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Potential Effects of Dendrobium Officinale Polysaccharide on Prostate Tumor Growth in Vivo

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Abstract

In recent years, Dendrobium officinale polysaccharide (DOP), a natural product, has garnered increasing attention due to its potential anticancer effect. To provide a theoretical basis for DOP as an anticancer drug or alternative treatment, we demonstrated the potential effects of DOP in a murine model of prostate cancer. The anticancer evaluation of excised tumor tissues showed that DOP resulted in specific growth inhibitory effects in a dose-dependent manner, which could be manifested by delayed tumor formation, reduced tumor size, improved hyperemia, and enhanced apoptosis. In addition, the Western blot results confirmed the upregulation of Bax, PCNA, and Bcl-2 expression. No significant changes in the body weight, including organ indices, were observed in DOP-treated groups. In conclusion, the results demonstrated that DOP inhibited the growth of prostate cancer in vivo by activating apoptosis towards a potential therapeutic or alternative drug.

Keywords

Prostate cancer, apoptosis, cell proliferation, dendrobium officinale polysaccharide.

1. Introduction

Prostate cancer (PCa), an age-associated degenerative disease in the elderly > 50 years of age, is one of the most common cancers among men, with an increasing incidence. Based on a data analysis over the past 5 years, the incidence of prostate cancer in China has increased at a rate of 2.6% per year. According to the Global Cancer Observatory (GCO), the number of patients in 2040 is expected to increase by 107.8% compared to 2018[1-2]. As an androgen-dependent disease, the clinical characteristics of adenocarcinoma include increased levels of prostate-specific antigen (PSA), prostate enlargement, as well as the symptoms of dysuria and spinal cord compression. During the development of PCa, the initial manifestations include the formation of prostatic intraepithelial neoplasia (PIN). Further, the loss of NKX 3.1, a member of the homologous gene superfamily, gradually results in high prostatic intraepithelial neoplasia (HPIN). Subsequently, the deletion of PTEN and Rb genes renders cancer cells highly invasive. Simultaneously, cell cycle disturbances and enhanced activity of telomerase accelerates the proliferation of the cancer cells[3-4]. After the diagnosis of HPIN, patients are generally treated with surgical excision of the tumor and subsequent radiotherapy in the early stages. In the advanced stages of PCa, luteinizing hormone-releasing hormone (LHRH) agonists are generally administered to patients after castration to lower serum androgen levels, thereby substantially controlling cancer proliferation and progression. Moreover, PSA levels are rose again and the cancer recurred after 2 years of treatment[5-6]. During this period, androgen receptors may undergo amplification, gene rearrangement, and mutations, resulting in highly aggressive androgen-independent prostate cancer (AIPC)[7-9]. In addition to these the above-mentioned treatments, several new therapies exist against PCa, such as immunotherapy and targeted therapy. The existing anti-cancer drugs, however, are associated with complications during or

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after the treatment, affecting patient quality of life. Therefore, it is inevitably required to search for effective and low-toxic PCa treatment drugs, which is a major focus of current research.

Several natural products of plant origin have gained importance with inhibitory effects on tumor growth, such as Ganoderma atrum polysaccharides, curcumin, thymoquinone, and tanshinone[10-14]. In addition to the tumor-suppressing effect, some of these plant-derived natural products have attracted attention for researchers due to their low toxicity. Dendrobium officinale Kimura et Migo is one of the most studied medicinal plants due to various pharmacologic effects, such as anti-oxidation, anti-fatigue, and immune regulation. Notably, several investigations indicated that the pharmacologic effects are due to the main ingredients (Dendrobium officinale polysaccharide [DOP]). In recent years, the role of DOP in cancer treatment has also begun to attract the attention of researchers. For instance, DOP has tumor-suppressive effects in a human breast cancer cell line (MCF-7) and gastric cancer models established with nude mice[15-16].However, , the investigations related to the effect of DOP on PCa in vivo are still at a blank stage in the infant state.

Therefore, we carried out the study to verify the possible therapeutic effect and mode of action of DOP on mouse prostate carcinoma cell line RM-1 in vivo. Based on these results, we speculate on whether DOP can become an anticancer drug or anticancer adjuvant drug and explore its mechanism of action.

2. Materials and Methods

2.1. Chemicals and Reagents

Dendrobium officinale was kindly provided by Guangdong Academy of Agricultural Sciences Crop Research Institute (Guangdong, China, 2017). RPMI-1640 medium was purchased from Gibco (USA). In situ cell death detection kit was procured from Roche Diagnostics Ltd. (USA). Bcl-2, Bax, GAPDH, PCNA, goat anti-rabbit IgG antibody (HRP-conjugated), and horse anti-mouse IgG antibody (HRP-conjugated) were obtained from Cell Signaling Technology (CST USA). ECL developer was purchased from Millipore (USA). BCA protein assay kit was obtained from Bioss Ltd. (China).

2.2. Cell Culture

The murine prostate cancer cell line RM-1was provided by Professor Hu of Southern Medical University (Guangdong, China). The RM-1 cell line was cultured in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5% CO2.

2.3. Animal and Tumor Xenograft Model

Healthy adult male C57BL/6 mice (SPF, 6 weeks old, and weighing 16-18 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). The experimental mice were raised in an adaptive environment (room temperature, 22~24 ℃; humidity, 40%~70%; and 12 hours of light and 12 hours of darkness) following the National Laboratory Animal Breeding Standards for 1 week before the experiments. All experiments were performed under the principles framed by the Experimental Animal Ethics Committee.

The C57BL/6 mice were randomly divided into four groups. Furthermore, the xenograft model of PCa was established by subcutaneous injection (s.c.) of 2 × 105 RM-1 cells. The day of cell inoculation was considered day 0, and further details were presented in Figure 1:

(a) Control group: mice administered intragastric (i.g.) saline for 21 days;

(b) DOP low-dose group: mice received 100 mg·kg-1 of DOP i.g. for 21 days;

(c) DOP medium-dose group: mice received 200 mg·kg-1 of DOP i.g. for 21 days;

(d) DOP high-dose group: mice received 400 mg·kg-1 of DOP i.g. for 21 days.

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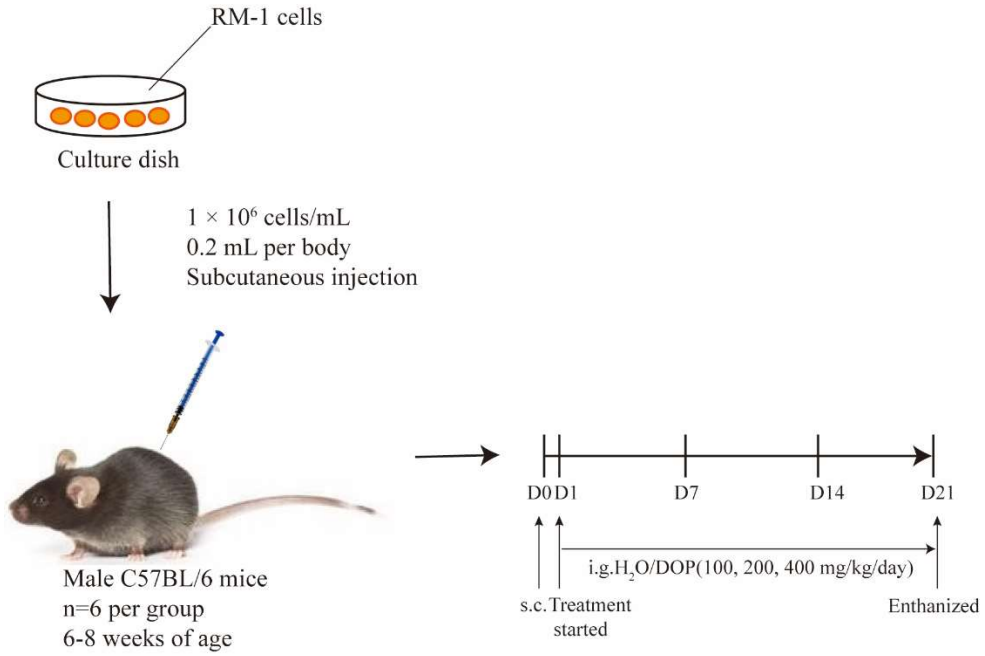

Figure 1. Grouping and processing of DOP treatment in the experimental mice.

2.4. Methods

2.4.1. Tumor Volume and Weight

The tumor volume (V) and body weight were monitored in all mice every 3 days during the treatment period. V was calculated by measuring the length (L) and width (W) of xenograft tumors using the formula $v = \pi / 6 (L \times w^2)$. After 21 days of i.g. DOP or saline administration, the mice were sacrificed and the weights of the excised tumor tissues were recorded.

2.4.2. Terminal Deoxynucleotidyl Transferase-Mediated Dutp Nick End Labeling Assay

The terminal deoxynucleotidyl transferase-mediated dUTP end labeling (TUNEL) assay was measured using an in situ cell death detection kit. Paraffin-embedded tumor tissue was heated at 60℃ for 30 min, dewaxed twice with xylene for 5 min, and rehydrated with absolute, 95%, 90%, 80%, and 70% ethanol for 3 min. After the water imbibition, the tissues were incubated for 15 min at 21-37℃ with 20 μg/ml proteinase K and the slides were twice-rinsed with PBS for 5 min. Then, the slides were incubated in the TUNEL reaction mixture at 37 °C for 60 min inside a humidified atmosphere in the dark and thrice-washed with PBS for 5 min. Finally, the samples were analyzed under an inverted fluorescence microscope to observe the apoptotic cell DNA (green fluorescence).

2.4.3. Western Blot Analysis

Tumors were lysed in ice-cold lysis buffer-RIPA containing a protease inhibitor for 30 min. Further, the lysate was centrifuged at 12000 g for 10 min at 4°C. Then, the supernatants were collected and the protein levels were determined using the BCA protein assay kit. The total proteins were separated by 12% SDS-PAGE and transferred to a PVDF membrane. The membranes were blocked using 5 % fat-free milk for 2 h at room temperature, then incubated with homologous primary antibodies overnight at 4°C. The membranes were then washed and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. The protein was examined using ECL developer and the pixel intensity was measured using Image J software.

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2.5. Statistical Analysis

Statistical analysis was performed using Graphpad Prism 7.0. Component differences were analyzed by the Student's t-test. The significance was determined as follows: * $P < 0.05$; ** $P < 0.01$; or *** $P < 0.001$, compared to the control treatment group.

3. Results

3.1. Effect of DOP Treatment on Tumor Growth

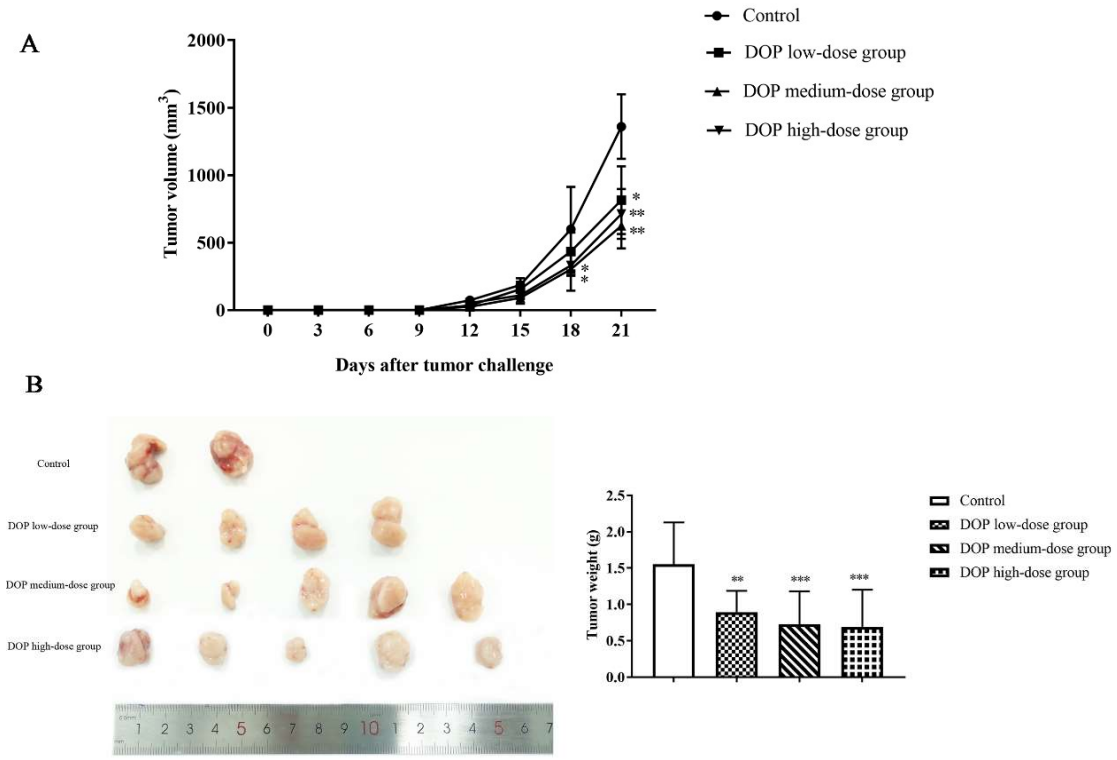

Figure 2. DOP treatment inhibited tumor growth. (A) Grouping and processing in experimental mice. (B) The changes in tumor volume in DOP-treated mice. (C) Photograph and weight of the excised tumor tissues from experimental mice.

Following the i.g. administration of saline or DOP, the tumor formation time in the control, DOP low-, and medium-dose treatment groups was 9-10 days, while the time of the DOP high-dose group was 11-12 days. From the 18th day of i.g. treatment, the tumor volume showed a downward trend in DOP medium- and high-dose groups. At the end of the 21st day, the difference in tumor volume between the experimental groups was more significant. Compared with the control group, the tumor volume of the DOP low-dose group was reduced by 40.07% ($P < 0.05$), while the DOP medium- and high-dose groups were reduced by 47.57% ($P < 0.01$) and 54.10% ($P < 0.01$), respectively (Figure 2A). In addition, the tumor epidermis of the control mice was severely damaged in the later stage of tumor formation accompanied by tissue fluid exudation; however, the phenomenon was improved in the DOP treatment group and rarely occurred in the DOP high-dose group.

In the control group, the tumor tissue was larger with apparent congestion, but there was no visible angiogenesis phenomenon, while in the DOP low-, medium-, and high-dose treatment groups, the size and weight of the tumor tissues were significantly reduced. Moreover, the congestion of tumors in the treatment groups has also been improved with tumor suppression

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rates reaching 41.94% (P < 0.01), 52.90% (P < 0.001), and 55.48% (P < 0.001), respectively. Tumor suppression was more significant in the medium- and high-dose DOP treatment groups compared to the control group, indicating that DOP interfered with the growth of prostate tumors (Figure 2B).

3.2. Effect of DOP Treatment on Tumor Proliferation

In fact, the cell proliferation protein, PCNA, is closely related to tumor growth and can be used as a marker for cancer diagnosis. Therefore, the expression of PCNA protein was detected to evaluate the effect of DOP treatment on tumor cell proliferation. Compared to the control treatment group, the relative expression of PCNA in the DOP low-dose group was slightly reduced with no statistical difference. To this end, the expression of PCNA protein in the DOP medium- and high-dose treatment groups was significantly downregulated, and the relative levels of PCNA expression were decreased to 37.57% (P <0.01) and 59.02% (P <0.001), respectively (Figure 3). Together, the Western blot results of PCNA protein expression confirmed that DOP treatment significantly inhibited proliferation by inhibiting the PCNA level and delaying the growth of prostate tumors.

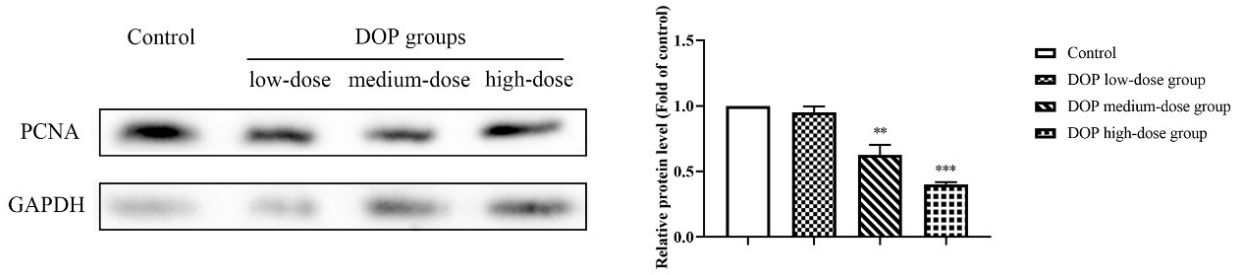

Figure 3. Effect of DOP on the expression of PCNA protein.

3.3. Effect of DOP Treatment on Tumor Apoptosis

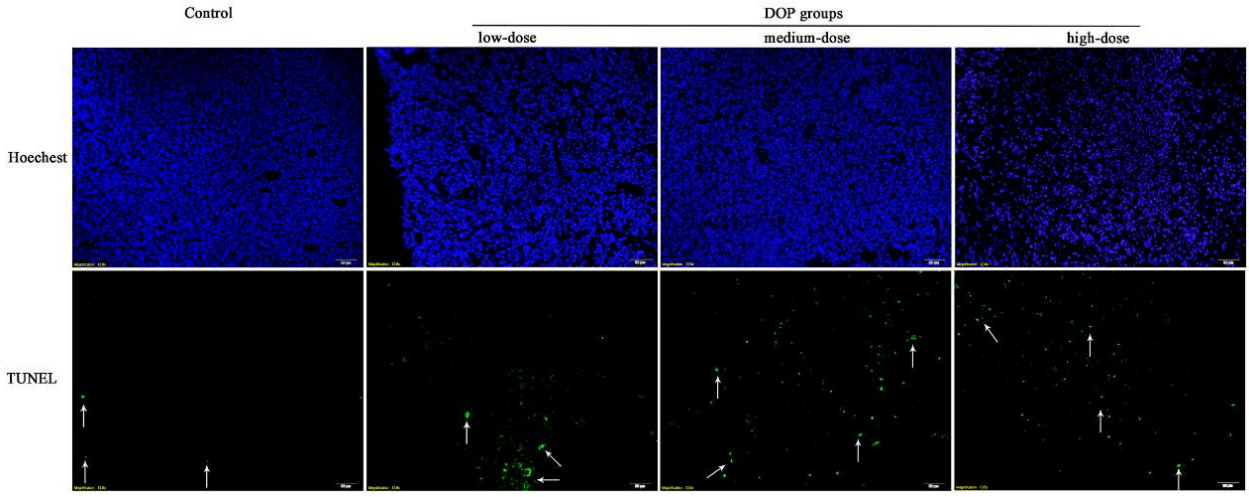

Figure 4. Fluorescent micrographs of tumor tissues with Hoechst and TUNEL staining to determine apoptosis. The white arrows in TUNEL staining indicate apoptotic cells. Scale bar = 50 μm

Apoptosis is one of the critical factors that maintains the dynamic balance of cell proliferation and tumor development. After excising the tumor tissues from the sacrificed mice, apoptosis in the tumor tissues of each experimental group was detected. There was a significantly greater number of nuclei, closely arranged, and fewer positive apoptotic cells in the control group,

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while in the DOP low-dose treatment group, a significant increase in the fluorescence signal was observed with more apoptotic cells in the tumor tissues. With an increase in DOP concentration, the number of cells that underwent apoptosis in tumor tissues was also increased, and there was already a significant apoptosis phenomenon in the medium-dose group of DOP, indicating that DOP inhibited the growth of prostate tumors by inducing tumor cell apoptosis (Figure 4).

In general, the Bcl-2 family plays an essential role during apoptosis, in which Bax and Bcl-2 exhibit pro- and anti-apoptotic functions. Compared with the control group, the relative expression of Bax and Bcl-2 were not significantly different in the DOP low-dose treatment group. With an increase in the concentration of DOP, the relative expression of the above proteins changed more obviously than the low-dose treatment group (Figure 5). Together, the experimental results showed that DOP interfered with tumor growth by regulating the translation of Bax and Bcl-2 in a dose-dependent manner.

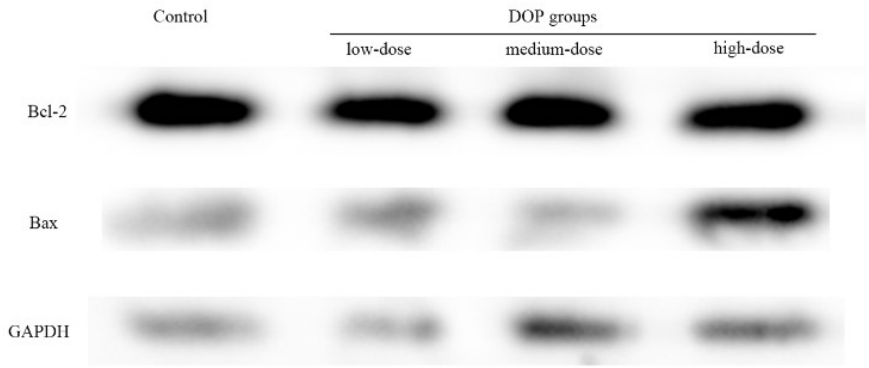

Figure 5. Effect of DOP treatment and its concentration on the expression of Bax and Bcl-2 protein.

4. Discussion

PCa is a lethal malignant tumor with continuous proliferation of prostate epithelial cells. The occurrence of PCa is often related to aging, androgens, dietary habits, and genetic factors, which may trigger oxidative stress, lead to intracellular ROS elevation, and DNA damage [17-18]. After DNA damage, the cells typically initiate the repair process. In the case where DNA repair cannot be completed, errors in DNA replication may occur, causing chromosomal mutations accompanied by an increase in dividing cells and resulting in a large number of dysfunctional cells [18-19]. The heterogeneity of tumor cells is characterized by breaking the original balance of proliferation-apoptosis, thus affecting the metabolism and proliferation of normal cells, and destroying homeostasis of the organism [20].

PCNA is a nuclear protein that exists in the late G1-S-early G2 phases and is involved in various cellular processes, such as DNA replication, repair of DNA damage, chromatin structure maintenance, chromosome segregation, and cell-cycle progression. In the DNA replication process, this nuclear protein controls cell proliferation through DNA polymerase δ and ε [21]. As a result, PCNA levels in cancer cells are several times higher than normal cells, and can be used as a biomarker for cancer diagnosis [22]. In this study, the expression of PCNA protein in tumor tissues treated with DOP was significantly inhibited. Moreover, we reasoned that the administration of DOP might block DNA replication, inhibiting the proliferation of prostate cancer cells. Similarly, the moderating of tumor growth curves and the decline in tumor weight demonstrated that the DOP substantially inhibited tumor cell growth in mice.

In addition to the uncontrolled cell proliferation, the apoptosis of PCa may also be impaired. Currently, there exist three apoptotic pathways in mammals, namely, the death receptor pathway, mitochondrial apoptosis pathway, and endoplasmic reticulum stress pathway [23-25]. In general, the stimulated cells result in the damage of DNA, causing the upregulation of the tumor suppressor gene, p53. Upregulation of the p53 gene, in turn, leads to several consecutive events in the disaggregation of the Bax/Bcl-2 heterodimer in mitochondria, alters the outer membrane permeability of mitochondria, and eventually executes apoptotic function through caspase-3 protein. The imbalance of anti- and pro-apoptotic of the Bcl-2 family and the change in the expression of caspase activity allow tumor cells to avoid death and the lesions to continue to expand. In this experiment, DOP affected the translation of apoptosis-related genes (Bax and Bcl-2), indicating that DOP could activate apoptotic and promote cell death. In this process, the level of upstream gene p53 expression was also likely to be upregulated.

In summary, we speculate that the DOP mechanism of action involves interferes with the growth of prostate tumors by inducing apoptosis. We believe that DOP may become a cancer therapeutic drug or an adjuvant, however, further investigations on the underlying mechanism and possible metabolic pathways involving DOP are warranted.

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