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Artesunate down-regulates immunosuppression from colorectal cancer Colon26 and RKO cells in vitro by decreasing transforming growth factor β_1 and interleukin-10

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ABSTRACT

Immunosuppression is the main source of ineffective treatment on tumor, and the study aimed to investigate the effect of artesunate on tumor immunosuppression. Supernatants of re-cultivated murine colorectal cancer cell Colon26 and human colorectal cancer cell RKO after pre-treatment with or without artesunate were enrolled, and their effects on five immune parameters were assessed, including killing activity of natural killer (NK) and lymphocyte proliferation, as measured by MTT, and expressions of interleukin 2 receptor (IL-2R)α, $CD3\varepsilon^{+}\zeta^{+}$ and $CD3\varepsilon^{-}\zeta^{+}$ on lymphocytes, as analyzed by flow cytometry. Six immunosuppressive factors were measured by ELISA, including transforming growth factor (TGF) β_1, vascular endothelial growth factor (VEGF), IL-4, IL-6, IL-10, and prostaglandin E_2 (PGE_2). Then, multiple linear regression analysis was applied to reveal the correlation between immunosuppression and immunosuppressive factors, and was used to confirm the findings. It was shown that Colon26 and RKO cells secreted immunosuppressive factors and inhibited these five immune parameters steadily. After pretreatment with artesunate, immunosuppression from the two cells was down-regulated significantly (all $P < 0.05$), and the concentrations of TGF-β_1 and IL-10 decreased greatly (all $P < 0.001$). There were positive correlations between the down-regulation of immunosuppression and the decrease in TGF-β_1 or IL-10. Their combined potency attributed to decreased TGF-β_1 and IL-10 with respect to the down-regulating effect of artesunate on immunosuppression of NK killing, lymphocyte proliferation and expressions of IL-2Rα and $CD3\varepsilon^{+}\zeta^{+}$, was about 60%–90%. The present analysis provides clues that artesunate reverses the immunosuppression from Colon26 and RKO colorectal cancer cells by decreasing TGF-β_1 and IL-10. This is probably one of the anti-tumor mechanisms of artesunate.

1. Introduction

Colorectal cancer is a malignancy with high incidence and mortality. Over the last decade, its clinical incidence has increased by 4% annually, and the age of patients with colorectal cancer has been decreasing, which has been attributed to changes in diet, work- and life-related stress, and environmental pollutants [1]. Thus, more attention has been focused on its therapeutics, and the development paradigm has gradually shifted from the single and popular model of surgery assisted with radiotherapy and chemotherapy to the multiple and individualized model of multi-targeted therapy, which arises from differences in anti-tumor immunity that can lead to the observed differences in tumor development and clinical prognoses.

Suppressed immune surveillance in patients occurs within both immune system and tumor tissue [2,3]. Studies on improving anti-tumor immunity have concentrated on promoting the activity of immunocytes and cytokines [4,5], but their clinical efficiency is still poor, because of the immunosuppressive factors secreted by tumor cells, such as transforming growth factor (TGF) β_1, vascular endothelial growth factor (VEGF), interleukin (IL) 4, IL-6, IL-10, prostaglandin (PG) E_2, etc. [6,7]. Immunosuppression attributed to these factors usually involves the

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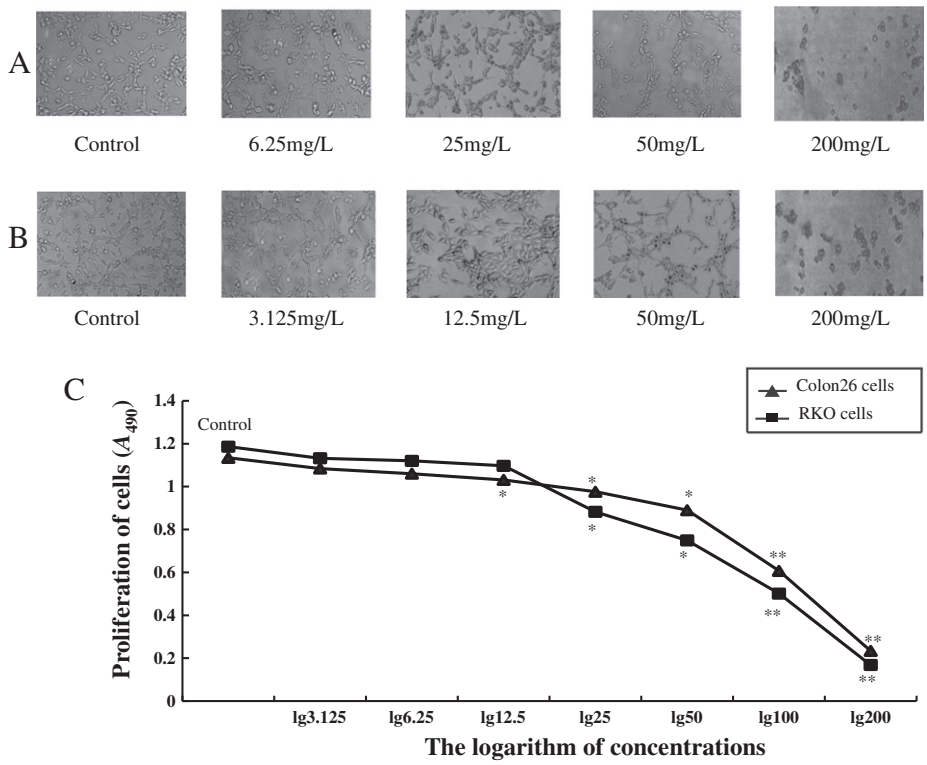

Fig. 1. Proliferation of Colon26 and RKO cells after treatment with ART (n = 15). To determine the appropriate experimental concentration of ART, Colon26 and RKO cells (2×10^8/L) were co-cultivated with ART at serial concentrations respectively (200 mg/L, 100 mg/L, 50 mg/L, 25 mg/L, 12.50 mg/L, 6.25 mg/L and 3.125 mg/L). The number of live cells and the value of A_{490} from cell proliferation decreased gradually along with the increased concentration of ART. (A) The growth of Colon26 cells after treatment with different concentrations of ART was observed under microscope (×200). (B) The growth of RKO cells after treatment with different concentrations of ART was observed under microscope (×200). (C) The values of A_{490} from cell proliferation were measured by MTT analysis. The X axis was the common logarithm of the serial concentrations of ART. The highest concentration of ART that did not reduce cell proliferation was used for subsequent experiments (6.25 mg/L ART for Colon26 cells, and 12.5 mg/L ART for RKO cells). Compared with Control (cells untreated with ART), *: $P < 0.05$; **: $P < 0.0005$.

suppression of T lymphocytes and natural killer (NK), which not only impedes the function of immunocytes in the tumor tissue but also devitalizes the activity of the normal and activated effector cells that are recruited to the tumor environment [8–14]. Furthermore, the strong immunosuppression makes endogenic and exogenic immunocytes unable to efficiently attack and eliminate tumor cells. However, few studies have been reported on tumor immunosuppression reversal.

Artesunate (ART), a monomer derivative of artemisinin, was approved for malaria treatment with good efficacy and minimal toxicity [15]. Nowadays, it also acts as an anti-tumor drug that can impede the proliferation of tumor cells and enhance the sensitivity to radiotherapy and chemotherapy [16–22], but its effect on tumor immunosuppression is unclear. Therefore, the effect of ART on immunosuppression attributable to colorectal cancer cells was analyzed in vitro. Experiments were designed to circumvent the disadvantage of passively improving anti-tumor immunity, by targeting immunosuppressive factors and reversing immunosuppression. Thereby, the surviving and activities of immunocytes were expected to be promoted along with actively rectified anti-tumor immunity, which will give great help on enhancing the efficiency of clinical therapy.

Table 1
Proliferation of Colon26 and RKO cells after treatment with ART (n = 15).

Groups	Concentrations (mg/L)	A_{490}	
		Colon26	RKO
Control	0	1.134 ± 0.111	1.186 ± 0.091
1	3.125	1.084 ± 0.108	1.132 ± 0.104
2	6.25	1.060 ± 0.094	1.120 ± 0.096
3	12.5	1.031 ± 0.098*	1.097 ± 0.124
4	25	0.976 ± 0.088*	0.882 ± 0.098*
5	50	0.890 ± 0.099*	0.749 ± 0.084*
6	100	0.607 ± 0.064**	0.501 ± 0.070**
7	200	0.234 ± 0.040**	0.168 ± 0.047**

Note: Compared with proliferation of Control (Colon26 or RKO cells untreated with ART).
* $P < 0.05$.
** $P < 0.0005$.

2. Materials and methods

2.1. Cells and animals

The murine colorectal cancer cell line (Colon26), the murine T-cell lymphoma cell line (YAC-1) and the human leukemia cell line (K_{562}) were obtained from the Tumor Research Center of the Fourth Hospital of Hebei Medical University, Shijiazhuang, China. The human colorectal cancer cell line (RKO) was obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences Institution, Shanghai, China. Peripheral

Table 2
Growth of Colon26 and RKO pre-treated with or without ART.

Groups	Number of live cells ($\times 10^8$/L)		A_{490}	
	(n = 3)		(n = 10)	
	Colon26	RKO	Colon26	RKO
Control-C	4.49 ± 0.23	5.07 ± 0.19	1.13 ± 0.11	1.19 ± 0.09
Control-C_1	4.53 ± 0.15	4.82 ± 0.21	1.09 ± 0.12	1.12 ± 0.11
Control-C_2	4.71 ± 0.19	4.97 ± 0.23	1.11 ± 0.10	1.08 ± 0.13
ART-C	4.87 ± 0.29	4.85 ± 0.25	1.10 ± 0.08	1.03 ± 0.21
ART-C_1	4.38 ± 0.18	4.79 ± 0.28	1.09 ± 0.11	1.08 ± 0.17
ART-C_2	4.95 ± 0.21	4.93 ± 0.29	1.08 ± 0.12	1.10 ± 0.19
	$F = 2.707$ $P =$ 0.073	$F = 1.525$ $P =$ 0.259	$F = 0.390$ $P =$ 0.846	$F = 0.582$ $P =$ 0.603