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Human urinary bladder cancer T24 cells are susceptible to the *Antrodia camphorata* extracts

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Abstract

Bladder cancer has been cited to result from the neoplastic lesion with environmental and/or occupational factors identified as causatives. Transitional cell carcinoma (TCC) is the most common type of bladder cancer. Most of the bladder cancer patients die from the invasive, metastatic TCC that has turned out to be resistant to chemotherapy. T24 cells, a cell line established from a human urinary bladder cancer patient, are high-grade and invasive TCC. T24 cells were found very susceptible to ACCE at concentration of 50 μg/mL. MTT assay showed that the cell growth and proliferation were inhibited to 50% of the control when treated with ACCE for 72 h, at which the cell proliferation suppressing rate revealed -4.4×10^3 cells/μg per day. Comparing the expressions of the cell cycle biomarkers Cdc2 and Cyclin B1 by the western blot analysis, a phase G_2M arrest was confirmed. Both the wound scratch assay and the transwell motility assay indicated that ACCE was very effective anti-metastatic against T24 cells. Furthermore, the active form of matrix metalloproteinase-9 (MMP-9) was also found totally suppressed as revealed by zymography at 72 h post-incubation with ACCE, while the light and electron microscopic images have apparently revealed cell membrane damages on T24 cells when treated with ACCE (50 μg/mL). Moreover, both the wound scratch and the transwell assays have demonstrated the migration capability of T24 cells has been significantly retarded to 1.5-fold at same dosage of ACCE used. In conclusion, ACCE is a good anti-cancer agent, being effective in inducing phase G_2M arrest, acting as an anti-proliferative, and an anti-metastatic agent against bladder cancer cell T24 cells.

Keywords: *Antrodia camphorata*; Transitional cell carcinoma (TCC); Migration; Matrix metalloproteinase (MMP)

1. Introduction

Bladder cancer, comprising a broad spectrum of tumors including transitional cell carcinoma (TCC), has been cited to result from the neoplastic lesion with environmental and occupational factors identified as causatives [1]. Epidemiological studies have demonstrated with sufficient evidence that the risk factors associated with bladder cancer involve smoking, and exposures to aromatic amines, paints and solvents, leather dust, inks, heavy metals, polycyclic aromatic hydrocarbons, and diesel combustion and exhaust fumes, etc. [2]. Generally, bladder cancer develops by a multistage process, the symptoms of which are very

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similar to the common infections at the initial phase, hence the diagnosis at the initial stage always has been very confusing and misled. Although majority of the patients may present with superficial bladder tumors, yet 20–40% of the bladder cancer either present with or develop invasive diseases [3]. In Taiwan, TCC has been ranked as the top seven common type cancer in male, and still is rising in incidence and prevalence [4]. Most of the bladder cancer patients die from the invasive, metastatic TCC that has turned out to be resistant to chemotherapy. T24 cells, a cell line established from a high-grade and invasive human urinary bladder cancer patient, belong to one category of urinary bladder epithelial transitional carcinoma cells. T24 cells have several documented characteristics: (1) having extremely low-level of alkaline phosphatase (AKL) isozyme pattern comparing to other four cell lines derived from human bladder carcinomas including RT4, RT112, J82, and EJ [5]; (2) resembling the liver-type AKL in electrophoretic mobility and sensitivity to heat denaturation [5]; (3) containing high proportion of β1–6 branched tri- and tetraantennary complex type glycans such as P-glycoprotien (P-gp) may facilitate the important function in adhesion and migration of T24 cells, a fact implying that oligosaccharides are involved in several steps of the metastatic process [6]. Several biomarkers for bladder cancer have been investigated in different bladder cancer cell lines including T24, RT4, TSGH 8301, and BFTC 905 [7]. Presence of wild type p53 was found only in cell lines derived from either a low-grade, papillary tumor, while the T24 cells were found to contain a novel p53 mutation in a panel of bladder carcinoma cell lines [8]. The subpopulations of human bladder cancer cell lines of T24, Hi-T24 and Lo-T24, were reported to exhibit similar gross morphology, cell growth rate, and adhesion activity to basement membrane extract (matrigel), but the Hi-T24 cells were 3.8-fold more haptotactic through matrigel than Lo-T24 cells. The amounts of MMP-2 protein secreted into the medium by Hi-T24 and Lo-T24 cells were 7.8 ± 0.2 and 3.8 ± 0.3 ng/mL, respectively, and correspondingly, the quantities of tissue inhibitor of metalloproteinase-2 (TIMP-2) protein secreted were 133.2 ± 4.3 and 168.7 ± 5.6 ng/mL, respectively. Furthermore, the expression of the matrix metalloproteinases (MMP) are imbalanced at the gene level in human urinary bladder cancer cells at the stage of tumor progression [9].

Recently, so-called 'complementary alternative medicines' have attracted attention from conventional medical therapeutists [10]. Some treatment courses have been observed to be more effective and versatile and less toxic than routine clinical treatments. *Antrodia camphorata* is a well-known traditional Chinese medicine. It usually grows only on the inner heartwood wall of the Taiwanese endemic evergreen *Cinnamomum kanehirai* Hay (Lauraceae), seldom found in the wild and more importantly, unable to be cultivated easily in laboratories. *A. camphorata* has long been used as a remedy for chemical intoxication, diarrhea, abdominal pain, hypertension, itchy skin, and hepatoma [11]. Recently, the culture broth of an AC strain (AC strain no. CCRC-35396) has been reported to possess an anti-HBV pyrrolidione, 3-isobutyl-4-{4-[(3-methyl-2-butenyl)oxy]phenyl}-1*H*-pyrrole-2,5-dione [12]. In addition, one of the constituent group, zhankunic acids (A–C) extracted by ethanol from the fruit bodies of AC, has shown to exhibit anti-inflammatory activities and immunomodulating effects in human leukocytes [13,14]. Another constituent group, polysaccharides extracted from the fruit bodies of *A. camphorata,* has shown anti-hepatitis B virus effects [15]. Recently, Nakamura et al. showed satisfactory cytotoxic effects of maleic and succinic acid derivatives from *A. camphorata* mycelia on the LLC tumor cell line, reaching ED50 (the 50% growth inhibition) at a level of 3.6 μg/mL [16]. Moreover, in treatment of human premyelocytic leukemia HL-60 cells with fermented *A. camphorata* culture fluid, researchers observed marked apoptosis, which was dose- and time-dependent [17]. To our knowledge, the application of *A. camphorata* to the treatment of bladder cancer has never been documented, in this study, we try to investigate the effect of *A. camphorata* crude extract on bladder cancer T24 cells that have been considered to be a high grade transitional cell carcinoma.

2. Materials and methods

2.1. Antrodia camphorata crude extract (ACCE)

The pulverized crude extract of *Antrodia camphorata* (ACCE) was provided by Well shine Biotechnology Development Co., Ltd (Taipei, Taiwan) following the preparation method as previously described [18], which contained 15–20% of triterpenoids and 1–2% of polysaccharides, the remaining of which was fungal cell wall components (HPLC and GC/MS data not shown). A stock of ACCE solution was prepared by dissolving the ACCE powder in absolute EtOH to make a final concentration of 80 mg/mL and stored at −20 °C. The concentrations of ACCE used in experimentation ranged between 10 and 150 μg/mL.