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Research paper

Gene expression profiling of mammary glands of cathepsin E-deficient mice compared with wild-type littermates

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Abstract

Cathepsin E is an endolysosomal aspartic proteinase predominantly expressed in cells of the immune system and has been implicated in various physiological and pathological processes. Because of physiological substrates of cathepsin E have not yet been identified, however, the physiological significance of this protein still remains speculative. To better understand the physiological significance of cathepsin E in the mammary gland, we investigated the effect of the deficiency of this protein on the gene expression profile of the tissue. Here we used mammary glands derived from multiparous and non-pregnant 11-month-old syngenic wild-type ($CatE^{+/+}$) and cathepsin E-deficient ($CatE^{-/-}$) mice for extraction of total RNA from each tissue and subsequent mRNA amplification, DNA fragmentation, and hybridization with cDNA mixroarray chips. A total of 654 genes were identified as overexpressed (>2-fold) in $CatE^{-/-}$ mammary glands compared with $CatE^{+/+}$ counterparts. These included genes related to signal transduction, immune responses, growth factor activity, and milk proteins, which occupied a large portion of the gene fragments identified as overexpressed. In contrast, a total of 665 known genes were identified as underexpressed in the mammary gland of $CatE^{-/-}$ mice compared with $CatE^{+/+}$ counterparts. These included genes related to cytoskeleton, cell differentiation, cell cycle arrest and apoptosis, which occupied the majority of the gene fragments identified as underexpressed. The results thus suggest that cathepsin E in mammary glands plays a crucial role in the regulation of proteins involved in signaling, development, differentiation and proliferation in the mammary gland.

Keywords: Aspartic proteinase; Cathepsin E; Mammary gland; Gene expression profiling

1. Introduction

Tissue remodeling is a key process involved normal development. The mammary gland undergoes extensive tissue remodeling during each lactation cycle. During pregnancy, the epithelial compartment of the gland is vastly expanded [1]. At the end of lactation the epithelial cells undergo apoptosis and adipocyte differentiation is induced [2]. Previous studies have demonstrated that ductal and alveolar growth during puberty and pregnancy, and the involution process are mediated by the action of proteases, including, matrix metalloproteinases (MMPs), plasminogen, membrane-peptidases, and cathepsin D, and the corresponding genes are activated during these periods [1,3,4]. MMPs are expressed in several cell types of the mammary gland, including stromal fibroblasts (e.g., MMP3, MMP2), epithelial cells (e.g. MMP7, MMP9), adipocytes (e.g., MMP2) and lymphoid cells (e.g. MMP9) [5,6]. Several knockout mouse strains, including MMP2-, MMP3- or plasminogen-deficient mice, have displayed alterations to mammary gland structure and impairment of lactation [5,6] and provided important insight into their functions in the mammary gland.

Cathepsin E is an intracellular aspartic proteinase of the pepsin superfamily, which is expressed predominantly in cells of the immune system [7–10] and highly secreted by activated

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phagocytes [11]. Like other pepsin family members, cathepsin E requires an acidic environment for optimal activity and is believed to be involved in unspecific bulk proteolysis in endo-lysosomal compartments, but there is growing evidence that this enzyme has specific, non-redundant functions [12,13]. On the other hand, cathepsin E possesses several unique properties not shown by other pepsin family members. Unlike other intracellular aspartic proteinases, cathepsin E forms a homodimer that is not necessarily required for expressing activity, correct intracellular localization, and carbohydrate modification, but is essential to structural stabilization of the molecule [14,15]. Besides their structural and immunological distinction [16–22], cathepsin E is different from other pepsin family members in tissue distribution and intracellular localization [7,8,10,23]. Differing from the definite localization of the analogous lysosomal aspartic proteinase cathepsin D, the intracellular localization of cathepsin E appears to vary with cell types [8,10,23]. In antigen presenting cells such as dendritic cells, microglia, and macrophages, cathepsin E is mainly found in endosomal structures mainly as a mature enzyme which is N-glycosylated mostly with complex-type oligosaccharides [8]. In some types of cells, including erythrocytes, renal proximal tubule cells, and osteoclasts, cathepsin E is exclusively confined to the plasma membrane [23,24] mainly as a proenzyme having complex-type oligosaccharides. In a variety of other cell types, cathepsin E is also detected in the endoplasmic reticulumn and Golgi complex [10,23,25]. Cathepsin E has been implicated in various physiological and pathological processes (see for review refs. [26,27]), the precise role of this protein remains largely unknown, because the physiological substrates of this protein have not yet been identified. Recent genetic approaches using mice lacking cathepsin E have provided important insight into their biological functions [28–30]. Cathepsin E-deficient ($CatE^{-/-}$) mice were shown to spontaneously develop atopic dermatitis-like skin lesions when reared under conventional conditions [28] and exhibit the increased susceptibility to bacterial infection accompanied by a marked decrease in killing of intracellular bacteria by macrophages [29]. Subsequent analysis of macrophages derived from $CatE^{-/-}$ mice revealed that the deficiency of this protein induced a novel form of lysosomal storage disorder manifesting the accumulation of major lysosomal membrane sialoglycoproteins such as LAMP-1 and LAMP-2 and the elevation of lysosomal pH [30]. Based on these observations, it has strongly been suggested that cathepsin E contributes to the maintenance of homeostasis by participating in host defense mechanisms.

The aim of the present study was to gain further insight into the mechanisms underlying cathepsin E functions in mammary glands in vivo, because this protein also exists in the reproductive system including mammary glands, besides the immune system. To better understand the physiological significance of cathepsin E in mouse mammary gland, the strategy was to take advantage of $CatE^{-/-}$ mice. We thus performed global gene expression profiling of mammary glands and conducted a screen that select specifically for genes that were significantly altered by cathepsin E deficiency.

2. Materials and methods

2.1. Animals

Wild-type and $CatE^{-/-}$ mice with C57BL/6 genetic background were housed as described previously [28]. All animals were maintained under specific pathogen-free conditions at Kyushu University Station of Collaborative Research animal facilities according to the guidelines of the Japanese Pharmacological Society. All animal experiments were approved with the Animal Research Committee of Graduate School of Dental Science, Kyushu University.

2.2. Real time RT-PCR

Total RNA was isolated with the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The yield and quality of RNA was evaluated by measuring its absorbance at A260/A280 and gel electrophoresis. cDNA synthesis was performed using an Ready-to-Go RT-PCR Beads (Amersham Biosciences Co., NJ, USA). A total of 1 μg of each sample was incubated in a 50 μl reaction mixture containing first-strand primers, forward and reverse primers (20 pmol), and RNase/DNase free water. RT was performed using a thermal program of 45 °C for 15 min, and 95 °C for 5 min. The specificity of each primer set was first tested by RT-PCR, followed by melting curve analysis using DyNAmo™ HS SYBR® Green qPCR Kit (Finnzymes, Espoo, Finland) with an Rotor-Gene™ 3000 (NIPPN TechnoCluster, Inc., Tokyo, Japan) and gel electrophoresis of the PCR products. Gene-specific forward and reverse cathepsin E primers were 5′-GTGCCCCTCAGA AGACATCA-3′ and 5′-GTATCCCAGACCCAGAATCC-3′, respectively. G3PDH expression was monitored as an endogenous control, during each PCR reaction. The forward and reverse primers for G3PDH were 5′-TCCACCACCCTGTTGC TGTA-3′ and 5′-ACCACAGTCCATGCCATCAC-3′, respectively. Each PCR reaction was carried out in triplicate in a total volume of 20 μl, containing master mix which contains hot start version of a modified *Tbr* DNA polymerase, SYBR Green I, optimized PCR buffer, 5 mM $MgCl_2$, dNTP mix including dUTP, forward primer, reverse primer, water, and cDNA. Thermocycling conditions were as follows: 95 °C for 15 min, followed by 45 cycles at 94 °C for 10 s, 57 °C for 30 s, and 72 °C for 30 s for cathepsin E, 95 °C for 15 min, followed by 35 cycles at 94 °C for 10 s, 60 °C for 20 s, and 72 °C for 30 s for G3PDH, with fluorescent readings at the end of each cycle. Total RNA from wild-type mouse stomach was used as a positive control.

2.3. Immunoblot analysis

SDS-PAGE and immunoblot analysis were performed as described previously [31]. After transfer of proteins fractionated by SDS-PAGE to a nitrocellulose membrane and blocking with 5% non-fat dried milk in Tris-buffered saline, the membrane was incubated overnight at 4 °C with primary antibody to cathepsin E (diluted 1/4000). After washing, the membrane