

Identification of a Mutation in the Human Raloxifene Response Element of the *Transforming Growth Factor-β3* Gene

The human transforming growth factor- $\beta 3$ (TGF- $\beta 3$) is an important cytokine to maintain bone mass by inhibiting osteoclast differentiation. Recently raloxifene response element (RRE), a new enhancer with a polypurine sequence for estrogen receptor (ER)-mediated gene activation, was identified on the *TGF- $\beta 3$* gene. Functional analysis of the RRE-mediated pathway has shown that this would be an important pathway for bone preserving effect. We found a novel mutation in the RRE sequence by single-strand conformational polymorphism analysis in one of 200 Korean women. Cloning and sequencing revealed a heterozygote in which one allele had an insertion of 20 nucleotides (AGAGAGGGAGAGGGAGA GGG) between nucleotide +71 and +72 and a point mutation at nucleotide +75 (G-A transition), and the other allele had normal sequence. The insertion was a nearly perfect tandem duplication of the wild type DNA sequence. The bone mineral density of the affected woman was not much lower than that of age-matched controls. Transient transfection of the mutant allele showed no significantly different activity compared with that of the wild type allele. These observations suggest that the heterozygote variation of the RRE sequence seems not to be operative in determination of bone mass.

Key Words : *Transforming Growth Factor- $\beta 3$; Raloxifene; Response Elements; Mutation*

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INTRODUCTION

A nonsteroidal benzothiophene derivative, raloxifene, has a tissue-selective estrogen agonist/antagonist profile. Raloxifene is a classical antiestrogen in the breast and uterus (1, 2) whereas it has a bone-preserving effect and a cholesterol lowering effect in nonreproductive tissues as an estrogen agonist (3, 4). Recently it was found that raloxifene sends its bone-preserving signals to the genes by a route, different from the one traced for estrogen in reproductive tissues (5). Activation of the gene encoding transforming growth factor - $\beta 3$ (TGF- $\beta 3$), an important cytokine in regulating osteoclast differentiation (5), demonstrated a distinct pathway from that of an estrogen response element (ERE) containing gene.

The novel DNA response element, raloxifene response element (RRE), was identified in the 5' untranslated region of *TGF- $\beta 3$* gene (6). RRE-mediated pathway might be one of the possible explanations for the wide spectrum of estrogen effects, especially in nonreproductive tissues including the bone. Moreover, it might have an answer for the tissue

specificity of raloxifene that acts as an antagonist in reproductive system, while acts as an agonist in non-reproductive system.

We, therefore, hypothesized that RRE of the *TGF- $\beta 3$* gene could be important in bone preservation and any mutants on the RRE sequence could affect the development of low bone mass. We investigated whether a polymorphic site or mutation exists on the RRE sequence of the *TGF- $\beta 3$* gene in postmenopausal Korean women.

MATERIALS AND METHODS

Two hundred healthy postmenopausal women of Korean ethnicity were investigated. Women with early menopause (before 40 yr of age) and those who had had an ovariectomy were excluded. None had a history of bone disease, illness, or drug use that might affect bone turnover. Bone mineral density (BMD) at the lumbar spine (L2-4) was measured by dual energy radiography absorptiometry using a XR-36 (Norland Co., Fort Atkinson, WI, U.S.A.). The precision

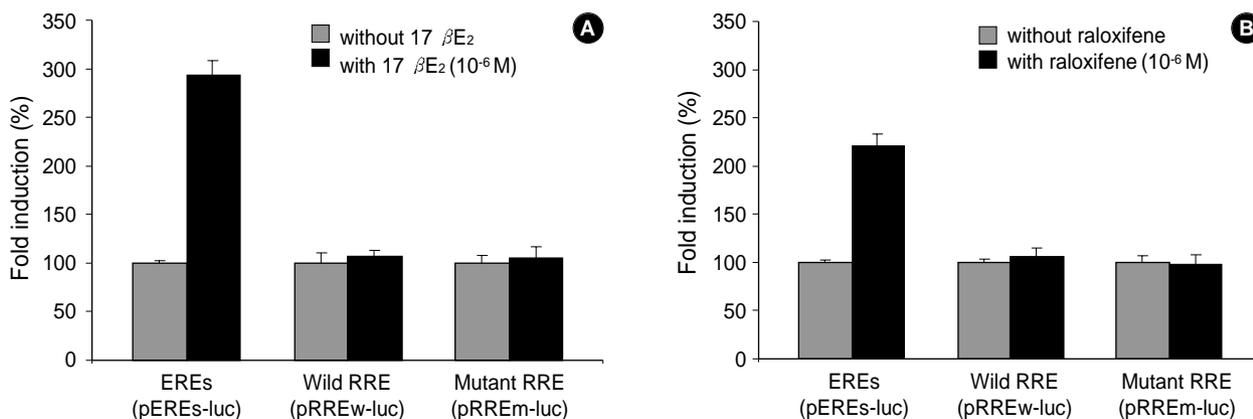


Fig. 3. Transient cotransfection study with human ER α (pCMVERa) and several reporter plasmids (pEREs-luc, pRREw-luc, or pRREm-luc) in Hela cells. Significant increase of luciferase activities can not be seen in either wild or mutant type RRE after treatment of estrogen (A) or raloxifene (B).

tion, which had a 19 base repeat sequence of the wild type DNA between nucleotide 53 and 71 (Fig. 1, 2).

A 55-yr-old woman with this mutant RRE was 160 cm tall. She had one daughter and one son. The morphology of the breast was normal and the external genitalia and ovaries were intact. She had had a hysterectomy because of myoma at the age of 45. The lipid profile was normal. Z score (the value of the SD obtained when the average of the data was adjusted to zero) of her spine BMD was -0.93.

The luciferase activity in pEREs-luc transfected cells were shown to increase significantly after treatment of estrogen or raloxifene whereas the luciferase activity in both wild and mutant type RRE did not increase even after treatment of estrogen or raloxifene. There were no significantly different activities between the wild type and mutant type RRE either before or after treatment of estrogen or raloxifene (Fig. 3).

DISCUSSION

We found a heterozygote with a 20 base insertion and one point mutation of the RRE site in the TGF- β 3 gene. Other polymorphic site could not be found in 200 women.

The functional significance of the mutant RRE remains unknown. BMD of the subject was comparable to that of the age-matched controls. These findings suggest that the RRE mutant of one allele is not likely to be responsible for the determination of BMD, and the mutant does not seem to have a dominant effect. However, the possibility can not be excluded that the homozygote may affect the bone preservation. In case of glucokinase gene, the heterozygote of the mutant in β -cell specific promoter was more frequently found in patients with glucose intolerance and showed a lower transcriptional activity. The homozygote of the variant was found only in non-insulin-dependent diabetic patients (7). Further investigation needs to be performed in a large

population to find the homozygote.

Transient transfection study of this heterozygote mutant RRE did not show any significant difference in activity compared with the wild type activity. Furthermore, both wild and mutant type RRE did not induce the luciferase activity even after the treatment of raloxifene. These findings suggest that the wild type RRE as well as the mutant type RRE is not influenced by raloxifene treatment. This suggestion can be supported by the report that demonstrated the RRE was not sufficient to mediate the full hormonal regulation of the TGF- β 3 gene (8).

Our data suggest that the novel RRE mutant seems not to be a functional mutant and the RRE promoter region may not be essential to mediate the target gene regulation.

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