysis

Analysis Statistic of Hardy-Weinberg equilibrium calculator with Chi-squared distribution (1 degree of freedom) by Santiago Rodriguez [10].
III. RESULTS

RLFP MspI enzymes showed two kinds of banding pattern with a size of 200 and 140 bp. Individuals with wild-type homozygotes have a band size of 340 bp. Individuals with variant (mutant) type homozygote have band size 140 and 200 bp, whereas heterozygous individuals will pick the pattern of the band are 340, 200 and 140 bp. The observation of all samples, showing the pattern of genotyping CYP1A1*2A varied (Fig 1).

Analysis of the genotype distribution and allele frequencies showed that homozygous mutant type is commonly found in patients, whereas heterozygous type is commonly found in controls (Table 1, 2).

IV. DISCUSSION

The earlier study had reported the association of lifestyle (especially cigarette) and genetic with colorectal cancer risk. Nisa et al showed that lifestyle and genetic factors have played in the role in colorectal carcinogenesis. They studied in comparison genetic of smoker and non-smoker colorectal cancer patients in Fukuoka, Japan used RFLP-MspI PCR Method [6].

CYP genes are member of large families of endoplasmic and cytosolic enzyme genes. CYP1A1*2A, part of CYP1A1 gene located on chromosome 15q, is a member of the CYP1 genes family with a major role in the oxidative activation and or deactivation of a wide range of xenobiotic and drug metabolism. The expression of this gene was consisting of the aryl hydrocarbon receptor and mutation on this gene reported has associated with cancer evidenced [11,12,13,14]. Two common polymorphisms in the CYP1A1 gene are a T6235C change within the 3' noncoding region of the gene (*2A, also known as the MspI restriction) and an A4889G change in the hems-binding domain of exon 7 (*2C). This polymorphism of the gene has been examined to cancer susceptibility [4,5,15].

Swinney RM et al (5), Cosman G et al [16] and Taioli E et al [17] reported that the CYP1A1*2A has been shown to vary among different ethnic groups and has an influence on risk to susceptibilities environmental toxins. Studies in Japan and other country showed the gene has been associated with a risk of colorectal cancer [6,14,18,19,20,21]. The other researcher was reported that CYP1A1 MspI was contributed to increased cancer susceptibility among Asians. The effect of this polymorphism is diverse according to by ethnicity and cancer types [8].

As described earlier, the CYP1A1 gene has an important role in the metabolism of xenobiotic and drugs. The presence of CYP1A1*2A polymorphism in our samples might be enable the risk of colorectal cancer, where polymorphism CYP1A1*2A in the form homozygote variant allele (C) might be increases the risk compared to wild-type form (T). Several studies have reported that the risk of the emergence of colorectal cancer is not only caused by the presence of variant alleles, but the environmental factors that are extrinsic factors are major contributors to the risk of cancer [22].
V. CONCLUSION

The research has shown the common genotyping type CYP1A1*2A of colorectal cancer from 40 samples were a homozygous variant type.

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

[1]. L. Yao, Y. Gyoung, B.P. Berman, P.J. Farnham, “Functional annotation of colon cancer risk SNPs”, Nature Communications, vol.5, no. 5114, pp. 1-13, Sept 2014. DOI: 10.1038/ncomms6114 www.nature.com/naturecommunications.

[2]. M.A. Murtaugh, C. Sweeney, Ma Khe-ni, B.J. Caan, M.L. Slattery, “The CYP1A1 genotyping may alter the association of meat consumption patterns and preparation with the risk of colorectal cancer in men and women,” J Nutr, vol. 135, no. 2, pp. 179-186, Feb 2005.

[3]. G. Xie, Peng Z, Raufman JP, "Src-mediated aryl hydrocarbon and epidermal growth factors receptors cross talk stimulates colon cancer cell proliferation," Am J Physiol Gastrointest Liver Physiol, vol. 302, no. 9, pp. G1006-1015, Feb 2012.

[4]. S. Hayashi, J. Watanabe, K. Nakachi, K. Kawajiri, “Genetic linkage of lung cancer-associated Mspl polymorphism with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene,” J Biochem, vol. 110, no. 3, pp. 407-411, Sept 1991.

[5]. R.M. Swinney, J. Beuten, A.B. Collier, T.L.T. Chen, N.J. Winick, B.H. Pollock, G.E. Tomlinson, “Polymorphisms in CYP1A1 and ethnic susceptibility to acute lymphoblastic leukemia in children,” Cancer Epidemiol Biomarkers Preiv, vol. 20, no. 7, pp. 1537-1542, May 2011.

[6]. H. Nisa, S. Konro, G. Yin, K. Toyomura, et al. “Cigarette smoking, genetic polymorphisms and colorectal cancer risk: the Fukushima colorectal cancer study,” BMC Cancer, vol. 10, no. 10, pp. 274-284, Jun 2010.

[7]. M.A. Pourhosseingholi, “Epidemiolog and burden of colorectal cancer in Asia-Pacific region: what shall we do now?”, Trand Gastrointest Cancer, vol. 3, no. 4, pp. 169-173, Oct 2014.

[8]. B. Wu, K. Liu, H. Huang, et al., “Mspl and Ile462Val polymorphisms in CYP1A1 and overall cancer risk: a meta-analysis,” PlosOne, vol. 8, no. 12, pp. e85166, Dec 2013.

[9]. M. Abdullah, A.W. Sudoyo, A.R. Utono, A. Fauzi, A.A. Rani, “Molecular profile of colorectal cancer in Indonesia: is there another pathway?”, Gastroenterol Hepatol Bed Bench, vol. 5, no. 2, pp. 71-78, 2012.

[10]. S. Rodriguez, T.R. Gaunt, I.N.M. Day, “Hardy-Weinberg Equilibrium Testing of Biological Ascertainment for Mendelian Randomization Studies,” Am J of Epid, vol. 169, no. 4, pp. 505-514, Feb 2009. http://www.oegoe.org/software/hwe-mr-calc.shtml.

[11]. Q. Nie, X.N. Yang, et al., “CYP1A1*2A polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI and its combined effects with EGFR intron 1 (CA)n polymorphism,” Eur J Cancer, vol. 47, no. 13, pp. 1962-1970, Sept 2011.

[12]. H.N. Honnman, E.M. De Capitani et al., “Polymorphism of the CYP1A1*2A gene and susceptibility to lung cancer in a Brazilian population,” J Bras Pneumol, vol. 35, no. 8, pp. 767-772, Aug 2009.

[13]. Murtaugh MA, Sweeney C, et al., “The CYP1A1 genotype may alter the association of meat consumption patterns and preparation with the risk of colorectal cancer in men and women,” J Nutr, vol. 135, no. 2, pp. 179-186, Feb 2005.

[14]. Q. Ma, A.Y. Lu, “CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies,” Drug Metabolism and Disposition, vol. 35, no. 7, pp. 1009-1016, Jul 2007.

[15]. M.L. Slattery, W. Samowitz, et al., “CYP1A1, Cigarette Smoking, and Colon and Rectal Cancer,” Am J Epidemiol, vol. 166, no. 9, pp. 842-852, Nov 2004.

[16]. G. Cosma, F. Crofts, D. Currie, I. Wirgin, P. Toniole, S.J. Garte, et al., “Racial differences in restriction fragment length polymorphisms and messenger RNA inducibility of the human CYP1A1 gene,” Cancer Epidemiol Biomarkers Preiv, vol. 2, no. 1, pp. 53-57, Jan-Feb 1993.

[17]. E. Taioli, F. Crofts, J. Truchman, S. Bayo, P. Toniole, S.J. Garte, et al., “Radical differences in CYP1A1 genotype and function,” Toxicol Lett, vol. 77. No. 1-3, pp. 357-362, May 1995.

[18]. L. Sivaraman, M.P. Leatham, J. Yee, et al., “CYP1A1 genetic polymorphisms and in situ colorectal cancer,” Cancer Res, vol. 54, no. 14, pp. 3692-3695, Jul 1994.

[19]. N. Ishibe, M. Stampafer, D.J. Hunter, et al., “A prospective study of cytochrome P450 1A1 polymorphisms and colorectal cancer risk in men,” Cancer Epidemiol Biomarkers Preiv, vol. 9, no. 8, pp. 855-6, Aug 2009.

[20]. H. Inoue, C. Kiyohara, T. Marugame, et al., “Cigarette smoking, CYP1A1 Mspl and GSTM1 genotypes, and colorectal adenomas,” Cancer Res, vol. 60, no. 14, pp. 3740-52, Jul 2000.

[21]. N. Song, W. Tan, D. Xing, et al., “CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China,” Carcinogenesis, vol. 22, no. 1, pp. 11-16, Jan 2001.

[22]. S. Wu, S. Powers, W. Zhu, Y.A. Hannun, “Substantial contributionof extrinsic risk factors to cancer development,” Nature, vol. 529, no. 7584, pp. 43-47, Jan 2016.