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# Danshen improves survival of patients with colon cancer and dihydroisotanshinone I inhibit the proliferation of colon cancer cells via apoptosis and skp2 signaling pathway

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## ARTICLE INFO

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Dihydroisotanshinone I (PubChem CID:89406)
Tanshinone I (PubChem CID: 114917)
Tanshinone IIA (PubChem CID: 164676)
Salvianolic acid B (PubChem CID: 6451084)
Oxaliplatin (PubChem CID: 5310940)

*Keywords:*
Dihydroisotanshinone I
Colon carcinoma
Skp2
National Health Insurance Research Database

## ABSTRACT

*Ethnopharmacological relevance:* Danshen (Salvia miltiorrhiza Bunge) is widely used in traditional Chinese medicine. However, it's definite clinical effect and mechanism on colon carcinoma is unclear.
*Aim of the study:* To test the hypothesis that the protective effect of danshen on colon cancer and discover the bioactive compounds through in vitro study.
*Materials and methods:* We conducted a nationwide cohort study by using population-based data from the Taiwan National Health Insurance Research Database (NHIRD). The study cohort comprised patients diagnosed with malignant neoplasm of colon (ICD-9-CM codes:153) in catastrophic illness database between January 1, 2000, and December 31, 2010. We used the Kaplan–Meier method to estimate lung cancer cumulative incidences. Next, human colon cancer cells (HCT 116 cells and HT29 cells) were used to investigate the effect of dihydroisotanshinone I (DT) on the proliferation and apoptosis of human colon cancer cells and the underlying mechanism through XTT assay and flow cytometry. The in vivo effect of DT treatment was investigated through a xenograft nude mouse model.
*Results:* In our study, the in vivo protective effect of danshen in the different stage of colon cancer patients was validated through data from the National Health Insurance Research Database in Taiwan. *In vitro*, we found that dihydroisotanshinone I (DT), a bioactive compound present in danshen, can inhibit the proliferation of colon carcinoma cells, HCT 116 cells and HT-29 cells. Moreover, DT induced apoptosis of colorectal cancer cells. DT also repressed the protein expression of Skp2 (S-Phase Kinase Associated Protein 2) and the mRNA levels of its related gene, *Snail1* (Zinc finger protein SNAI1) and *RhoA* (Ras homolog gene family, member A). In addition, DT also blocked the colon cancer cells recruitment ability of macrophage by decreasing CCL2 secretion in macrophages. DT treatment also significantly inhibited the final tumor volume in a xenograft nude mouse model.
*Conclusion:* Danshen has protective effects in colon cancer patients, which could be attributed to DT through blocking the proliferation of colon cancer cells through apoptosis.

## 1. Introduction

Colorectal cancer (CRC) is the third most common malignant disease in worldwide (Schreuders et al., 2015). While resection is often curative, as many as 45% of CRC patients die as a result of the disease, despite treatment (Ferlay et al., 2010). In these advanced tumors show recurrences in distant organs such as the liver, lung, lymph node, bone or peritoneum even after complete resection of the primary tumors

*Abbreviations:* NHIRD, National Health Insurance Research Database; NHI, National Health Insurance; CRC, Colorectal cancer; Skp2, S-phase kinase-associated protein 2; CCL2, CC chemokine ligand 2; TAMs, tumor-associated macrophages; TCM, Traditional Chinese Medicine; FHPs, Finished herbal products; EMT, tumor epithelial-mesenchymal transition; Snail1, Zinc finger protein SNAI1; RhoA, Ras homolog gene family, member A; DT, dihydroisotanshinone I; TI, Tanshinone I; T2A, Tanshinone IIA; SA, Salvianolic acid B

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(Itatani et al., 2016). Therefore, developing treatment regimens with superior effectiveness and minimal adverse effects for CRC remain apriority in colorectal adenocarcinoma research.

S-phase kinase-associated protein 2 (Skp2) belongs to the F-box protein family and is one of the components of the SCF E3 ubiquitin ligase complex. Skp2 overexpression has been observed in human colorectal cancer and may have critical downstream effects in CRC development (Shapira et al., 2005; Tian et al., 2013). In addition, the previous reports showed that inhibition of skp2 could be a potential strategy for colorectal cancer (Bochis et al., 2015; Uddin et al., 2008). Chronic inflammation, including inflammatory bowel disease, plays an important contributing role to the development of colorectal cancer (Choi et al., 2016; Ekbom et al., 1990; Nowacki et al., 2015; Rogler, 2014; Yashiro, 2014). In the inflammation environment, tumor-associated macrophages (TAMs) are derived from peripheral blood monocytes that are recruited into the tumor. TAMs also potentiate the seeding and establishment of metastatic cells (Guo et al., 2010). Chemokines are also one of the key players that promote cancer cell metastasis in CRC (Itatani et al., 2016). The expression of CC chemokine ligand 2 (CCL2, also known as monocyte chemoattractant protein-1) in CRC cells and TAM accumulation are strongly correlated with advanced CRC stages and a poor prognosis (Bailey et al., 2007; Hu et al., 2009). Moreover, the neutralizing antibody against CCL2 was reported to inhibit development of malignant pleural effusion of CRC in the animal model (Marazioti et al., 2013). These reports suggested CCL2 and skp2 could be the novel targets for the anti-CRC treatment.

In Traditional Chinese Medicine (TCM), the dried root of *Salvia miltiorrhiza* Bunge (danshen) is used to treat numerous cardiovascular and endocrine diseases and cancers (Chen et al., 2001). However, the clinical effects and mechanism of danshen in colon cancer treatment remains unclear. The National Health Insurance Research Database (NHIRD) of Taiwan owned the almost complete information of patients in Taiwan, including the clinical drugs and TCM and is widely used to investigate the clinical effort of these drugs and TCM on patients in Taiwan (Chang et al., 2017; Hung et al., 2017; Liao et al., 2017; Lin et al., 2017; Liu et al., 2016). Finished herbal products (FHPs), a modern form of decoctions in which herbal formulae and single herbs are concentrated into granulated compounds, are widely prescribed by TCM physicians because of their convenience and quality. The National Health Insurance program in Taiwan reimburses claims for FHPs. In this study, we used NHIRD to discover the clinical protective effect of danshen on colon cancer patients. In addition, we observed an inhibitory effect of dihydroisotanshinone I (DT) (Fig. 3A), extracted from the dried root of *S. miltiorrhiza* Bunge, on the proliferation of colon carcinoma cells, HCT 116 cells and HT-29 cells. DT also can induce apoptosis of HCT 116 cells and diminished the ability of macrophages to recruit HCT 116 cells. Mechanistically, DT could interrupt Skp2 and the tumor epithelial-mesenchymal transition (EMT) gene expression, including *Snail1* (Zinc finger protein SNAI1) and *RhoA* (Ras homolog gene family, member A). DT also reduced the secretion of CCL2 from macrophages to inhibit the ability of recruiting colon tumor cells. Moreover, we found that DT treatment (30 mg/kg) significantly inhibited the final tumor volume on xenograft nude mice. Our result suggests that DT could be the novel candidate for anti-colorectal cancer in the further.

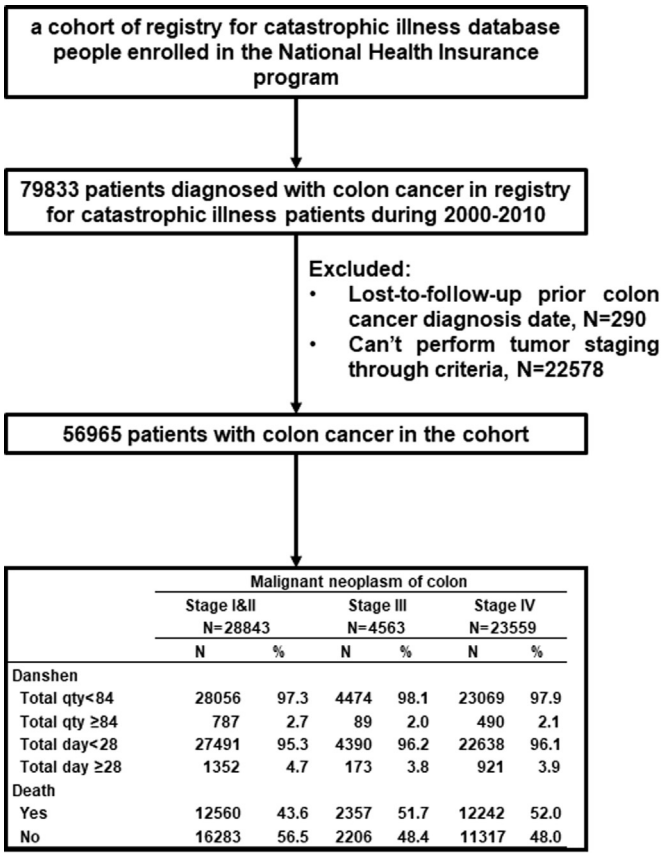
## 2. Material and methods

### 2.1. Data source

We conducted a nationwide cohort study by using population-based data from the Taiwan National Health Insurance Research Database (NHIRD). Because National Health Insurance is a compulsory universal program for all residents in Taiwan, the NHIRD is a comprehensive health care database that covers nearly the entire 23.7 million populations of this country. We used databases for admissions and outpatient visits, both of which included information on patient characteristics such as sex, date of birth, date of admission, date of discharge, dates of visits, and up to five discharge diagnoses or three outpatient visit diagnoses (according to International Classification of Diseases, Ninth Revision (ICD-9) codes). The data files also contained information on patient prescriptions, including the names of prescribed drugs, dosage, duration, and total expenditure. Following strict confidentiality guidelines in accordance with personal electronic data protection regulations, the National Health Research Institutes of Taiwan maintains an anonymous database of NHI reimbursement data that is suitable for research. Meanwhile, this study was approved by the Ethics Review Board of Chang Gung Memorial Hospital, Chia-Yi Branch, Taiwan (201601433B1).

### 2.2. Study subjects

This study cohort was obtained from the Taiwanese National Health Insurance research database, which included all patients who received diagnosis of malignant neoplasm of colon (ICD-9-CM codes:153) in catastrophic illness database between January 1, 2000, and December 31, 2010. Patients who apply for a cancer catastrophic illness certificate are required to provide pathological reports or other supporting documents, such as laboratory and image studies. The date of the initial colon cancer diagnosis was defined as the index date of colon cancer. Patient with other cancer diagnosed before colon cancer or missing data were excluded. A total of 56,965 patients were included in the study cohort. Then these patients were categorized into three groups according to clinical cancer staging: stage I & II, stage III, stage IV (Fig. 1) depending on previous study (Kuan et al., 2017). These patients accrued follow-up time beginning on January 1, 2000 and ended on the date of death, or withdrawal from the registry or on December 31, 2010.




| | Malignant neoplasm of colon | | | | | |
|---|---|---|---|---|---|---|
| | Stage I&II N=28843 | | Stage III N=4563 | | Stage IV N=23559 | |
| | N | % | N | % | N | % |
| Danshen | | | | | | |
| Total qty<84 | 28056 | 97.3 | 4474 | 98.1 | 23069 | 97.9 |
| Total qty ≥84 | 787 | 2.7 | 89 | 2.0 | 490 | 2.1 |
| Total day<28 | 27491 | 95.3 | 4390 | 96.2 | 22638 | 96.1 |
| Total day ≥28 | 1352 | 4.7 | 173 | 3.8 | 921 | 3.9 |
| Death | | | | | | |
| Yes | 12560 | 43.6 | 2357 | 51.7 | 12242 | 52.0 |
| No | 16283 | 56.5 | 2206 | 48.4 | 11317 | 48.0 |

**Fig. 1.** Patient of colon cancer disposition.