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MCPH1 protein expression and polymorphisms are associated with risk of breast cancer

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ABSTRACT

Microcephalin 1 (MCPH1) has a crucial role in the DNA damage response by promoting the expression of checkpoint kinase 1 (CHK1) and breast cancer susceptibility gene 1 (BRCA1). MCPH1 containing BRCT domain has been suggested as a tumor suppressor in breast and ovarian cancers. We analyzed the effect of both protein expression and MCPH1 polymorphisms in breast cancer patients. Low nuclear expression of microcephalin was present in 52.4% of breast cancers and was associated with allele T in rs2912010 ($p = 0.046$). However, cytoplasmic microcephalin expression increased with increasing grade ($p = 0.010$). An association between low nucleus microcephalin expression and allele T was identified in rs2912010 ($p = 0.046$). After data analysis, allele distribution of the MCPH1 polymorphisms was not different between breast cancer patients and healthy controls. But the polymorphism was associated with negative status for ER (rs2912010/C2302T; $p = 0.032$, rs1057090/C2358T; $p = 0.027$, rs2912016/C2494A; $p = 0.024$), and allele T in both rs2912010 and rs1057090 was associated with increasing tumor grade (rs2912010; $p = 0.040$, rs1057090; $p = 0.043$) in breast cancer. We are first to report that association of MCPH1 protein expression and its polymorphisms in breast cancer. The MCPH1 polymorphisms and protein expression were associated with tumorigenesis in breast cancer and may be a useful biomarker for identification of the aggressive types of breast cancer.

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1. Introduction

Breast cancer is one of the most common cancers and leads to most of the cancer deaths among women worldwide. Breast cancer is a multifactorial disease, involving a complex interplay between genetic and environmental factors. Approximately 7% of breast cancer cases are estimated to be related to cancer susceptibility genes (Claus et al., 1996). BRCA1 and BRCA2 are well-known as inherited breast cancer susceptibility genes and are considered to be involved in over 50% of all familial breast cancer cases. In early 1990s, BRCA1 and BRCA2 are discovered, intensive efforts have largely been made to discover further breast cancer susceptibility genes, but they were not successes. There is a reason why novel high penetrant genes, unidentified genes and many low penetrant genes had a mixed effect to increase the risk for breast cancer. This polygenic model of breast cancer susceptibility has focused on tumor suppressor gene expression and association studies of single nucleotide polymorphisms (SNPs) in candidate genes, to identify such low penetrant genes. Genes involved in both DNA damage response and cell cycle regulation are suitable candidates, as possible breast cancer susceptibility genes and as controlled risk factors for breast cancer.

The MCPH1 protein called microcephalin 1 is also known as BRIT1 (BRCT-repeat inhibitor of hTERT expression), which was initially identified as a transcriptional receptor of human telomerase reverse transcriptase (Lin and Elledge, 2003). It has three BRCT domains, including the single BRCT domain in the N-terminal and two tandem BRCT domains in the C-terminal. BRCT domain-containing proteins are involved in multiple cellular responses, including cell cycle checkpoint controls, DNA repair and transcription regulation; the main BRCT domain-containing proteins are BRCA1, BRCA2, 53BP1, XRCC1, Rad9, NBS1, MDC1, and DNA polymerase λ (Caldecott, 2003; Goldberg et al., 2003; Lou et al., 2003; Stewart et al., 2003; Wu et al., 1996; Xu et al.,

Abbreviations: MCPH1, microcephalin1; BRCA1, breast cancer susceptibility gene 1; BRCT domain, BRCA1 C-terminus domain; BRIT1, BRCT-repeat inhibitor of hTERT expression; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TMA, tissue microarray.

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2002). The BRCT domains are known phosphopeptide binding motifs that are able to discriminate the phosphorylation status of their protein partner (Manke et al., 2003; Yu et al., 2003). MCPH1 is related to the DNA damage-induced cellular response, cell cycle regulation, chromosome condensation and apoptosis (Rai et al., 2006; Xu et al., 2004; Yang et al., 2008). Recently, it has been reported that MCPH1 acts as a downstream of CHK1 in regulating cell division cycle 25A (Cdc25A) stability, and consequently prevents the cell from premature entry into mitosis (Alderton et al., 2006). By forming a complex with E2F1, MCPH1 regulates a subset of crucial E2F target genes involved in DNA repair, checkpoint activation and apoptosis. During DNA damage and E2F1-dependent apoptosis, MCPH1 leads to activation of p73, caspases and BRCA1 (Yang et al., 2008).

The function of MCPH1 in DNA repair and as a checkpoint control marker is mainly accomplished by acting as a potential tumor suppressor (Lin et al., 2013; Rai et al., 2006). Thus, deletions at the MCPH1 loci (8p23) have been associated with tumor development and poor prognosis in various types of cancer, including breast cancer (Qin, 2002). Breast cancer cell lines are decreased in MCPH1 DNA copy number and show deceased MCPH1 RNA and protein expression. These findings support the hypothesis that MCPH1 is a tumor suppressor gene (Rai et al., 2006). Therefore, we investigated whether the reduction or alteration in microcephalin protein expression may be associated with any clinicopathologic significance or MCPH1 polymorphism in breast cancer tissues.

In this study, we investigated MCPH1 protein expression pattern analysis and correlated with their polymorphisms at the BRCT domain in Korean breast cancer patients, as compared to normal healthy women. We examined the MCPH1 protein expression in breast cancer tissues using tissue microarray. In addition, we screened the SNPs of BRCT domains, and selected the 8 candidate SNPs, i.e. rs2912010 (C2302T), rs35999761 (C2332T), rs1057090 (C2358T), rs75204744 (C2408T), rs117059261 (A2475G), rs7017210 (C2476T), rs45540031 (A2477G) and rs2912016 (C2494A). The correlation between the MCPH1 polymorphism, MCPH1 protein expression and clinicopathological findings in breast cancer patients was investigated.

2. Materials and methods

2.1. Patients and healthy subjects

This study included breast cancer diagnosed 101 women and 102 healthy control participants having the same ethnic background between 2000 and 2008, in Keimyung University Hospital (Daegu, Korea) and Kyunghee University Hospital (Seoul, Korea). We used three selection criteria for tissue samples: 1) histologically proven diagnosis of breast cancer, 2) the proportion of tumor tissues exceeded 50% on microscopic slides and 3) preservation of fresh frozen samples for DNA study. Clinicopathological data was obtained from a retrospective chart review and included histological type, tumor grade, lymph node status and several biomarkers, i.e. ER, PR, HER2, p53 and Ki-67. All patients and healthy controls agreed to genetic testing, as approved by the hospital Institutional Review Board.

2.2. Tissue microarray construction and immunohistochemistry

To assess the immunoexpression of microcephalin, tissue microarrays (TMA) were constructed from specimens of 82 breast cancer patients at Keimyung University Hospital. Paraffin wax blocks were punched with a 3.0-mm diameter from the selected area in the donor blocks, and then transferred and arrayed in the recipient blocks, using a manual tissue microarray device (UNITMA, Seoul, Korea). The presence of cancer cells on the arrayed samples was verified on hematoxylin- and eosin-stained sections. Immunostaining was performed on 4-μm sections. After deparaffination and rehydration, sections were immersed for 10 min in methanol containing 3% hydrogen peroxide to block endogenous peroxidase activity. All slides were pretreated with citrate buffer (10 mM; pH 6.0) for antigen retrieval by heating the slides in a microwave oven at 97 °C for 2 min. A cooling period of 15 min preceded the incubation of the polyclonal anti-MCPH1 antibody (A300-368A, Bethyl Laboratories, TX, USA) diluted 1:200 at 37 °C, for 4 h. Thereafter, the EnVision™ system (Dako, Glostrup, Denmark) was used for MCPH1 staining, according to the manufacturer's instructions. All staining procedures were developed with diaminobenzidine. Before the slides were mounted, all sections were counterstained for 30 s with hematoxylin and dehydrated in alcohol and xylene. Whole sections of breast cancer tissues with strong expression of the examined protein were used as positive controls.

2.3. Evaluation of IHC staining

All immunohistochemistry (IHC) slides were examined by two independent investigators (J.-Y.S. and J.L.), who were blinded to the clinical data. IHC for ER (6F11, Novocastra, Bannockburn, IL, USA), PR (1A6, Novocastra, Bannockburn, IL, USA), Her2 (A0485, DAKO, Carpenteria, CA, USA), Ki-67 (MIB-1, DAKO, Carpenteria, CA, USA) and p53 (DO-7, DAKO, Carpenteria, CA, USA) was performed using a Bond Polymer Intense Detection System (Vision Biosystems, Victoria, Australia), according to the manufacturer's instructions. ER immunoactivity and PR immunoactivity were evaluated according to the Allred scoring method (Allred et al., 1998). Membranous Her2 staining was scored according to the HercepTest (Dako) protocol (Jacobs et al., 1999). The tissue was considered p53-positive and Ki-67-positive when more than 10% of the cells showed strong and distinctive nuclear immunostaining. Nuclear and cytoplasmic expression of microcephalin was scored as a percentage of positive cells. Based on a 30% cut-off value, a dichotomous analysis into low- and high-expression was performed.

2.4. DNA extraction

Genomic DNA was isolated from breast cancer tissue. The sliced tissue was put into 500 μl lysis buffer and 50 μl SDS/Proteinase K and samples were incubated at 48 °C for 48 h. One volume of P/C/I (phenol/chloroform/isoamyl alcohol = 25:24:1) was added and mixed well for 10 min by vortex, then centrifuged at 12,000 rpm for 10 min. The supernatant was moved to a new 1.5 ml tube. One volume of C/I (chloroform/isoamyl alcohol = 24:1) was added and mixed well for 10 min by vortex, then centrifuged at 12,000 rpm for 10 min. The supernatant was again moved to a new 1.5 ml tube. 0.1 volume of 3 M sodium acetate and 2 volumes of EtOH were added, then invert mixed. Genomic DNA was isolated from peripheral blood samples using the Core-one™ Blood genomic DNA isolation kit (Core-bio system, Korea).

2.5. Polymerase chain reaction and SNP analysis

The oligo nucleotide primers for the 3rd BRCT domain of MCPH1 were: forward: 5′-CAC CTC TGT AAT TCT ATC TCT GTC-3′ and reverse: 5′-CAC CAC AGC GTC TGT GTG CCT GG-3′. The PCR conditions for MCPH1 were, as follows: 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s, followed by final extension at 72 °C for 10 min. PCR products purified by 95% ethyl alcohol were used as template DNA for cycle sequencing. The PCR for sequencing was performed using BigDye Terminator (version 3.1) cycle sequencing and analyzed using the ABI Prism® 3730 Automated DNA sequencer (Applied Biosystems, Foster City, CA).

2.6. Statistical analysis

The chi-square test (SPSS for Windows version 18.0, SPSS Inc., Chicago, IL) was used to assess correlations between clinicopathological features (i.e. tumor grade, lymph node status and biomarkers) and

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