

Mutational Analysis of 17β -hydroxysteroid dehydrogenase type 2 gene in Breast Cancers

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Purpose : The 17β -hydroxysteroid dehydrogenases (17HSDs) play an important role in the regulation of the physiologic activities of sex steroid hormones. The predominance of 17HSD type 1 in the malignant breast tissue could increase the estrogen-dependent proliferation and stimulate the cancer progression. On the other hand, the oxidative 17HSD type 2 may protect the normal breast cells from an excessive estradiol effect. To identify the role of 17HSD type 2 in the carcinogenesis of breast cancer, we investigate the mutation of 17HSD type 2 in 35 breast cancers.

Methods : We analyzed the entire coding region of the 17 HSD type 2 gene for detection of the somatic mutations in 35 invasive ductal carcinomas of the breast by polymerase chain reaction, single strand conformation polymorphism, and DNA sequencing.

Results : We found one missense mutation in exon 6 (2.86%). It revealed the CCT→CTT (Pro→Leu) transition type at codon 262 in exon 6.

Conclusion : In present study, we found only a mutation of the 17β -HSD type 2 gene in breast cancer and could not demonstrate the direct relationship between the mutation of the 17β -HSD type 2 gene and the development of breast cancer. These results suggest that the mutation of 17HSD type 2 doesn't play a major role in the development of breast cancer.

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Key Words Breast cancer, 17HSD type 2
중심단어 유방암, 17HSD type 2

INTRODUCTION

Breast cancer is the second most common cancer for women in the world. Estrogen is the most powerful factor in breast carcinogenesis. Estrogens are well recognized to play an important role in the regulation of cell growth and differentiation in normal mammary gland as well as in hormone-sensitive breast carcinomas.

An enzyme group affecting the availability of biologically active estrogens and androgens is the family of 17β -hydroxysteroid dehydrogenases (17HSDs). The 17 HSDs play an important role in the regulation of the physiologic activities of sex steroid hormones by catalyzing the oxidation or

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continued for 5 minutes at 72 °C. After amplification, PCR products were denatured 5 minutes at 95°C at a 1:1 dilution of sample buffer containing 98% formamide/5 mmol/L NaOH and were loaded onto a single-strand conformation polymorphism (SSCP) gel (FMC Mutation Detection Enhancement System; Intermountain Scientific, Kaysville, UT) with 10% glycerol. Samples were electrophoresed at 8W at room temperature overnight. After electrophoresis, the gels were transferred to 3-mm Whatman paper (Whatman International Ltd, Maidstone, England) and dried, and autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY). For the detection of mutations, DNAs showing mobility shifts were cut out from the dried gel and reamplified for 30 cycles using the same primer set. Sequencing of the PCR products was performed using the cyclic sequencing kit (Perkin-Elmer, Foster city, CA) according to the manufacturer's recommendation.

RESULTS

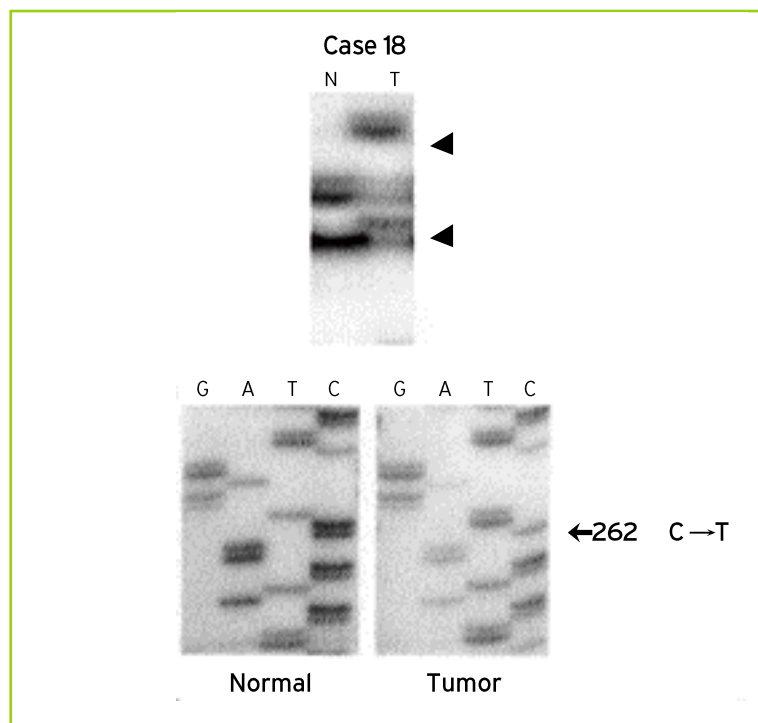


Fig 1. PCR-SSCP of exon 6 of the 17HSD type 2 shows abnormally shifted bands (arrow heads) in case 18 (N, normal; T, tumor). Sequencing analysis reveals C→T transition at codon 262 in case 18 (arrow).

Through the microdissection technique, we selectively procured tumor cells from histological sections of 35 invasive ductal carcinomas. We performed PCR-SSCP and Sequencing analyses. We found one missense mutation in exon 6 of 17HSD type 2 gene. Case 18 revealed the CCT→CTT (Pro→Leu) transition type at codon 262 in exon 6 (Fig 1). Five silent mutations were also found.

DISCUSSION

It has been shown that estrogens play an important role in the development and progression of breast cancer in epidemiological and experimental studies.⁽¹¹⁾ Excessive and longer exposures to estrogen result in an increased risk of breast cancer.

17HSD enzymes play pivotal roles in the regulation and maintenance of circulating estrogens. 17HSDs catalyze the interconversion of 17-ketosteroids and 17-hydroxysteroids, such as estrone and estradiol, and androstenedione and testosterone. 17HSD type 2 cDNA encodes a predicted protein of 387 amino acids with a molecular mass of 42782. This enzyme catalyzed the conversion of estradiol into estrone. 17HSD type 2 may protect target tissues from excessive estrogen exposure by catalyzing estrogens into less-active form. 17HSD type 2 is expressed in a wide variety of tissues, such as breast, uterus, prostate, placenta, liver and kidney.⁽¹²⁾ There are previous studies of a role of 17HSD type 2 enzyme in various tumors. 17HSD type 2 enzyme is expressed in the surface epithelium of gastrointestinal tract⁽¹³⁾ and development of colon cancer is associated with a decrease of 17HSD type 2.⁽¹⁴⁾ Lin et al⁽¹⁵⁾ suggest that loss of 17HSD type 2 and the subsequent loss of estrogen inactivation might contribute to the hepatocarcinogenesis. We presumed that the loss of the genetic function in 17HSD type 2 could not protect the target tissue of the breast from the exposure of excessive estrogen and eventually would play some roles in the carcinogenesis of breast cancers. Mannermaa et al⁽¹⁶⁾ analyzed 17HSD type 2 gene in human familial and sporadic breast cancer. They detected no mutation in coding regions of exon 1-5