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17beta-estradiol induces both up-regulation and processing of cyclin E in a calpain-dependent manner in MCF-7 breast cancer cells

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ABSTRACT

In the current study, we investigated whether 17beta-estradiol (E2) induces cyclin E expression and triggers cyclin E processing via calpain in MCF-7 breast cancer cells. We found that E2 induced increased expression of cyclin E in a slow and persistent manner, and a rapid yet sustained processing of cyclin E. In addition, estrogenic ethanol was able to stimulate cyclin E truncation. Calpeptin or ALLN greatly suppressed the E2-triggered cyclin E processing and its expression, suggesting a calpain-mediated action for E2. Finally, the E2-induced effects could also be significantly suppressed by BAPTA or U0126, indicating involvement of calcium/ERK signaling. Taken together, these results show that estrogen may contribute to both up-regulation and proteolysis of cyclin E through calpain in MCF-7 cells.

1. Introduction

Cyclin E plays a key role in the G1 to S phase transition of the cell cycle [1]. Deregulation of cyclin E in breast tumors is characterized by over-expression of total cyclin E and appearance of its truncated forms [2]. It has been shown that over-expression of cyclin E is associated with tumor progression and that high levels of total or processed cyclin E strongly predicts poor prognosis of breast cancer patients [3–5]. Moreover, truncated cyclin E is also observed in other kinds of tumors and is indicative of poor survival [6–8]. Recently, it is shown that resistance to letrozole in breast cancer is due to over-expression of proteolyzed cyclin E [9]. The mechanism(s) whereby cyclin E is deregulated, however, remains unsolved [10–13].

We previously reported that cyclin E can be truncated in breast cancer cells into its low-molecular-weight (LMW) forms via calpain by calcium stimulation [10]. Another report shows that over-expressing cyclin E in breast cancer cells may result in cyclin E processing [11]. Yet, other literature suggests that intracellular elastase is responsible for cyclin E proteolysis [12,13]. It is likely that at least two kinds of intracellular proteases may be responsible for cyclin E processing. However, it is unclear how these proteases are activated to deregulate cyclin E in living cancer cells. As estrogen plays an important role in hormone-dependent breast cancer, we hypothesized that deregulation of cyclin E might be attributable to estrogen through calpain that is over-expressed and highly active in breast cancer cells and tissues [10].

In the present study, we used estrogen receptor(ER)-positive MCF-7 breast cancer cells to address whether E2 induces deregulation of cyclin E via calpain. We found that E2 caused increased expressions of cyclin E mRNA and protein and triggered cyclin E processing in a sustained manner and that both of these E2-induced effects were calpain-dependent.

2. Materials and methods

2.1. Estrogen treatment

Cells were grown under normal conditions until reaching 60%–70% confluence, and then cultured in phenol red (PR)-free medium without serum for 24 h. Cells were grown in PR-free medium with 2.5% CS-FBS for 12 h before experiment. During treatment, cells were kept in medium plus 1%CS-FBS for observation of proteolysis or 5%CS-FBS to measure the levels of gene and proteins.

2.2. RT-PCR

PCR was performed with gene-specific primers followed by PCR amplification; beta-actin was used as a reaction standard. Each PCR product was analyzed by 2% agarose gel electrophoresis. Authentic

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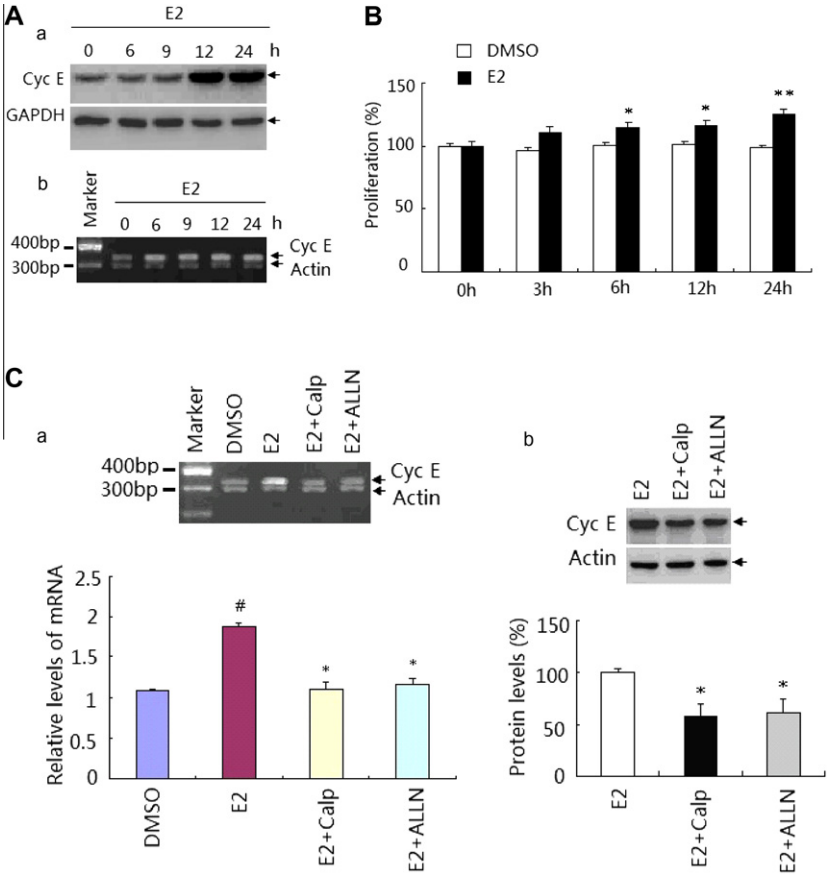

Fig. 1. E2-enhanced increased levels of cyclin E are greatly suppressed by calpain inhibitors in MCF-7 cells. (A) Cells were treated with E2 (10 nM) for 0–24 h. Immunoblotting was used to determine the levels of protein (a); RT-PCR was performed to observe the levels of mRNA (b). (B) At indicated times after E2 (10 nM) treatment, cells were prepared for MTT assay to measure proliferation. (C) Cells were pretreated with calpeptin (10 μM) or ALLN (10 μM) for 30 min where indicated, followed by treatment with E2 (10 nM) for 12 h. The ratios of cyclin E/actin were calculated to evaluate the mRNA or protein levels, with DMSO as vehicle control ($^{\#}P < 0.01$ vs. DMSO; $^{*}P < 0.01$ vs. E2).

bands were detected by GelDoc2000 imaging system (Bio-Rad) and determined by Quantity One Software (Bio-Rad).

2.3. Western blot analysis

Cells were lyzed in RIPA lysis buffer. Cell lysates were separated on a 12% SDS–PAGE and transferred to a PVDF membrane (Millipore). Protein bands were visualized by using an enhanced chemiluminescence kit (Milipore). Digital images of blots were produced by Syngene Imaging System and quantified using the GeneSnap software (Syngene).

2.4. Statistical analysis

The Student's *t*-test or one-way ANOVA was used to calculate the statistical differences. Data are expressed as mean ± S.E.M. and $P < 0.05$ was considered significant.

3. Results

3.1. E2-upregulated expression of cyclin E was mediated by calpain in MCF-7 cells

To confirm whether E2 causes up-regulation of cyclin E, we treated MCF-7 cells with E2 for 0 h–24 h. We observed that the protein level of wild type cyclin E was greatly increased in response to E2 stimulation, which occurred in a delayed yet sustained manner (Fig. 1Aa) and was concurrent with enhanced cell proliferation (Fig. 1B), indicating increased cyclin E expression promotes cell growth. To test whether cyclin E protein expression results from its increased gene transcription, we employed RT-PCR to measure its mRNA levels. We noticed that mRNA levels of cyclin E increased prior to protein elevation after E2 treatment (Fig. 1Ab). We further accessed whether calpain is involved in E2-triggered up-regulation of cyclin E using a pharmacologic method. It was found that both mRNA and protein expressions of cyclin E stimulated by E2 could be effectively inhibited by calpain inhibitors calpeptin or ALLN (Fig. 1C). These observations indicate that E2 may up-regulate cyclin E expression through calpain.

3.2. E2 induced processing of cyclin E in MCF-7 cells

We first observed that 10%CS-FBS in the culture led to cyclin E processing, while lower serum rendered cyclin E intact (Fig. 2A), indicating a growth factor-induced effect. To test the E2 action, we treated cells with E2 of varied concentrations and found that E2 effectively triggered truncation of wild type cyclin E into an LMW band similar in size to that observed in breast cancer tissues. E2 at a concentration of ~0.1nM was enough to trigger cyclin E truncation(Fig. 2B), implying a very sensitive response to E2 stimulation. E2-induced cyclin E truncation occurred in a rapid (~15 min) yet sustained manner, peaked at ~1 h and lasting for hours, and E2 at ~10 nM achieved a maximum effect (Fig. 2C). Additionally, truncated cyclin E appeared coincident with processing of focal adhesion kinase (FAK, positive control [11]), but not of cyclin D1 (negative control). We further tested a possible