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Anti-angiogenesis effect of trichosanthin and the underlying mechanism

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ABSTRACT

The growth and metastasis of tumors depend on angiogenesis. Tumor angiogenesis is initiated by the secretion of growth factors from tumor cells; downstream signals are then triggered in pre-existing blood vessels to sprout a new vascular network. Trichosanthin (TCS) is a type I ribosome-inactivating protein that has anti-tumor activity, but the underlying mechanism remains unclear. In this study, we found that a non-toxic dose of TCS decreased the wound-healing and the migration of H5V mouse heart capillary endothelial cells (ECs) induced by human choriocarcinoma (JAR) cells, as well as the JAR-induced angiogenesis of rat third-order mesenteric arteries. TCS was effective on both tumor cells and ECs/arteries. First, TCS decreased vascular endothelial growth factor transcription and secretion by JAR cells. Second, TCS consequently inhibited the tumor cell-induced, extracellular signal-regulated kinase-mediated angiogenic signal in ECs and blood vessels. In conclusion, the ability of TCS to inhibit tumor angiogenesis contributes to its anti-tumor activity.

1. Introduction

Angiogenesis is the formation of a new vascular network from pre-existing vessels [1]. It is a vital process in the growth of blood vessels and wound healing. Furthermore, angiogenesis plays a key role in tumor development. Tumor cells are able to activate a process very similar to normal angiogenesis. Tumor cells can secrete a large number of growth factors, such as interleukin (IL)-8 and vascular endothelial growth factor (VEGF), which stimulate new blood vessel formation by nearby endothelial cells (ECs) from pre-existing vessels. Angiogenesis is a crucial step in cancer growth, providing nutrients to tumor cells, thus enabling the progressive enlargement and metastatic dissemination of tumors.

A series of positive and negative factors [2] control the process of angiogenesis. VEGF is the most well-studied angiogenic factor. In humans, VEGF mRNA is alternatively spliced and the proteins are classified as $VEGF_{121}$, $VEGF_{121b}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$ [3]. VEGF stimulates angiogenesis by binding to VEGF receptors (VEGFRs) on the plasma membrane of ECs. Upon activation, VEGFRs elicit a proliferative signal in the ECs and promote endothelial cell–cell interactions and capillary formation.

Trichosanthin (TCS) is a 27-kDa protein isolated from the root tuber of the Chinese medicinal herb *Trichosanthes kirilowii* Maximowicz. TCS is a type I ribosome-inactivating protein (RIP) and has been used to treat trophoblastic tumors, but the underlying mechanism remains unclear. Nevertheless, evidence shows that TCS at a low dose can interfere with humoral immunity and immune-related processes [4,5]. Because tumor growth, the immune response, and angiogenesis are co-dependent and share key regulatory growth factors and cytokines [6], in this study, the effect of TCS at non-toxic levels on choriocarcinoma cell line-induced angiogenesis was explored. Our results demonstrated that TCS profoundly decreased the angiogenic response of ECs and angiogenesis induced by tumor cells, and the effect of TCS is likely to be mediated through its functional interference with VEGF production in tumor cells and the extracellular regulation of protein kinase activation in ECs.

2. Materials and methods

2.1. Cells, viruses and reagents

The H5V mouse heart capillary endothelial cells and human choriocarcinoma JAR cells were obtained from the ATCC (Rockville, MD, USA). H5V cells were grown in DMEM and JAR cells in RPMI 1640. TCS was from Shanghai Jinshan Pharmaceutical Co., Ltd. (Shanghai, PRC).

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2.2. Cell viability assessment by MTT assay

MTT was used to assess the viability of cells in 96-well plates following the kit protocol (Roche Applied Science). Briefly, H5V and JAR cells were treated with TCS (0.16–5 μg/mL in twofold serial dilutions) for 48 h. After the treatment, to measure the number of viable cells, 10 μL MTT was added to form formazan crystals, which were later dissolved in solubilization solution. Absorbance was read at 570 nm.

2.3. Wound-healing assay

Cell motility was investigated by measuring cell migration into a wound made in a confluent monolayer. Briefly, H5V and JAR cells were separately cultured in 12-well plates and transwell inserts (0.4 μm pore size; Falcon, USA) in complete media with 10% FBS. After overnight growth and attachment, both media were replaced with serum-free ones for another 24 h. A wound was then made by scraping with a P10 pipette tip on the monolayer of H5V cells; floating cells were washed off with PBS. The transwells with JAR cells were then placed into the culture plate wells to co-culture with H5V cells for 48 h. During certain treatments, 1.5 μg/mL TCS and 80 nM of the extracellular signal-regulated kinase (ERK) inhibitor U0126 (Cell Signaling, USA) or 0.1 μg/mL of the ERK activator epidermal growth factor (EGF, Cell Signaling, USA) were added into both the wells and transwell inserts.

The ability of cells to migrate into the wound gap was observed and recorded photographically. Healing was analyzed by measuring the percentage of wound reduction with ImageJ software (NIH, USA).

2.4. Migration assay

H5V migration was assessed with transwell inserts (5.0 μm pore size; Corning, USA). Briefly, JAR cells were cultured in 24-well plates and allowed to grow and attach. Culture medium was then replaced with serum-free medium. The transwell inserts were placed into the wells of the cell culture plates. H5V cells (10^6 cells/ml) were then harvested, washed twice with PBS and resuspended in serum-free DMEM with 0.2% BSA. The cell suspension (200 μL) was then seeded into each upper chamber of the inserts and co-cultured with JAR cells. During certain treatments, 1.5 μg/mL TCS or 5 μg/mL of the pan-VEGF inhibitor GW654652 (Glaxo-SmithKline, Collegeville, PA) was added. FBS (10%) without JAR

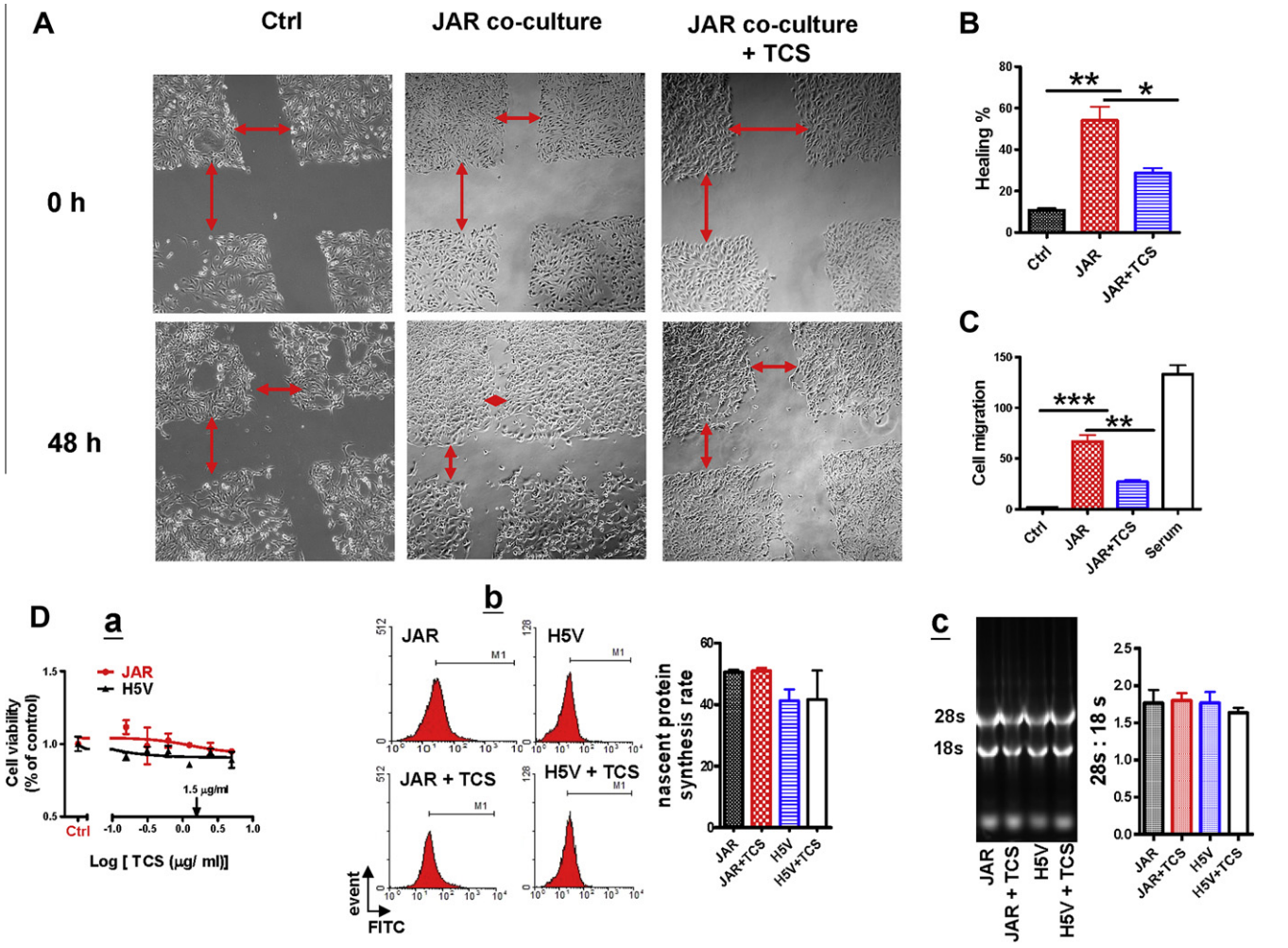

Fig. 1. TCS suppresses JAR cell-derived endothelial migration. (A and B) Representative images and statistics of the effect of TCS on the JAR cell-induced wound-healing response in H5V cells. A wound was generated by scraping the monolayer of H5V cells, and JAR cells were co-cultured with H5V cells in transwell inserts. Untreated or with 1.5 μg/mL TCS for 48 h, the ability of cells to migrate into the wound was observed and analyzed. (C) Statistics summarizing the effect of TCS on JAR-induced migration in H5V cells. H5V cell suspensions were seeded in transwell inserts and co-cultured with pre-seeded JAR cells with or without 1.5 μg/mL TCS for 18 h. H5V cells that migrated through the filter were stained with crystal violet and counted under a microscope. (D) Cytotoxicity in H5V and JAR cells assessed by MTT assay (a). RIP activity of TCS was analyzed by status of nascent protein synthesis (b) or ribosome RNA agarose gel (c). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).