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BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

The Anti-Proliferative Effect of Genistein on Antioxidant System, Cell Proliferation, and Apoptosis: Implications for Hormone-Refractory Prostate Cancer Therapy

Genisteinin Antioksidan Sistem, Hücre Çoğalması ve Apoptoz Üzerindeki Anti-Proliferatif Etkisi: Hormon Refrakter Prostat Kanser Tedavisinde Uygulamalar

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Abstract

Objectives: This study aims to examine the effect of different genistein concentrations on apoptosis, estrogen receptor beta, and manganese superoxide dismutase (MnSOD) protein expressions in PC3 hormone-resistant metastatic prostate cancer cell lines.

Materials and Methods: Genistein concentrations of 0, 0.01, 0.1, 0.5, 1, 5, 10, and and 50 µM were applied to PC3 hormone-resistant metastatic prostate cancer cell lines for 48 hours and water soluble tetrazolium salt-1 cell proliferation assay was performed. Protein was extracted from the cells and cleaved poly (ADP-ribose) polymerase, estrogen receptor beta (ERβ), and MnSOD protein expression levels were examined using the Western blot protocol. One-way analysis of variance (ANOVA) and Student's t-test statistical analysis methods were applied to determine whether there are statistically significant differences.

Results: Genistein was found to change ERβ expression in PC3 cells depending on concentration. While ERβ protein expression increases observed at low concentrations (0.01-0.5 µM), it sometimes decreased to baseline or increased modestly at high concentrations (1-50 µM). MnSOD protein expression showed a stimulating effect on the protein expression level at high concentrations (1-50 µM).

Conclusion: This research study investigates how different concentrations of genistein affect cell proliferation, apoptosis, estrogen receptor beta, and MnSOD protein expression levels in PC3 hormone-refractory prostate cancer cells.

Keywords: Prostate cancer, genistein, apoptosis, ERβ, MnSOD

Öz

Amaç: Bu çalışmanın amacı hormona dirençli olan PC3 metastatik prostat kanser hücre serilerinde farklı genistein konsantrasyonlarının apoptoz, östrojen reseptör beta ve manganez süperoksit dismutaz (MnSOD) protein ekspresyonlarındaki etkisini incelemektir.

Gereç ve Yöntem: PC3 hormona dirençli olan metastatik prostat kanser hücre serilerine 0, 0,01, 0,1, 0,5, 1, 5, 10, 50 µM genistein konsantrasyonları 48 saat uygulanmıştır ve suda çözünebilen tetrazolyum tuzu-1 hücre proliferasyon yöntemi uygulanmıştır. Hücrelerden protein ekstrekte edilip, Western blot yöntemi kullanılarak cleaved poli (ADP-riboz) polimeraz, östrojen reseptörü beta (ERβ) ve MnSOD protein ekspresyon seviyeleri incelenmiştir. Tek yönlü varyans analizi (ANOVA) ve Student's t-test istatistiksel analiz metodları uygulanmıştır.

Bulgular: Genistein PC3 hücrelerinde konsantrasyona bağlı olarak ERβ ekspresyonunu değiştirdiği saptanmıştır. Düşük konsantrasyonlarda (0,01-0,5 µM) ERβ protein ekspresyonu artarken, yüksek konsantrasyonlarda (1-50 µM) bazen temel seviyeye düşerek ya da ılımlı artarak farklılık göstermiştir. MnSOD protein ekspresyonu ise yüksek konsantrasyonlarda (1-50 µM) protein ekspresyon seviyesi üzerinde uyarıcı etki göstermiştir.

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Sonuç: Bu araştırma çalışmasında, PC3 hormonuna dirençli prostat kanseri hücrelerinde farklı genistein konsantrasyonlarının hücre çoğalmasını, apoptozu, östrojen beta reseptörünü ve MnSOD protein ekspresyon düzeylerini nasıl etkilediği araştırılmıştır.

Anahtar Kelimeler: Prostat kanseri, genistein, apoptoz, ERβ, MnSOD

Introduction

Prostate cancer, being one of the most prevalent cancers in men worldwide, is a significant public health challenge (1). The mainstay options in the treatment of prostate cancer include traditional methods such as surgical removal, hormone ablation therapy, and radiotherapy. However, these treatments come with limitations, especially in treating androgen-independent prostate cancers that exhibit chemoresistance and radioresistance (2,3).

Recent interest has surged around genistein, a natural compound isoflavone found in soy products, due to its potential therapeutic benefits in cancer prevention and treatment. The complex methods by which genistein inhibits cell proliferation in prostate cancer involve modifying several signaling pathways that regulate cell growth and division (4).

Specifically, genistein can alter the expression of proteins involved in cell cycle regulation, halting cancer cells' uncontrolled growth (5,6). Additionally, genistein induces apoptosis, which helps eliminate damaged or malignant cells, consequently suppressing tumor growth and progression (7).

The development of prostate cancer is significantly influenced by estrogen receptor signaling, with both estrogen receptors alpha (ERα) and beta (ERβ) being implicated in the pathogenesis of the disease. The expression of these receptors in prostate cancer tissues contributes to the activation of estrogen-signaling pathways that promote cancer cell proliferation and survival (8). Genistein interacts with ERβ, mimicking estrogen's effects, which include regulating cellular growth, differentiation, and apoptosis in prostate cancer cells (9). The abnormal activation of estrogen signaling can lead to dysregulated gene expression patterns that drive tumor progression. Hence, targeting estrogen receptor signaling pathways offers promising avenues for the development of novel therapeutic strategies to be used in the treatment of prostate cancer (4).

Manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme, regulates reactive oxygen species (ROS) levels in cells by detoxifying superoxide radicals generated during cellular metabolism (10). Dysregulation of MnSOD expression in prostate cancer can lead to an imbalance in ROS homeostasis, which in turn may cause tumorigenesis (11). Some studies suggest MnSOD is one of the mediators of the antioxidant benefits of genistein on prostate cancer (12). This research study investigates how genistein affects cell proliferation, apoptosis, estrogen receptor beta, and MnSOD protein expression levels in PC3 hormone-refractory prostate cancer cells.

Materials and Methods

The cells used in the study are commercially available, so ethics committee approval is not required. The study protocol was appropriate for the Declaration of Helsinki.

Cell Culture

RPMI 1640 (Gibco, Carlsbad, CA, US) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, US) and 1% penicillin/streptomycin (Gibco, Carlsbad, CA, US) were utilized for growing human hormone refractory-PC3 prostate cancer cells. These cells do not rely on androgen signaling for growth and survival, making them a valuable model for studying castration-resistant prostate cancer (CRPC)-an advanced stage of prostate cancer that progresses despite androgen deprivation therapy. The cells were maintained in a humidified incubator with 5% CO_2 at 37 °C. The vehicle was used as the control and around 60-70% of confluent cells were treated with varying dosages of genistein (Sigma, St. Louis, MO, US) dissolved in dimethyl sulfoxide.

Cell Proliferation Assay

Utilizing the water soluble tetrazolium salt-1 (WST-1) calorimetric cell proliferation assay kit, the impact of genistein treatment on PC-3 cell growth was examined. Cells were seeded in a 96-well plate at 1×l04 cells/well density and overnight incubated in 100 µL culture media. Afterward, the cells were treated with different concentrations of genistein (0.01, 0.1, 0.5, 1, 5, 10, and 50 µM). These concentrations were selected based on literature, our previous trial experiments. Following 48 hour, each well was treated with 10 µl of WST-1 reagent at 37 °C for 4 h. The micro-plate reader (Thermo Scientific, Multiscan GO) was used to measure absorbances at 450 nm oxide dismutase. The experiment was conducted three times.

Western Blotting

The analysis of protein expression was evaluated by Western blotting. Seeded 1×10^6 cells were subsequently treated for 48 h with 0.01, 0.1, 0.5, 1, 5, 10 and 50 µM of genistein. Following harvesting, treated and vehicle cells were lysed in 1X cell lysis buffer (Cell Signaling Technology, USA) containing diluted 1 mM PMSF (Sigma, Germany) in distilled water.

Bicinchoninic acid assay kit (Thermo Fisher Scientific, England) was utilized to measure protein concentrations in

MultiSkan GO Microplate Spectrophotometer (Thermo Fisher Scientific, England). Proteins (20 µg) were separated by sodium dodecyl sulfate-polyacrylamide gels before being transferred to a polyvinylidene fluoride membrane. Then, the blots were incubated with the primary antibodies against; Cleaved poly ADP-ribose polymerase (PARP) (Cell Signaling Technology, USA), ERβ (Invitrogen, Paisley, UK), MnSOD (Abcam, Cambridge, US) and β-actin (Cell Signaling Technology, USA). Following secondary antibody incubations, the blots were subjected to a chemiluminescence solution to visualize the specific binding. The protein bands were densitometrically measured by ImageJ (NIH, Bethesda, USA). The data was normalized by comparing the results with the expression of β-actin in every concentration group.

Statistical Analysis

Experiment findings are displayed as a mean ± standard error of the mean. Student's t-test and one-way ANOVA were applied to evaluate the group differences.

The group differences were analyzed by using one-way ANOVA and Student's t-test. The software system GraphPad Prism 7® (La Jolla, CA, USA) was used to assess all the results. A p-value ≤0.05 or p-value ≤0.0001 was accepted as statistically significant.

Results

Effects of Genistein on Cell Proliferation

Incubation of PC3 cells with 0.01 µM and 0.1 µM genistein treatments resulted in the absorbance values slightly lower than the vehicle control. However, regarding the effect of genistein on cell proliferation, incubation with 0.1 µM genistein caused a significant increase, suggesting a stimulatory effect on cell proliferation. This might be due to a biphasic dose-response relationship, where low doses of certain compounds can stimulate cell growth while higher doses inhibit it. At 0.5 µM concentration, there is a notable increase in cell proliferation compared to vehicle control, indicating a potential proliferative effect of genistein at this concentration. At 1 µM and 5 µM concentrations, the cell proliferation levels are similar to the vehicle control (Figure 1). This suggests that these concentrations do not significantly affect cell proliferation in PC3 cells, indicating a possible threshold effect where genistein neither stimulates nor inhibits cell growth significantly within this range. At higher concentrations (10 µM and 50 µM), there is a noticeable decrease in cell proliferation compared to vehicle control. This aligns with the known anti-proliferative effects of genistein at higher concentrations, likely due to its ability to induce apoptosis and inhibit cell cycle progression in prostate cancer cells.

Cleaved PARP Protein Expression

There was a moderate cleaved PARP protein expression level at vehicle control genistein concentrations with some variability in PC3 cells. At 0.01 µM genistein concentration, there was a slight increase in cleaved PARP protein levels compared to the control, indicating some apoptotic activity. At 0.1 µM genistein concentration, the levels decreased, suggesting a reduction in apoptosis. There was a significant increase in cleaved PARP protein levels at 0.5 µM genistein concentration, indicating a strong apoptotic response. At 1 µM genistein concentration, there was a decrease in cleaved PARP, indicating reduced apoptosis. At 5 µM, levels were like the control genistein concentrations while at 10 µM level, there was an increase, and at 50 µM, the levels were back to low again (Figure 2).

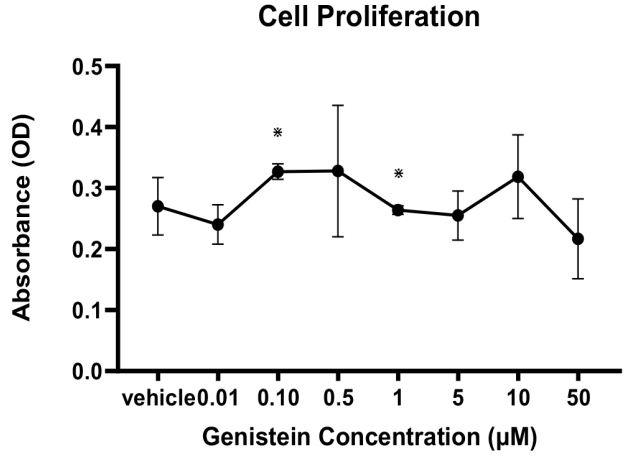

Figure 1: Cell proliferation was determined by the WST-1 assay. Triplicate wells containing 1×10^4 cells/well of PC-3 were exposed to 0.01, 0.1, 0.5,1, 5, 10 and 50 µM concentrations of genistein. Growth curves were obtained at 48 h. Error bars represent the SEM values, p<0.05

*Represents statistically significant, OD: Optical density, SEM: Standard error of the mean

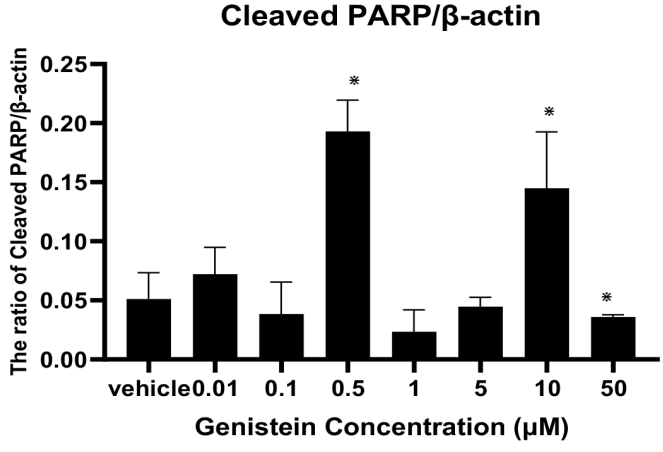

Figure 2: The cleaved PARP protein expression level was determined by Western blot. Error bars represent the SD values, p<0.0001

*Represents statistically significant, PARP: Poly ADP-ribose polymerase, SD: Standard deviation

ERβ Protein Expression

The baseline expression level of ERβ in untreated PC3 cells was approximately 0.6. At 0.01 μM genistein concentration, the expression level increased to approximately 1.2, indicating a significant upregulation of ERβ at this low concentration. At 0.1 μM genistein concentration, the expression level peaked at around 1.4, suggesting that this concentration induced the highest ERβ expression among the tested doses. At 0.5 μM genistein concentration, the expression level remained elevated at approximately 1.2, similar to the 0.01 μM treatment. At 1 μM genistein, there was a noticeable decrease in ERβ expression returning to baseline levels. At 5 μM genistein concentration, the expression level remained low at around 0.6, consistent with the vehicle control and 1 μM treatment. At 10 μM genistein concentration, the expression level increased slightly to around 0.8, indicating a modest upregulation compared to the control. At 50 μM, the highest genistein concentration, the expression level rose to approximately 1.0, showing a moderate increase in ERβ expression (Figure 3).

MnSOD Protein Expression

At 0.01 μM concentration of genistein, the MnSOD/β-actin ratio is 0.326, indicating a baseline or slightly reduced activity of MnSOD relative to β-actin protein. The MnSOD/β-actin ratio increased to 0.469 at 0.1 μM concentration, suggesting a potential stimulatory effect on MnSOD protein expression level compared to the lower concentration. At 0.5 μM concentration, the MnSOD/β-actin ratio decreased slightly to 0.424, indicating a possible reduction in protein expression of MnSOD relative to β-actin. The MnSOD/β-actin ratio increased notably to 0.519 at 1 μM concentration, suggesting a potential stimulatory effect on MnSOD protein expression level compared to lower concentrations. At 5 μM, the MnSOD/β-actin ratio continued to increase to 0.553, indicating a further potential increase. The MnSOD/β-actin ratio increased to 0.645 at 10 μM concentration, indicating a continued stimulatory effect on MnSOD protein expression level. At 50 μM concentration, the MnSOD/β-actin ratio increased further to 0.703, suggesting a potentially enhanced stimulatory effect on MnSOD activity compared to lower concentrations (Figure 4).

Discussion

There are conflicting results about the potential protective versus adverse effects of soy and genistein in breast and prostate cancer animal model studies (13-15). In our previous study, it was exhibited that genistein exerts its biphasic beneficial and harmful effects through various molecular mechanisms and pathways (16). The effects of physiological (0.01, 0.1, 0.5, 5, and 10 μM) and pharmacological (50 μM) concentrations of genistein on PC-3 cells regarding cell proliferation, apoptosis, estrogenic effects, and antioxidant defense interactions were examined in this current study. The WST-1 cell proliferation assay demonstrated a significant increase with 0.1 μM genistein treatment, indicating a stimulatory effect on cell proliferation at this low concentration. This is also demonstrated in a study by Li et al. (17) where genistein's biphasic effect on cell proliferation was exhibited, as low concentrations of the compound were shown to stimulate growth while higher concentrations inhibited it. The cell proliferation assay results showed that the cell growth levels were similar to the vehicle control at 1 μM and 5 μM genistein concentrations suggesting that these concentrations do not significantly affect cell proliferation in PC3 cells, therefore indicating a possible threshold effect where genistein neither stimulated nor inhibited cell growth significantly (18). Conversely, a noticeable decrease in cell proliferation relative

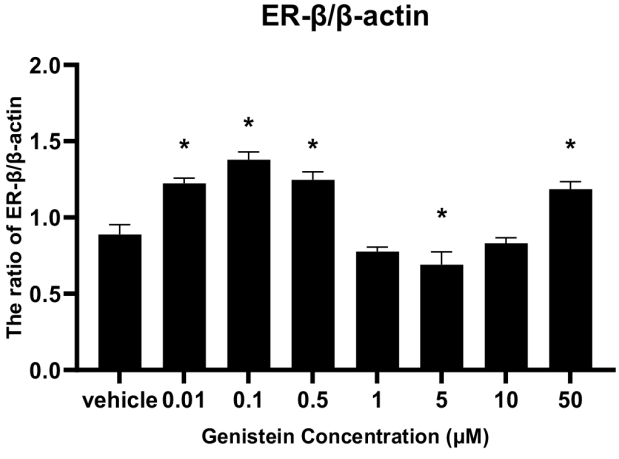

Figure 3: ERβ protein expression level was determined by Western blot. Error bars represent the SD values. p<0.0001

*: Represents statistically significant, ERβ: Estrogen receptor beta, SD: Standard deviation

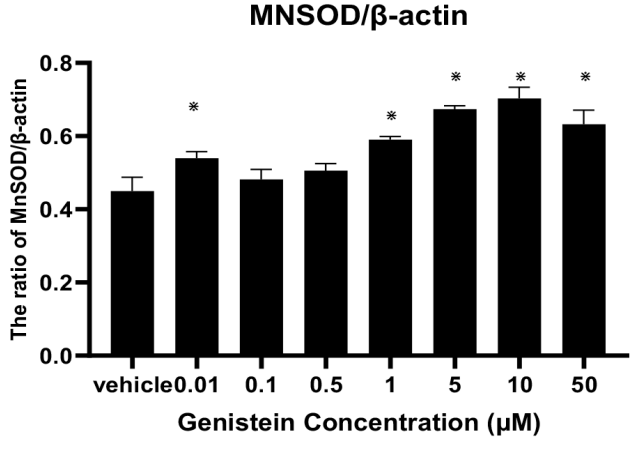

Figure 4: MnSOD protein expression level was determined by Western blot. Error bars represent the SD values. p<0.0001

*Represents statistically significant, MnSOD: Manganese superoxide dismutase, SD: Standard deviation

to the vehicle control was observed at higher concentrations (10 µM and 50 µM). This finding aligns with the known anti-proliferative effects of genistein at higher concentrations, likely owing to the compound's ability to induce apoptosis and inhibit cell cycle progression in prostate cancer cells (19). In addition to this study, the induction of apoptosis has also been shown by various other studies as one of the most reported biological activities of genistein (16,17,20). It was observed that genistein had a notable impact on apoptosis in PC3 cells through the examination of cleaved PARP protein, which is an early indicator of apoptosis. The data suggests a dose-dependent response of cleaved PARP protein levels to genistein treatments, with certain concentrations (e.g., 0.5 µM and 10 µM) showing significant increases in apoptosis markers, while others (e.g., 1 µM and 50 µM) exhibited lower levels. In terms of therapeutic potential, genistein's activation of ERβ offers a promising strategy. The ability of genistein to enhance ERβ activity suggests it could be beneficial in managing prostate cancer via the promotion of tumor suppression and reduction of metastasis. Studies have shown that loss of ERβ expression is associated with higher-grade, more aggressive prostate cancers, making the activation of this receptor a potential therapeutic target. ERβ is also known to modulate oxidative stress, a key factor affecting prostate cancer progression. Higher levels of ERβ are associated with increased expression of antioxidant enzymes, reducing oxidative stress and potentially slowing cancer progression (4,21). This study suggests that genistein modulated ERβ expression in a concentration-dependent manner in PC3 prostate cancer cells. Low concentrations (0.01-0.5 µM) significantly upregulated ERβ, while higher concentrations (1-50 µM) showed a varied response, with some concentrations returning to baseline levels or showing moderate increases indicating ERβ has anti-metastatic properties (20). By upregulating ERβ, genistein may inhibit key pathways involved in cancer cell migration and invasion, both of which are critical in the metastatic spread of prostate cancer (22). The observed upregulation of ERβ at low concentrations of genistein implies that genistein could help re-sensitize prostate cancer cells to hormonal therapies or delay the progression to hormone-refractory status by maintaining or enhancing ERβ expression and improving survival rates in return (23). A study regarding the prognostic significance of ERβ expression in prostate cancer indicated that patients with ERα-negative/ERβ-positive prostate cancer had a 5-year biochemical recurrence-free survival rate of 85.7 whose PCa tissue has ERα (-)/ERβ (+) staining results, indicating a lower risk of recurrence in this group (24). In metastatic prostate cancer, combining genistein with conventional treatments (e.g., chemotherapy, radiation) could enhance overall treatment efficiency (25). For example, the combination of genistein with AR-targeted therapies might improve outcomes in patients with CRPC by leveraging the anti-proliferative effects mediated by ERβ (4,21,23,26). Studies suggest that genistein can modulate oxidative stress pathways by improving antioxidant defenses, including upregulation of enzymes like MnSOD, to mitigate cell oxidative damage (27). Genistein's ability to enhance MnSOD activity may involve its antioxidant properties and regulation of mitochondrial function, which are crucial in maintaining cellular redox balance and protecting against oxidative stress-induced damage (28). According to the MnSOD protein expression levels, genistein concentrations have a dose-dependent effect on MnSOD/β-actin ratios in PC3 prostate cancer cell lines. Lower concentrations (0.01 to 0.5 µM) generally demonstrated variable effects on MnSOD protein expression level, with potential stimulatory or neutral effects. Higher concentrations (1 to 50 µM) consistently demonstrated an increase in MnSOD/β-actin ratio, indicating a stimulatory effect on MnSOD protein expression. Variances in measurements decreased with higher concentrations, suggesting more consistent effects at these levels. This study is in line with the results of the study conducted by Van der Eecken et al. (29) where lower concentrations of genistein had variable effects, while higher concentrations consistently increased MnSOD expression, representative of the stimulatory effect on MnSOD activity in prostate cancer cells. The modulation of MnSOD could reduce oxidative stress by enhancing cellular response, which contributes to the therapeutic potential of genistein in prostate cancer.

Studies have shown that there is a complex interaction between genistein, estrogen receptor signaling, and MnSOD in prostate cancer highlighting possibilities for the development of novel therapies.

Understanding the mechanisms through which genistein exerts its effects on prostate cancer cells could pave the way for the development of more effective treatments. Furthermore, accounting for how MnSOD and estrogen receptors contribute to the development of prostate cancer stresses the significance of targeting several pathways and variables in cancer treatments. Clarifying the interactions between genistein, MnSOD, and estrogen receptors may make it possible to create innovative treatments for prostate cancer. Furthermore, the combination of genistein with other treatments may be particularly beneficial in treating CRPC, a disease for which standard therapeutic strategies frequently prove ineffective. Moreover, genistein in combination with other therapies may be especially helpful in treating CRPC, a condition for which conventional treatment approaches are known to be ineffective.

Conclusion

In conclusion, this study highlights the biphasic effects of genistein on prostate cancer cells, demonstrating concentration-dependent modulation of proliferation, apoptosis, estrogen receptor signaling, and oxidative stress response. Findings in

this research article underscore genistein's complex molecular interactions and potential therapeutic relevance in prostate cancer, particularly in combination therapies for castration-resistant cases, warranting further investigation into its optimized clinical application.

Ethics

Ethics Committee Approval: The cells used in the study are commercially available, so ethics committee approval is not required.

Footnotes

Financial Disclosure: The author declared that this study has received no financial support.

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