

RESEARCH ARTICLE

Design of novel multifunctional targeting nano-carrier drug delivery system based on CD44 receptor and tumor microenvironment pH condition

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Abstract

In this study, to develop a multifunctional targeting nano-carrier drug delivery system for cancer therapy, the novel pH-sensitive ketal based oligosaccharides of hyaluronan (oHA) conjugates were synthesized by chemical conjugation of hydrophobic menthone 1,2-glycerol ketal (MGK) to the backbone of oHA with the histidine as the linker of proton sponge effect. The multifunctional oHA conjugates, oHA-histidine-MGK (oHM) carried the pH-sensitive MGK as hydrophobic moieties and oHA as the target of CD44 receptor. The oHM could self-assemble to nano-sized spherical shape with the average diameters of 128.6 nm at pH 7.4 PBS conditions. The oHM nanoparticles (oHMN) could release encapsulated curcumin (Cur) with 82.6% at pH 5.0 compared with 49.3% at pH 7.4. The results of cytotoxicity assay indicated that encapsulated Cur in oHMN (Cur-oHMN) were stable and have less toxicity compared to Cur suspension. The anti-tumor efficacy *in vivo* suggested that Cur-oHMN suppressed tumor growth most efficiently. These results present the promising potential of oHMN as a stable and effective nano-sized pH-sensitive drug delivery system for cancer treatment.

Keywords

Drug delivery, nanoparticles, oligosaccharides of hyaluronan (oHA), oHA-histidine–menthone 1,2-glycerol ketal (oHM), pH-sensitive

History

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Introduction

Hyaluronan (HA), a naturally negatively charged and biodegradable polysaccharide, has been studied as a good targeting moiety in drug delivery system (Morra, 2005; Stern et al., 2006; Gerecht et al., 2007). Because various tumor cells can over-express the HA receptor, CD44, HA and its derivatives can possess good biocompatibility and unique biological characteristics in the use of tumor targeting drug delivery systems (Toole, 2004; Jaracz et al., 2005; Götte & Yip, 2006; Choi et al., 2011).

Many studies about HA and its derivatives were reported, which can be divided into two types. One type was HA–drug conjugates, also called prodrugs (Coradini et al., 2004; Auzenne et al., 2007; Lee et al., 2007). The other type was the micro/nanoparticles loading the different drugs, such as siRNA (Lee et al., 2007), doxorubicin (Eliaz et al., 2004), and mitomycin C (Peer & Margalit, 2004). It has been reported that both the types of HA can obtain some enhanced tumor

targeting and therapeutic efficacy. However, some new ideas presented that the native high molecular weight hyaluronan (nHA) and oligosaccharides of hyaluronan (oHA) could produce distinct biological effects upon binding to CD44 (Yang et al., 2012). It has been reported that nHA binding to CD44 selectively induces CD44 clustering, which could be inhibited by oHA (Yang et al., 2012), which is very important and interesting for the future use of HA in drug delivery.

After irritated by highly generated HA, CD44 clustering is a unique character which can influence cell response (Sleeman et al., 1996). Due to the “Coating Saccharide” (CD44 clustering) of tumor surface and the slightly acidic condition of tumor, it is difficult to target the chemotherapeutic drugs to the tumor site and achieve satisfying effect for targeting drug. To solve this problem, based on our previous basic work of pH-sensitive nano-carrier (Chen et al., 2010, 2011, 2012, 2013; Yu et al., 2013; Chen & Wang, 2014), we will design a multifunctional oHA-histidine (oHA)–menthone 1,2-glycerol ketal (MGK) nano-drug delivery system with CD44 receptor as target. As shown in Figure 1, once oHA-histidine-MGK (oHM) nanoparticles meet the CD44 receptor, the coating of CD44 clustering will not appear. Due to the protonation of histidine imidazole group, it is useful for the cellular uptake. After endocytosis, oHM nanoparticles (oHMN) will release the encapsulated

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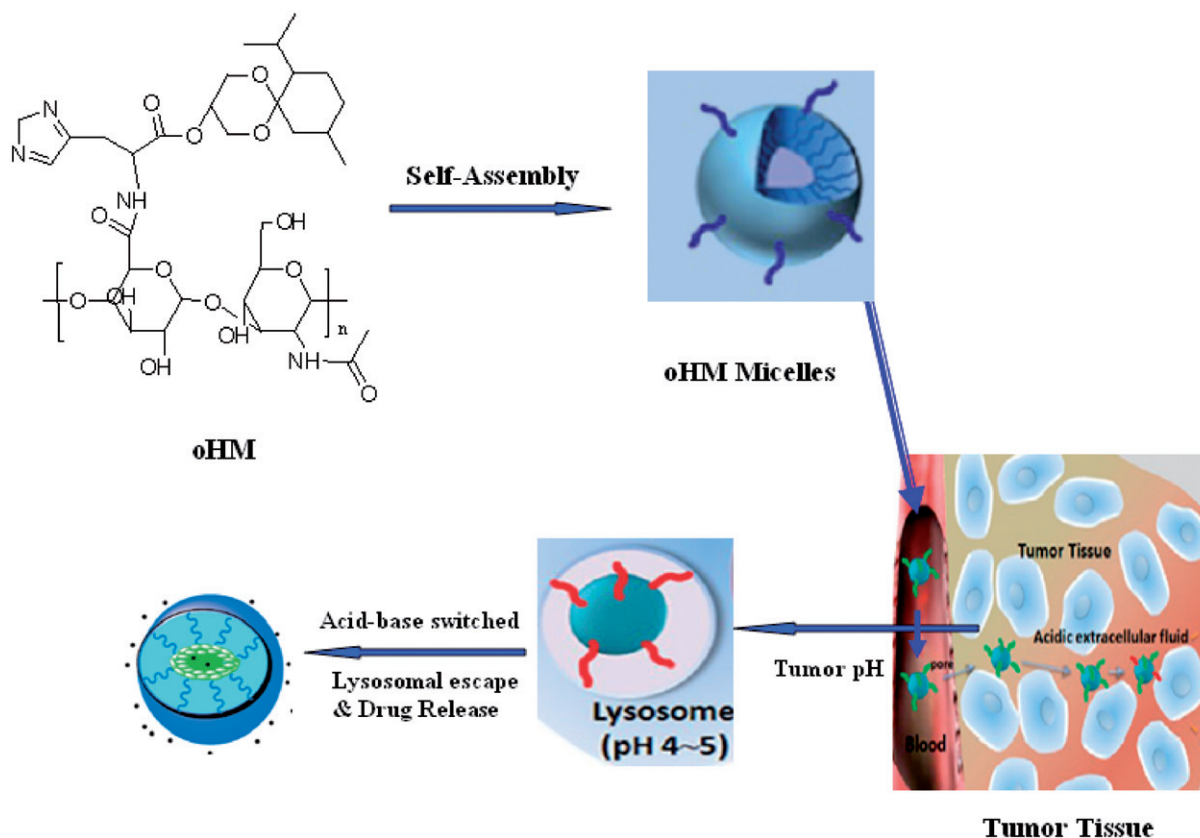


Figure 1. Schematic illustration of the formation, accumulation at tumor site and drug delivery of self-assembled oHM nanoparticles (oHMN).

drugs under the slightly acidic condition of tumor rapidly with the degradation of micellar hydrophobic ketal group. Then, lysosomal membrane will be destroyed via proton sponge effect of histidine imidazole group. The neutral ketone, alcohol degradation products of oHM can also help the escape of drug in lysosome. The encapsulated drugs will escape from lysosome to cytoplasm with good stability, produce efficacy and reduce side effects. The synthesis, characterization, and anti-tumor efficacy of oHMN were under investigation. Because of curcumin (Cur) with low non-specific toxicity to normal cells, it is a good candidate for anti-tumor drug delivery. In our previous study, we have described the Cur. Thus, we choose the Cur as the model drug.

Materials and methods

Materials

Cur was supplied by Yantai Science & Biotechnology Co. Ltd. (Yantai, China). oHA was provided by the FRED A Group Co. Ltd. (Jinan, China). MGK was provided by the Adamas Reagent Co. Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 4-(dimethylamino) pyridine (DMAP), and Pd/C were obtained from Aladdin Chemistry Co. Ltd. All other reagents were of analytical grade and supplied by Sinopharm Group Chemical Reagent Corp. All the animal experiments were in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

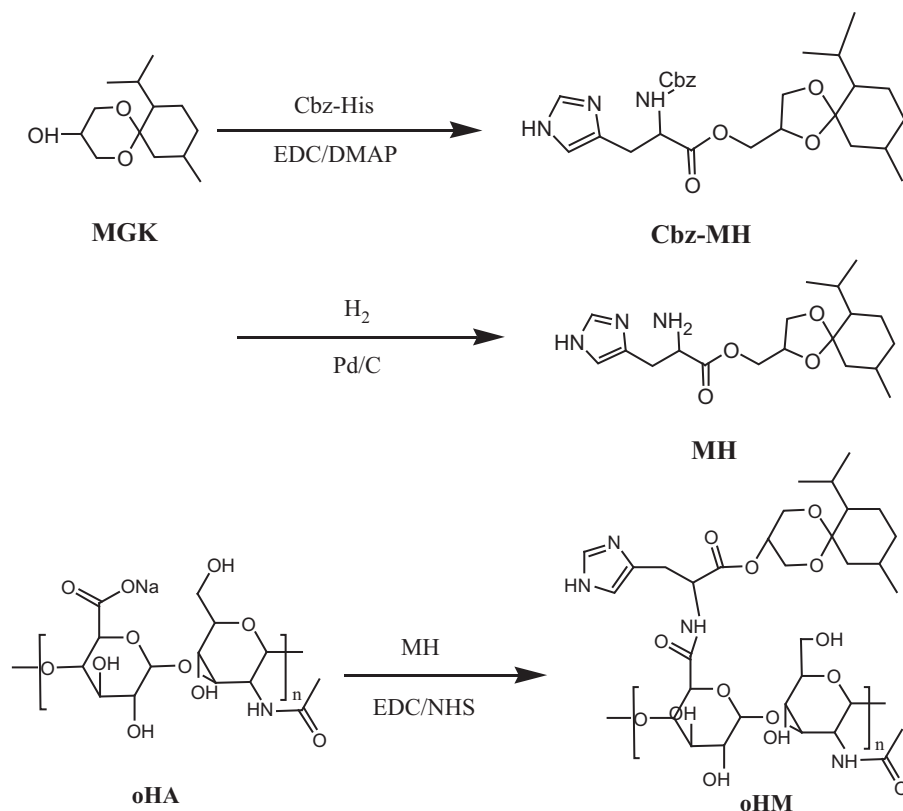
Synthesis of oHM

As shown in Figure 2, water-soluble oHA was modified by chemical conjugation of hydrophobic MGK to the backbone of oHA with the histidine as the linker. oHM was synthesized with three steps. First, Cbz-His (0.5 g, 1.72 mmol), DMAP (0.3 g, 2.58 mmol) and EDC (0.36 g, 1.89 mmol) were dissolved in 15 ml of DMF and stirred for 30 min. Then MGK (0.43 g, 1.88 mmol) was added. After reaction of 8 h, the reaction solution was extracted by 40 ml ethyl acetate and 70 ml water. The yellow oil product of Cbz-His-MGK was obtained under vacuum at 60 °C overnight. Second, Cbz-His-MGK (200 mg), methanol (5 ml) and tetrahydrofuran (1 ml) were added in the 25-ml flask. After reaction of 20 h with the H₂ protection and Pd/C, the solvent removed and the residue was concentrated and purified with column chromatography. The purified His-MGK was obtained. Third, oHA (0.012 g, 0.03 mmol), EDCI (0.016 g, 0.08 mmol), NHS (0.010 g, 0.08 mmol) and His-MGK (0.029, 0.08 mmol) were suspended in 5-ml water, stirring at room temperature for 24 h. After dialysis of 4 h and being freeze-dried, a faint yellow oHM powder was obtained.

Preparation of multifunctional Cur-oHMN

The multifunctional Cur-oHMN were prepared by the enhanced thin-film hydration method. Briefly, the oHM conjugate and curcumin (4:1) were dissolved in chloroform. The organic phase was removed at 40 °C on a rotary flask. The flask was evaporated under reduced pressure. The dry lipid formed was hydrated with phosphate buffer saline

Figure 2. Synthesis of multifunctional oHM.



(pH 7.4). The solution was filtrated through 0.45- μ m filters and stored at 4 °C until use.

Characterization of Cur-oHMN

The size and zeta potentials of Cur-oHMN were measured by a Zetasizer 3000 HS instrument. Atomic Force Microscope (AFM) and Transmission electron microscopy (TEM) observation carried out in the Cur-oHMN solution. The concentration solution was kept constant at 1 mg/ml.

In vitro drug release assay

The *in vitro* release assay of Cur-oHMN was determined using a dialysis bag placed in a sealed glass vial under constant shaking. The powder of Cur-oHMN (containing curcumin 3 mg) and 2 ml PBS (pH 7.4) were put into a dialysis tube. Then, the tube was introduced into 250 ml PBS (0.1 M, pH 7.4 and pH 5.0) containing 0.1% (w/v) Tween 80 with stirring at 120 rpm and 37 °C. The 20- μ L sample was filtrated and analyzed by HPLC after different time intervals (Chen et al., 2011, 2012; Yu et al., 2013).

In vivo anti-tumor efficacy

The *in vivo* anti-tumor efficacy of Cur-oHMN was evaluated in tumor-bearing BALB/c mice. The mice model was carried out with inoculation of 1×10^6 SCC7 cells at the right flank of the mice using a 1.0-ml syringe. When the tumor volume grow about 100–300 mm³, 0.1 ml Cur-oHMN (20 mg/kg) were injected by tail vein every other day. Normal saline and free Cur suspension injected for the control groups. Each individual tumor size was measured with a caliper and the tumor volume was calculated every other day using the following equation: $(W^2 \times L)/2$, where W is the tumor

measurement at the widest point and L is the tumor dimension at the longest point (Chen et al., 2010, 2011, 2012; Yu et al., 2013).

Statistical analysis

The results were expressed as the mean \pm standard deviation ($n=3$) and the statistical analysis was performed using SPSS software (Chicago, IL). $p < 0.05$ was considered to be statistically significant.

Results

Characterization of oHM

The chemical structures of the oHM determined by ¹H-NMR (400 MHz) (Figure 3). The molar ratio of oHA and ketal group moiety was about 5:1. The oHA of the oHM conjugate was confirmed by ¹H-NMR with the peak at 4.38–4.40 ppm (2H), 1.77 ~ 2.13 ppm (4H). The peak at 0.83 ~ 0.94 ppm of ketal group was confirmed by the MGK conjugated to oHM. The peaks at 7.33, 8.55 ppm were confirmed by the histidine conjugated to oHM.

Preparation and characterization of oHM

As shown in Figure 4, the Cur-oHMN could self-assemble to nano-sized spherical shape with the diameters of 128.6 nm at pH 7.4 PBS conditions. The entrapment efficiency (EE) was determined to be about 57.6% (Table 1). The zeta potentials of Cur-oHMN were negative and ranged from –28 to –16 mV, indicating that the Cur-oHMN surface were covered by the charged oHM conjugates (Table 1). This negatively charged surface of Cur-oHMN might offer a long-term stability in water.

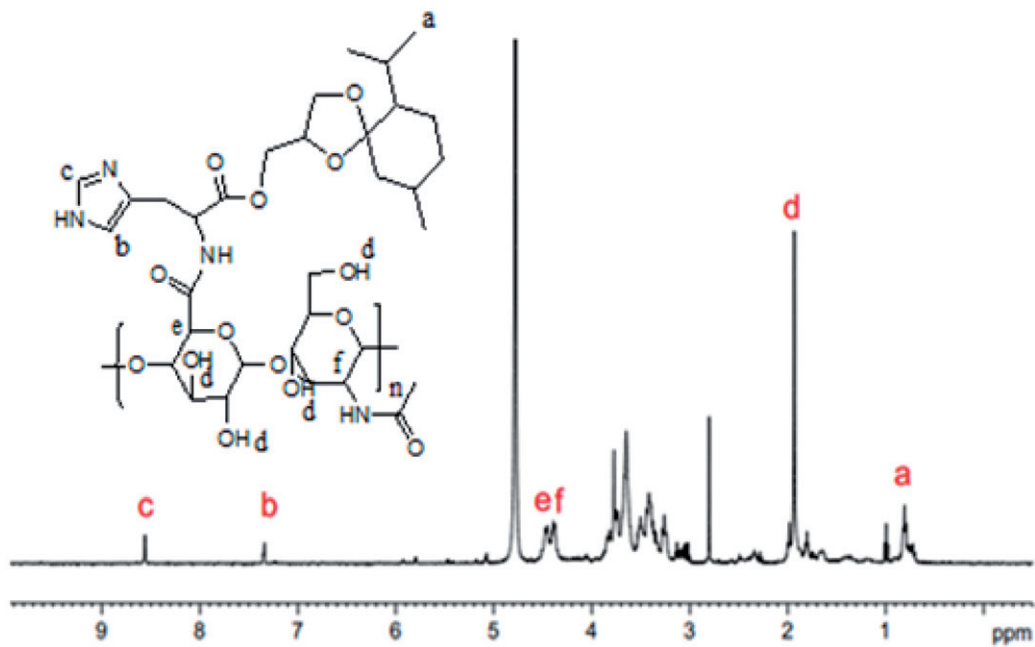


Figure 3. ¹H NMR spectra of oHM.

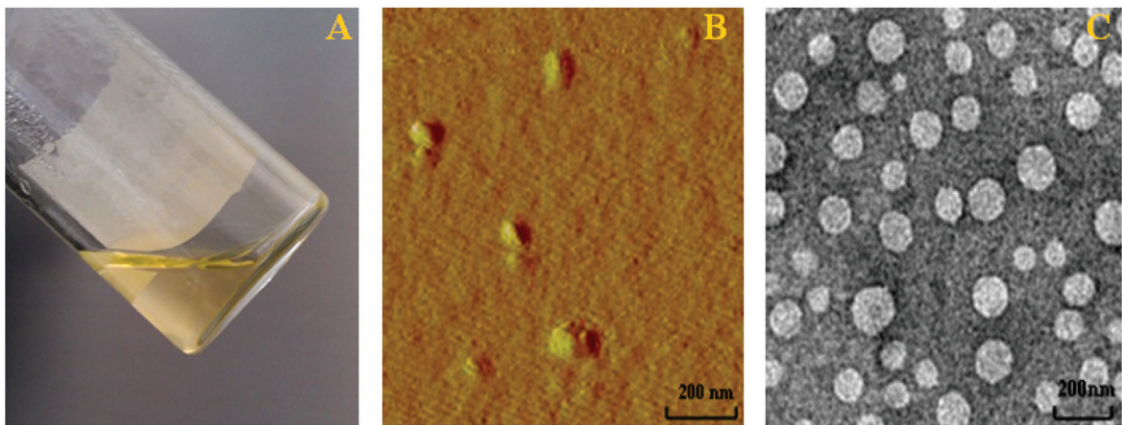


Figure 4. The external physical properties (A), AFM (B), TEM (C) of oHM nanoparticles (oHMN).

Table 1. The physicochemical parameters of Cur-oHMN before freeze-drying and after freeze-drying.

Parameters	Before freeze-drying	After freeze-drying
Entrapment efficiency (%)	57.6 ± 4.8	42.3 ± 6.5
Particle size (nm)	128.6 ± 7.4	165.2 ± 8.3
Zeta potential (mv)	−27.7 ± 2.3	−16.2 ± 2.6

In vitro releasing assay and in vivo anti-tumor efficacy of Cur-oHMN

In vitro pH-sensitive test were presented at 37 °C for 24 h in different pH buffers. The Cur-oHMN could release encapsulated curcumin with 82.6% at pH 5.0 compared with 49.3% at pH 7.4, shown in Figure 5. As shown in Figure 6, the tumor volume of the saline control group was excessively enlarged (>1000 mm³), while the other groups were much smaller. The Cur-oHMN group suppressed tumor growth most efficiently,

followed by the free Cur group (*p* < 0.05) (Chen et al., 2010, 2011, 2012; Yu et al., 2013).

Discussion

CD44 is a major cell surface receptor for the HA. nHA and oHA display different biological effects upon binding to CD44. It has been reported that the biological effect of HA depends on its molecular weight (Camenisch & McDonald, 2000; Stern et al., 2006; Kothapalli et al., 2008). Interestingly, oHA could promote cell proliferation (Slevin et al., 2006), whereas nHA could represent inhibitory effect (Kothapalli et al., 2008). However, there is no report about the oHA nanoparticles in drug delivery system, which is interesting and important.

In this work, we have prepared a multifunctional targeting nano-carrier drug delivery system for curcumin, which is novel and interesting. The oHM conjugates were synthesized by chemical conjugation of pH-sensitive MGK to the

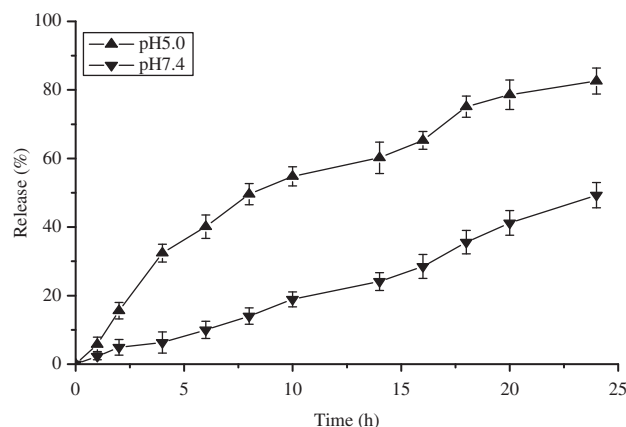


Figure 5. Percentage of curcumin released from oHMN at different pH condition.

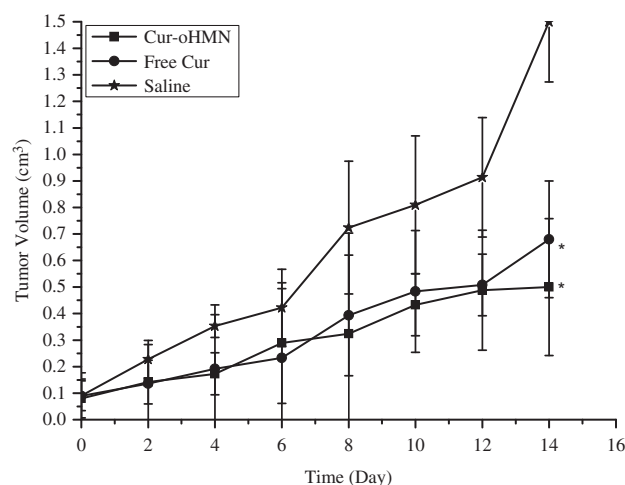


Figure 6. Effect on the inhibition of tumor growth of Cur formulations. Each data represent the mean \pm SD ($n = 6$). * $p < 0.05$, compared with the free saline.

backbone of oHA with the histidine as the linker. The MGK has the pH-sensitive ketal moiety, which can selectively degrade under tumor microenvironment pH condition and its pH degradation products of ketal could avoid inflammatory problems. Simultaneously, the linker between the oHA and MGK has the proton sponge effect of histidine imidazole group, which can destroy the lysosomal membrane and help the intracellular drugs avoiding destruction. In our previous study (Chen et al., 2010, 2011, 2012, 2013; Yu et al., 2013; Chen & Wang, 2014), novel pH-sensitive mPEG-Chitosan-Ketal (PCK) conjugates were also synthesized and were used to potential pH-sensitive drug delivery system. The similar research was studied in our lab. However, compared to the PCK conjugates, the oHM conjugates possess multi multi-function, such as the CD44 targeting and proton sponge effect of histidine imidazole group.

Conclusion

The oHM could self-assemble to nano-sized spherical shape with the diameters of 120–200 nm at pH 7.4 PBS conditions. The Cur-oHMN could release encapsulated curcumin with 82.6% at pH 5.0 compared with 49.3% at pH 7.4. The results

of cytotoxicity assay indicated that encapsulated curcumin in Cur-oHMN were stable compared to curcumin suspension. The anti-tumor efficacy *in vivo* suggested that Cur-oHMN suppressed tumor growth most efficiently. Much more details are under our research. These results present the promising potential of oHM as a multifunctional targeting nano-carrier in drug delivery system for cancer treatment.

Declaration of interest

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