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Solanum incanum extract (SR-T100) induces human cutaneous squamous cell carcinoma apoptosis through modulating tumor necrosis factor receptor signaling pathway

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ABSTRACT

Background: The *Solanum* species herbs have been used to treat cancer for centuries; however, the underlying mechanisms and effectiveness *in vivo* remain unclear.
Objectives: SR-T100, extracted from the *Solanum incanum*, contains solamargine alkaloid as the main active ingredient. Here, we investigated the apoptosis-inducing effects of SR-T100 for targeting squamous cell carcinoma (SCC) *in vitro* and *in vivo*.
Methods: We elucidated the mechanism by which SR-T100 induces apoptosis of human SCCs (A431, SCC4, SCC9, and SCC25) cells. The efficacy and safety issues were addressed regarding topical treatment of SR-T100 on UVB-induced cutaneous SCC of hairless mice and actinic keratoses (AKs) of human.
Results: SR-T100 induces apoptosis in human SCCs cell lines by up-regulating the expressions of tumor necrosis factor receptors (TNFRs) and Fas, and downstream adaptors FADD/TRADD of the TNF-α and Fas ligand signaling cascades. SR-T100 also triggered the mitochondrial apoptotic pathway, as up-regulated cytochrome *c* and Bax, down-regulated Bcl-X_L. Animal experiments showed that all papillomas (35/35) and 27 of 30 UVB-induced microinvasive SCCs in hairless mice disappeared within 10 weeks after once-daily application of topical SR-T100. Furthermore, 13 patients, who suffered with 14 AKs, were treated with once-daily topical SR-T100 gel and 10 AKs cured after 16 weeks, showing negligible discomforts.
Conclusion: Our studies indicate that SR-T100 induces apoptosis of SCC cells via death receptors and the mitochondrial death pathway. The high efficacy of SR-T100 in our preclinical trial suggests that SR-T100 is a highly promising herb for AKs and related disorders.

1. Introduction

Solanum species extracts have been shown to possess anticancer properties for centuries around the world, including China [1–3]. Several active ingredients, including solamargine, solasodine and solasonine, suppress cancer growth both *in vitro* and *in vivo* [2–5]. However, the underlying mechanisms for their activities remain to be elucidated. Different *Solanum* plants contain various amount and percentages of the above mentioned components. Solamargine is the glycoalkaloid that is found in *Solanum incanum* and at least in

Abbreviations: 5-FU, 5-fluorouracil; AKs, actinic keratosis; FADD, Fas-associated death domain; FasL, Fas ligand; FDA, Food and Drug Administration; MISCC, microinvasive squamous cell carcinomas; NMSC, non-melanoma skin cancers; PCNA, proliferating cell nuclear antigen; PI, propidium iodide; RT-PCR, reverse transcription-polymerase chain reaction; SCC, squamous cell carcinoma; TNFRs, tumor necrosis factor receptors; TRADD, TNFR-1-associated death domain; TUNEL, terminal nucleotidyl transferase-mediated nick and labeling; UV, ultraviolet.

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100 other *Solanum* species [6]. *S. incanum* extract is an important traditional Chinese medicine in Taiwan for the treatment of various chronic liver diseases since 1973. It exhibits liver protective effect against CCL_4 (carbon tetrachloride)-induced liver damage [7]. Another *Solanum* species, an Australia local plant *Solanum sodomaeum* (*linnaeanum*), was effective in the treatment of actinic keratosis (AKs), squamous cell carcinoma (SCC) and basal cell carcinoma, which has been marketed in Australia under the name Curaderm for almost 20 years [2].

Previously, we demonstrated that solamargine increases the expression of tumor necrosis factor receptors (TNFR1 and TNFR2) [6,8–10], along with activation of the mitochondrial pathway of apoptosis, in human hepatocellular carcinoma (HepG2 and Hep3B), lung cancer cells (A549, H441, H520, H661 and H69) [11,12], and breast cancer (SK-BR3, MCF-7, HBL-100 and ZR-75-1) cell lines [13]. Compared with paclitaxel, cisplatin, gemcitabine and etoposide, solamargine was found to have a superior effect in suppressing human lung cancer cell growth [12]. Also, solamargine inhibits the growth of a variety of cultured human solid tumor cell lines HT-29 (colon), HCT-15 (colon), LNCaP (prostate), PC-3 (prostate), T47D (breast) and MDA-MB-231 (breast) *in vitro* [14].

Up-to-date, pure solamargine is only for academic research and still not available for clinical use, probably because of availability for mass production, water solubility of the chemical, and marketing considerations of pharmaceuticals. Chemical synthesis of solamargine is not yet available because of its specific oligosaccharide side chain. SR-T100 is a water-soluble product extracted from *S. incanum*, which contains solamargine alkaloid as the main active ingredient. An analytic method for quality control was developed (US patent 7,078,063, EU patent 1,058,334 and Japan patent 3,940,928). The consistency of SR-T100 preparation can be well controlled by high performance of liquid chromatography (HPLC), which made it possible to carry out the current study.

SCC of the skin, including its clinical precursor lesions such as AKs, is one of the most common malignancy in human [15]. Furthermore, incidence of the diseases is still increasing [16]. Several stages of SCC can be defined microscopically, including slight epidermal squamous cell dysplasia, moderate to severe squamous cell dysplasia (SCC *in situ*), invasive SCC, and metastasis [17]. AKs is the clinical precursor lesions associated with cutaneous SCC and displays squamous cell dysplasia confined to the epidermis. Although mortality from cutaneous SCC is rare, there is significant morbidity associated with the natural course of cancer development and failed treatment for SCC [18]. At present, prevention of SCC, or the treatment of its precursor lesions, AKs, is necessary and important [18], because not only of its high prevalence but also for the low per-patient cost and far more efficacious of its management [19]. It is not yet possible to determine which AK lesions will evolve into SCC. The current consensus is to treat AK lesions whenever and wherever they are present. This practice would ensure best treatment outcome and lessen medical expenditures [18–20]. However, it should be noted that all current topical field therapies are associated with substantial pain, tissue destruction, scarring, pigmentary changes, inflammatory reactions, and frequent recurrence [21]. Thus, there is still a need for new topical treatment modality with excellent tolerability, high response rate, as well as good cosmetic acceptability.

Uncontrolled growth and loss of apoptotic death are two important biological events responsible for the tumorigenesis [22]. In the present work, we assessed whether the water-soluble SR-T100 induces growth inhibition and/or apoptosis in skin SCC cells *in vitro*. *In vivo* experiments using UVB-induced SCC in hairless mice were also carried out to assess the safety, efficacy, and underlying mechanisms of SR-T100. Our observations showed that SR-T100 is a potent inducer of apoptosis of cutaneous SCC, probably via activation of death receptors and the mitochondrial pathway of apoptosis. Most encouragingly, topical SR-T100 exhibited more than 80% clearness rate with only negligible cutaneous side effects on UVB-induced papillomas and microinvasive SCCs in hairless mice and AKs in patients. The high efficacy of SR-T100 in our preclinical trial of human subjects suggests that SR-T100 is of great therapeutic values in treating AK and related skin cancers.

2. Materials and methods

2.1. Materials and cell lines

SR-T100 was manufactured from *S. incanum* according to the patent (US patent 7,078,063, EU patent 1,058,334, and Japan patent 3,940,928). In brief description, the active component of solamargine was extracted by acid–base precipitation and followed by the different ratios of ethanol/H_2O extraction. The final extraction was then dried by lyophilization. Solamargine in SR-T100 extract was quantified by reverse-phase high performance liquid chromatography. The chromatographic fingerprint of SR-T100 revealed two major components, solamargine and solasonine, as identified by comparisons with reference standards. SR-T100 contains solamargine and solasonine with the ratio approximately 65 to 35, and less than 20% of hydrophilic minor components. Previous studies have demonstrated that solamargine, and to a lesser extent of solasonine, was the major active component of *S. incanum* against various tumor cells [23] (also see Supplementary Fig. S1). Furthermore, other components within SR-T100 did not show cytotoxicities to tumor cells (see Supplementary Fig. S2). Thus, solamargine serves as standard in the present study for quality control. SR-T100 was quantified and diluted to a concentration of solamargine 10 mg/ml with pure water as a stock solution. Human epidermoid carcinoma (A431) and immortalized keratinocytes (HaCaT) were cultured in DMEM medium (Gibco). SCC4, SCC9 and SCC25 were cultured in DMEM/F12 medium (Gibco) supplemented with 0.4 μg/ml hydrocortisone. All culture cells were purchased from American Type Culture Collection and maintained in medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in 5% CO_2. The source of A431 (ATCC no. CRL-1555) provided by American Type Culture Collection was derived from epidermoid carcinoma of skin. The source of SCC4 (ATCC no. CRL-1624), SCC9 (ATCC no. CRL-1629) and SCC25 (ATCC no. CRL-1628) cell lines in this study were derived from squamous cell carcinoma of tongue.

2.2. Cell viability and morphological change

Cells (1.5×10^4 cells/well) were seeded in 96-well plates and treated with SR-T100 for 18 h, and the effects on cell growth were determined by MTS [3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] assay as described previously [11]. SR-T100-treated cells were washed with PBS, and morphological alterations were visualized by contrast inverted light microscopy (Nikon, TE2000-U, Japan).

2.3. Cell cycle analysis

Cells (1.5×10^5 cells/ml) were seeded in 24-well plates and incubated with SR-T100 (IC_{50} and IC_{80}) for 18 h. After treatment, the cells were stained with propidium iodide (PI) and analyzed by flow cytometer as described previously [11]. The cell population in each phase of the cell cycle was determined using WinMDI software.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from SR-T100 (IC_{80})-treated and untreated cells for 1 and 2 h using the Qiagen RNeasy Kit (Hilden, Germany). One μg of total RNA was reverse-transcribed using the