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RESEARCH ARTICLE

Anti-cancer characteristics of mevinolin against three different solid tumor cell lines was not solely p53-dependent

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Abstract

Mevinolin (MVN) has been used clinically for the treatment of hypercholesterolemia with very good tolerance by patients. Based on epidemiological evidences, MVN was suggested strongly for the treatment of neoplasia. Early experimental trials suggested the mixed apoptotic/necrotic cell death pathway was activated in response to MVN exposure. Herein, the cytotoxic profile of MVN was evaluated, compared to the robust and frequently used anti-cancer drug doxorubicin (DOX), against breast (MCF-7), cervical (HeLa) and liver ($HepG_2$) transformed cell lines. MVN was showed comparable results in cytotoxic profile with DOX in all tested solid tumor cell lines. In addition, the MVN-induced cytotoxicity was inferred to be multi-factorial and not solely dependent on p53 expression. It was concluded that molecular and genetic assessment of MVN-induced cell death would be useful for developing cancer therapeutic treatments.

Keywords: Mevinolin, Doxorubicin, Solid tumors, natural products, p53

Introduction

In the developing world mortality rates due to cancer, including solid tumors, have remained constant over more than five decades which has urged intensified efforts to discover local agents for the treatment of cancer.[1] Natural products of plants provide an abundant source of potentially active compounds for the treatments of different disorders[1,2]. Far East, Middle East, Saharan, and tropical regions were among the richest sources of natural products in the world. The isolation and purification of the active fractions and active ingredients amongst potentially active natural products have received increased scientific and industrial interest[2].

Red yeast rice (RYR), a Chinese dietary product made by fermenting ordinary rice with the mould *Monascus purpureus,* has been widely used as a food condiment and colorant in several Asian countries[3]. RYR has been used for centuries without any reports of health hazards or long-term toxicity[4]. Several medicinally active ingredients were isolated from RYR including monacholin-K, mevinolin (lovastatin), γ-aminobutyric acid, dimerumic acid, sterols (β-sitosterol, campesterol, stigmasterol and sapogenin), isoflavones and monounsaturated fatty acids[3,4].

Mevinolin (MVN) or lovastatin was a potent HMGCo-A reductase enzyme inhibitor that interfered with *de novo* steroidogenesis[5]. MVN was used clinically for the treatment of hypercholesterolemia with very good patient tolerance profiles[6,7]. In the last decade, several epidemiological evidences have drawn attention to possible beneficial roles of

[1] Cancer remains the leading cause of death over 50 years (Source: 1950 mortality data - CDC/NCHS, NVSS, mortality Revised. 2002 mortality Data-NVSR-Death final Data 2002- Volume 53, No. 5. Cost data from american cancer Society Cancer & Figures 2005).

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HMGCo-A reductase inhibitors (statins), such as MVN, in neoblastic disorders. Some members of the statin group may reduce the recurrence of cancer after radical prostatectomy[8]. Also, dramatic reduction in the incidence of lipoma was observed for statin treated patients[9]. Most interestingly, a negative association was reported between the use of HMGCo-A reductase inhibitors and cancer incidence in veteran populations[10]. Many researches focused on the ability of MVN and other statins to sensitize tumor cells for conventional chemotherapeutics[11]. Some experimental reports manifested a potential anti-cancer activity of MVN and other HMGCo-A reductase inhibitors *per se*[12]. However, the exact signaling mechanism of MVN - induced cell death remain controversial. Few reports attribute the anti-cancer activity of MVN to the induction of apoptosis[13], while others negate any role of apoptosis in MVN-induced cell death[14]. Whether the apoptosis pathway is involved in MVN-induced cytotoxicity, or not, remained an open issue by 2011. The resolution of the mechanism of MVN might improve understanding of its anti-cancer effects and infer the likelihood of the emergence of resistance among cancer cell lines.

Doxorubicin (DOX) was a cytotoxic anthracycline originally isolated from *Streptomyces peucetius* which has been used for the past four decades for the treatment of several hematologic as well as solid malignancies[15,16]. However, DOX shows unique cardiotoxicity on the top of its regular chemotherapeutic toxicities such as myelosuppression, hyperemesis, diarrhoea, mucositis, impotence and alopecia[17,18].

Herein, the cytotoxic profile of the promising HMGCo-A reductase inhibitor, MVN, was compared to the clinically used chemotherapeutic agent, DOX against breast, cervix and liver cancer cell lines. Further, the expression of markers of apoptosis were compared in response to MVN and DOX treatment in solid tumor-like cell lines.

Materials and methods

Chemicals and drugs

DOX, sulfarhodamine (SRB) and MVN were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI-164 media, fetal bovine serum and other cell culture materials were purchased from Fisher Scientific Cell Culture(Houston, TX, USA). Other reagents were of the highest analytical grade available.

Cell culture

Human transformed cell lines, from liver (hepatocellular; HepG2), breast (MCF-7) and cervical (HeLa) lines were obtained from Vaccera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/mL streptomycin, 100 µg/mL penicillin and 10% (w/v) heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity assays

The cytotoxicity of MVN was tested against MCF-7, HeLa and HepG2 cells by the SRB assay as previously described[19]. Exponentially growing cells were collected using 0.25% (w/v) Trypsin–EDTA plated in 96-well plates at 1,000–2,000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10% (w/v)) for 1 h at 4°C. After several washings, cells were exposed to 0.4% (v/v) SRB solution for 10 min in the dark and subsequently washed with 1% (v/v) glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm[19].

Data analysis

The dose-response curve of compounds was analyzed using E_{max} model (Eq. 1).

$$\%\ \text{Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d{}^m + [D]^m}\right) + R \qquad (1)$$

Where R was the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce absorbance to 50% of that of the control (i.e. $K_d = IC_{50}$ when $R = 0$ and $E_{max} = 100 - R$)[31].

RNA extraction and real time PCR analysis for gene expression quantification

To assess the effect of MVN and DOX on the apoptosis pathway, total RNA isolation from cells was performed using RNeasy Mini Kit® (Qiagen Inc. Valencia, CA, USA). Reverse transcription was undertaken to construct a cDNA library from different treatments using the High-Capacity cDNA Reverse Transcription Kit™ (Applied Biosystems, Foster City, CA, USA). Real time quantitative PCR reactions were performed as previously described using SYBR green (Fermentas Inc., Glen Burnie, MD, USA) labeled probes[20]. Primer sequences were as follows; Bcl2 forward primer GGG-TAC-GAT-AAC-CGG-GAG-AT and reverse primer CTG-AAG-AGC-TCC-TCC-ACC-AC; BAX forward primer TCT-GAC-GGC-AAC-TTC-AAC-TG and reverse primer TGG-GTG-TCC-CAA-AGT-AGG-AG; p53 forward primer CCT-CAC-CAT-CAT-CAC-ACT-GG and reverse primer CTG-AGT-CAG-GCC-CTT-CTG-TC. GAPDH was used as reference with forward primer TGC-ACC-ACC-AAC-TGC-TTA-G and reverse primer GAT-GCA-GGG-ATG-ATG-TTC[32].

Statistical analysis

Data are presented as mean ± SEM. Analysis of variance (ANOVA) with LSD *post hoc* test was used for

testing the significance using SPSS® for windows, version 17.0.0. $p < 0.05$ was taken as a cut off value for significance.

Results

Evaluating the anti-cancer effect of DOX and MVN against solid tumor cell lines

SRB-U assay was used to assess the cytotoxicity of DOX and MVN against three different solid tumor cell lines. DOX showed cytotoxicity against the solid tumor cell lines with IC_{50} that ranged from 0.28 to 0.42 μg/ml. HeLa cells were the most susceptible cell lines to DOX while HepG2 cells were least susceptible (Figure 1A). MVN, showed comparable cytotoxic profiles against the tested solid tumor cell lines with IC_{50} that ranged from 0.6 to 1.1 μg/ml. HeLa cells were the most susceptible cell line to MVN and HepG2 cells were the least susceptible (Figure 1B).

Comparting treatments with the IC_{50} of DOX against HepG2 and MCF-7 were 0.42 ± 0.05 and 0.42 ± 0.11 μg/ml, respectively. Despite the fact that HeLa cells were the most susceptible cell lines to DOX (IC_{50} = 0.28 ± 0.07 μg/ml), they showed the highest occurrence of resistance amongst the cell lines (7.7 ± 0.5%). HepG2 and MCF-7 showed resistance at 5.3 ± 2.2% and 2.6 ± 0.9%.

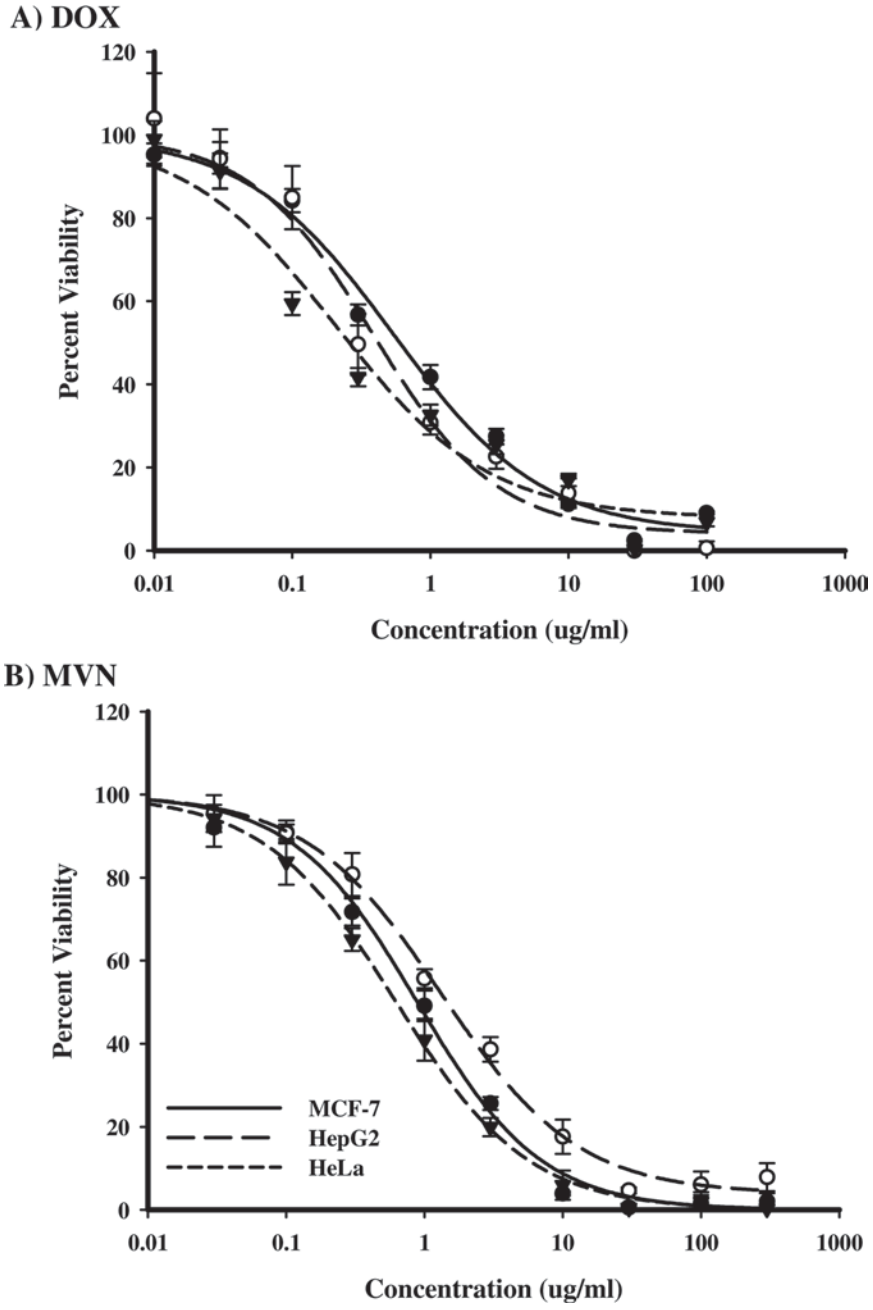

Figure 1. The effect of MVN and DOX on different solid tumor cell lines. Dose-response curves of DOX (A) and MVN (B) in solid tumor cell line cultures of HeLa (•) HepG2 (○) and MCF-7 (▾) cells. Cells were exposed to serial dilutions of DOX or MVN for 72 h. Cell viability was determined using SRB-U assay and data are expressed as mean ± S.D. ($n = 3$).

The IC_{50} of MVN against both MCF-7 and HeLa were very similar (at 0.67 ± 0.15 and 0.61 ± 0.02 μg/ml, respectively). Also the frequency of resistance of both MCF-7 and HeLa cells were similarly low (1.03 ± 0.15 and 0.33 ± 0.09%, respectively). In contrast to DOX, HepG2 cells were the most resistant in terms of IC_{50} (1.1 ± 0.12 μg/ml) and resistant frequency (5.1 ± 0.82%) among all tested cell lines. In general, MVN showed about two fold less potency than DOX in all the tested cell lines. However, resistance to MVN was much lower than to DOX (2–20 fold (Table 1).

Assessment of apoptosis in solid tumor cell lines after treatment with DOX and MVN

In order to examine the effect of DOX and MVN on the apoptosis pathways in the solid tumor cell lines, the

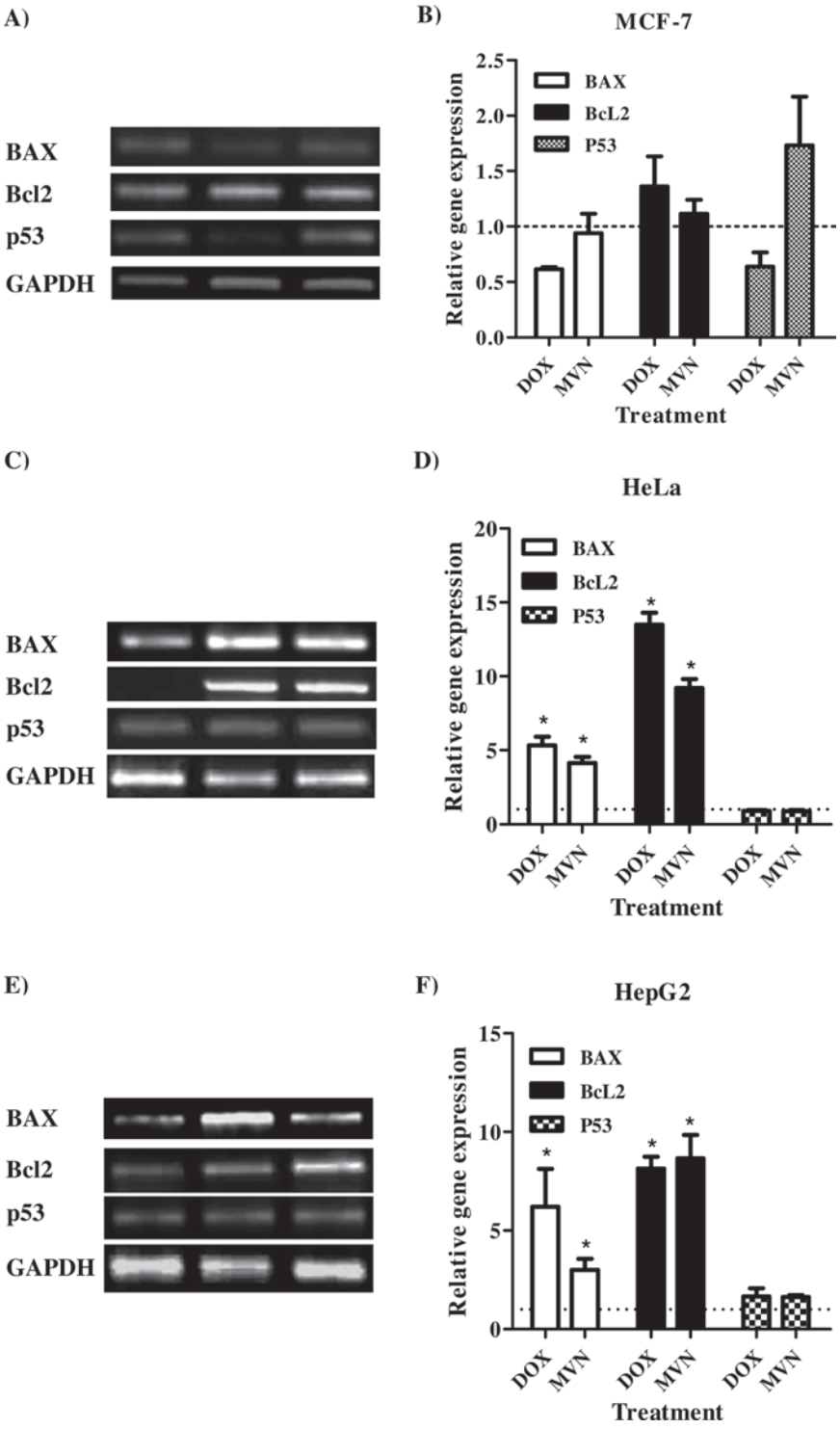

Figure 2. Effects of MVN and DOX on the apoptosis pathway in solid tumor cells. Transcript abundances of BAX, Bcl2 and p53 using RT-PCR in MCF-7 (A and B), HeLa (C and D) and HepG2 (E and F) cells after treatment with DOX or MVN. Data were expressed as means ± S.D.s ($n = 3$). The * indicated significant differences from controls at $p < 0.05$.

Table 1. The cytotoxicity parameters of MVN and DOX in different solid tumor cell lines.

	DOX		MVN	
	IC_{50} (μg/ml)	Resistant fraction (%)	IC_{50} (μg/ml)	Resistant fraction (%)
MCF-7	0.42 ± 0.11	2.6 ± 0.9	0.67 ± 0.15	1.03 ± 0.09
HepG2	0.42 ± 0.05	5.3 ± 2.2	1.1 ± 0.12	5.1 ± 0.82
HeLa	0.28 ± 0.07	7.7 ± 0.5	0.61 ± 0.02	0.33 ± 0.09

transcript abundance of a pro-apoptotic gene (BAX), an anti-apoptotic gene (BcL2) and the key gene of apoptosis (p53) were quantified using RT-PCT technique in cells treated for 72 h with the IC_{50} of DOX and MVN. In MCF-7, DOX increased BcL2 transcript abundance but decreased BAX transcript abundance. In contrast, MVN treatment did not change the transcript abundance of BAX, but did decrease BcL2 transcript abundance. Consequently, the p53 transcript was increased in MVN treated cells and decreased in DOX treated cells (Figure 2A and B). Both DOX and MVN dramatically increased the transcript abundances of BAX and BcL2 in HeLa cells (up to 4–5 fold for BAX and up to 10–15 fold for BcL2). The effective apoptosis marker gene, p53 was not changed in transcript abundance due to either treatment with DOX or MVN in the HeLa cell lines (Figure 2C and D). Similar to HeLa cell lines, DOX and MVN dramatically increased the transcript abundance of BAX and BcL2 in HepG2 cells (up to 2–5 fold in BAX and up to 7–10 fold in BcL2). However, the transcript abundance of p53 the gene was increased by both DOX and MVN treatments (Figure 2E and F).

Discussion

The slow advances in modifying the epidemiological mortality of solid tumors has warranted wider screening for new drugs with potential anti-cancer activity[1,2]. Rather than searching for new chemical moieties, the discovery of new applications for drugs with known clinical and toxicological profiles would cut down the time required for scaling-up to clinical stages. MVN was shown to be a clinically safe drug of natural origin that is known to inhibit the HMGCo-A reductase activity and interfere with steroidogenesis[4]. Here the cytotoxic profile of MVN was compared to DOX in three solid tumor-like cell lines, MCF-7, HeLa and HepG2. In addition, the transcript abundance of some key apoptosis markers was measured.

Steroidogenesis and cholesterol transport were suggested to be essential for the growth and proliferation of tumor cells[21]. Steroidogenesis inhibition and the disruption of geranylgeranyl pyrophosphate-dependent survival pathways were attributed to the anti-proliferative effects of simvastatin, another HMGCo-A reductase inhibitor[22]. Additionally, the association between the statins in general and the low incidence of carcinogenesis supported this hypothesis[10]. The interference with mevalonate pathway (prenylation) was known with its complexity to be affecting several apoptotic signaling pathways[23]. Moreover, MNV and other statins have shown to affect cell viability via mixed apoptosis and necrosis pathways in the same time[24]. This explains the observed low resistant cell fractions in all cell types treated with MNV compared to DOX (Table 1). Similar efficacy of MVN against all the cell lines might be partly attributed the multiplicity of its target signaling pathways (Figure 1).

In comparison with DOX, which was well-known as an anti-cancer agent and used clinically, MVN showed comparable cytotoxicity and less potential to allow the development of resistance (Table 1). Additionally, DOX-induced toxicity was a major limitation in patient compliance and chemotherapeutic course completion[16,17]. The safety profile of MVN both in experimental and clinical stages were encouraging for further clinical trials for the treatment of various types of tumors. The dose of MVN suggested for anti-cancer treatments was believed to be clinically safe[6,7]. Therefore, very high doses of MVN administered every four hours to patients have been found tolerable[23]. Consequently, MVN and other natural statins were believed to be better treatment option for cancer than synthetic statins by many factors[26].

Apoptosis has been receiving great attention as a major mechanism of cell death in normal as well as tumor cells. However, the programmed cell death might be interrupted due to defective signaling pathway nonetheless in tumor cells with higher rate of mutation[27]. Defective apoptosis has been reflected in the form of cell resistance to apoptotic inducing agents and, consequently, treatment failure. MVN has been suggested to induce cell death via multiple apoptotic[13], necrotic[24] and autophagic pathways[14]. In the current work, MCF-7 seemed to undergo apoptosis by the p53-dependent pathway; however both Bcl_2 and BAX were not significantly affected (Figure 2). That agrees with previous work of Lee and coworkers showing ameliorated cytotoxic effects of simvastatin in p53 knockdown clones of HCT116 colon cancer cell lines[26]. On the other hand, HeLa cell lines showed no significant change in p53 transcript abundance after MVN treatment, despite the very high BAX transcript abundance (Figure 2). However, the anti-apoptotic marker Bcl_2 was also increased in transcript abundance in HeLa cells after MVN and DOX treatments which might explain the unchanged p53 transcript abundance. Similarly, MVN cytotoxic effects in more than one cancer cell lines was p53 independent in nature[29,30]. In contrast to DOX, there was very low resistant fraction of HeLa cells to MVN indicative of proceeding via non-apoptotic cell death pathway such as necrosis or autophagy. This may also explain the ability of MVN to overcome K-Ras

mutation in human non-small lung cancer[31]. In $HepG_2$ cells, both apoptotic (BAX) and anti-apoptotic (Bcl_2) signals were increased in transcript abundance. However, p53 was marginally increased in transcript abundance as well (Figure 2). This may explain the relatively higher fraction of resistant cells in $HepG_2$ cells treated with MVN. In future, gene silencing studies would be recommended to understand the exact molecular mechanism of MVN and other statins-induced cytotoxicity in tumor cells.

Conclusion

In conclusion, MVN showed promising cytotoxic profile comparable to DOX in breast (MCF-7), cervix (HeLa) and liver ($HepG_2$) solid tumor cell lines. MVN-induced cytotoxicity was inferred to be multi-factorial and not solely dependent on p53 molecules.

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Declaration of interest

The authors report no conflicts of interest.

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