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•Review•

Toosendanin: upgrade of an old agent in cancer treatment

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[ABSTRACT] Toosendanin (TSN), a tetracyclic triterpenoid derived from *Melia toosendan* and *M. azedarach*, demonstrates broad application prospects in cancer treatment. Although previously employed as a pesticide, recent studies have revealed its potential therapeutic value in treating various types of cancer. TSN exerts an anticancer effect *via* mechanisms including proliferation inhibition, apoptosis induction, migration suppression, and angiogenesis inhibition. However, TSN's toxicity, particularly its hepatotoxicity, significantly limits its therapeutic application. This review explored the dual nature of TSN, evaluating both its anticancer potential and toxicological risks, emphasizing the importance of balancing these aspects in therapeutic applications. Furthermore, we investigated the incorporation of TSN into novel therapeutic strategies, such as Proteolysis-targeting chimeras (PROTAC) technology and nanotechnology-based drug delivery systems (DDS), which enhance treatment efficacy while mitigating toxicity in normal tissues.

[KEY WORDS] Toosendanin; Cancer therapy; Hepatotoxicity; PROTAC; Nano-delivery systems
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Introduction

Toosendanin (TSN), a tetracyclic triterpenoid, is a unique chemical isolated from the fruit or bark of *Melia toosendan* Sieb. et Zucc. and *M. azedarach* L., both members of the Neem family. As shown in Fig. 1, the chemical structure of TSN mainly consists of the following components: 1) two acetoxy groups; 2) three free hydroxyl groups, one of which exhibits an allylic alcohol relationship with the double bond; 3) a carbonyl group in close proximity to the acetoxy group; 4) *β*-mono-substituted furan rings and 1,2 epoxy groups [1]. As the main component of neem, TSN is widely present in Fructus Toosendan, and its content serves as a crucial indicator for the quality evaluation of Fructus Toosendan. According to the *Pharmacopoeia of the People's Republic of China*, the content of TSN in Fructus Toosendan should be 0.06%–0.20%. Traditional techniques such as high-performance liquid chromatography-ultraviolet (HPLC-UV) and high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD), as well as innovative approaches like ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) [2, 3].

Historically, TSN was primarily employed as a pesticide in China, appreciated for its sterilizing and insecticidal capabilities because of its intrinsic toxicity [4, 5]. Nonetheless, the anticancer activity of TSN has garnered more attention in recent years (Supplementary table 1). Substantial research has underscored TSN's efficacy against a variety of cancers, including gastric cancer [6, 7], ovarian cancer (OC) [8, 9], glioma [10, 11], kidney cancer [12], melanoma [13], colorectal cancer [14], and breast cancer (BC) [15, 16]. Mechanistically, TSN exerts antitumor effects through the induction of apoptosis,

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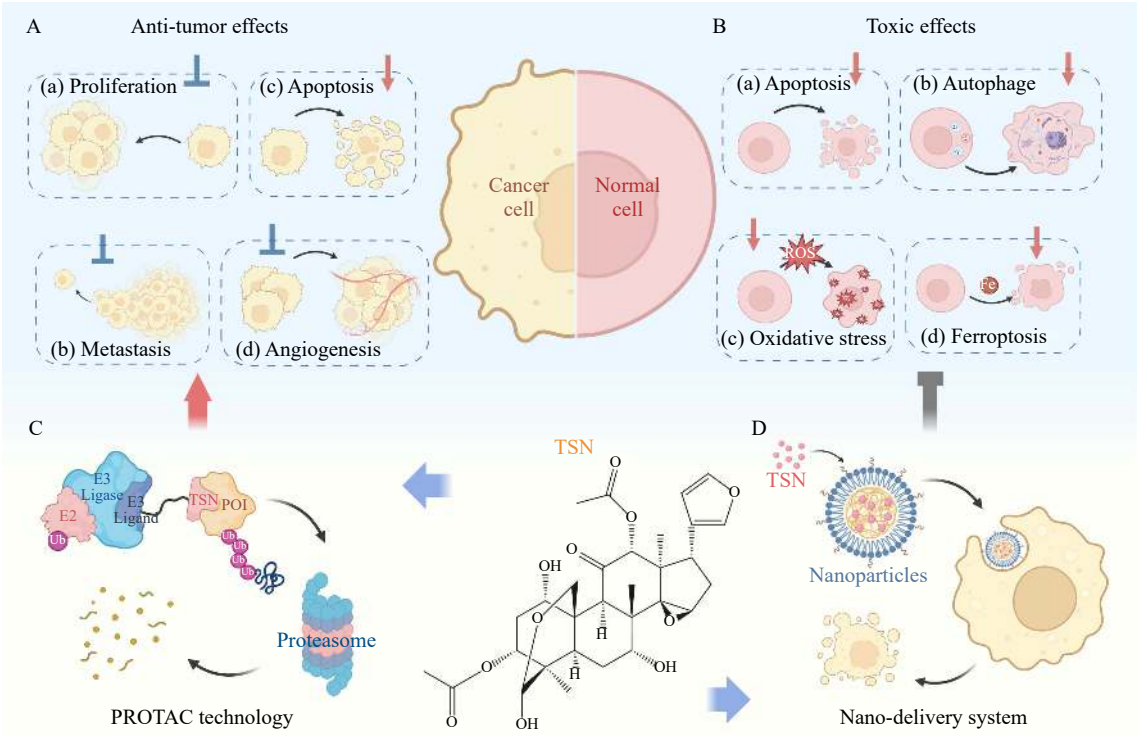

Fig. 1 Overview: The antitumor effects, toxic effects, and potential applications of TSN. (A) TSN exerts antitumor effects *via* inhibiting cell proliferation and metastasis, anti-angiogenesis, and promoting cell apoptosis; (B) TSN showed toxic effects on normal cells through triggering apoptosis, inducing autophagy and oxidative stress, and triggering ferroptosis; (C) PROTAC technology of TSN; (D) nano-delivery system of TSN.

suppression of migration and invasion, and inhibition of angiogenesis. Furthermore, TSN may improve the efficacy of other anticancer medications when used in combination. Despite these promising therapeutic characteristics, the application of TSN is limited due to its inherent toxicity. As a natural chemical, TSN demonstrates considerable deleterious effects, particularly hepatotoxicity, necessitating a reevaluation of its use in specific medical contexts. Due to TSN's dual role as a potential therapeutic agent and a source of toxicity, it is imperative to thoroughly elucidate its mechanisms of action and any associated adverse effects. A critical challenge lies in strategically balancing the dynamic interplay between the therapeutic effects and toxicity of TSN.

This review provides a comprehensive analysis of the molecular mechanisms underlying the anticancer effects, pharmacokinetic properties, and practical constraints associated with TSN. We further emphasize the importance of enhancing anticancer efficacy while mitigating toxicities in the clinical application of TSN, suggesting that innovative targeted-delivery technologies may facilitate more effective and safer cancer treatment approaches utilizing this compound.

TSN and cancer

TSN exerts anticancer activity by targeting various proteins and regulating signaling pathways related to cancer progression. Based on a series of natural product target discovery technologies, such as Drug Affinity Responsive Target Stability and Cellular Thermal Shift Assay, research has successfully identified lots of targets of TSN, including signal transducer and activator of transcription protein 3 (STAT3) in osteosarcoma [17], eukaryotic translation elongation factor 2 (eEF2) in esophageal squamous cell carcinoma (ESCC) [18], and sonic hedgehog (Shh) in colorectal cancer (CRC) [19]. Moreover, TSN is also involved in the modulation of many signaling pathways in cancer, including JAK/STAT3 [17], Wnt/*β*-catenin [20], phosphatidylinositol-3-kinase-AKT (PI3K/AKT/mTOR) [21], and Hedgehog (Hh) [22] (Fig. 2). By targeting above proteins and modulating related signaling pathways, TSN regulates a series of biological processes such as cell proliferation, apoptosis, and metastasis, thereby exerting antitumor effects.

TSN inhibits the growth of cancer cells

Abnormal cell proliferation and cell death resistance are both the hallmarks of cancer [23]. Some signaling pathways, including PI3K-AKT, mitogen-activated protein kinase, and mammalian target of rapamycin pathways, were activated during cancer progression and supported the abnormal proliferation of cancer cells. Common therapeutic approaches include attenuating proliferative signaling and inducing cell apoptosis [24]. Research has shown that TSN exerts an anticancer effect by inhibiting abnormal proliferative pathways. In the context of gliomas, TSN elevates the expression of microRNA-608 (miR-608) while reducing the expression of target genes, such as Notch1 and Notch2, thereby disrupting the proliferative process [11]. TSN further impedes the proliferation of glioblastoma (GBM) cells U87MG and LN18 by inhibiting the PI3K/Akt/mTOR signaling pathway [10]. In CRC

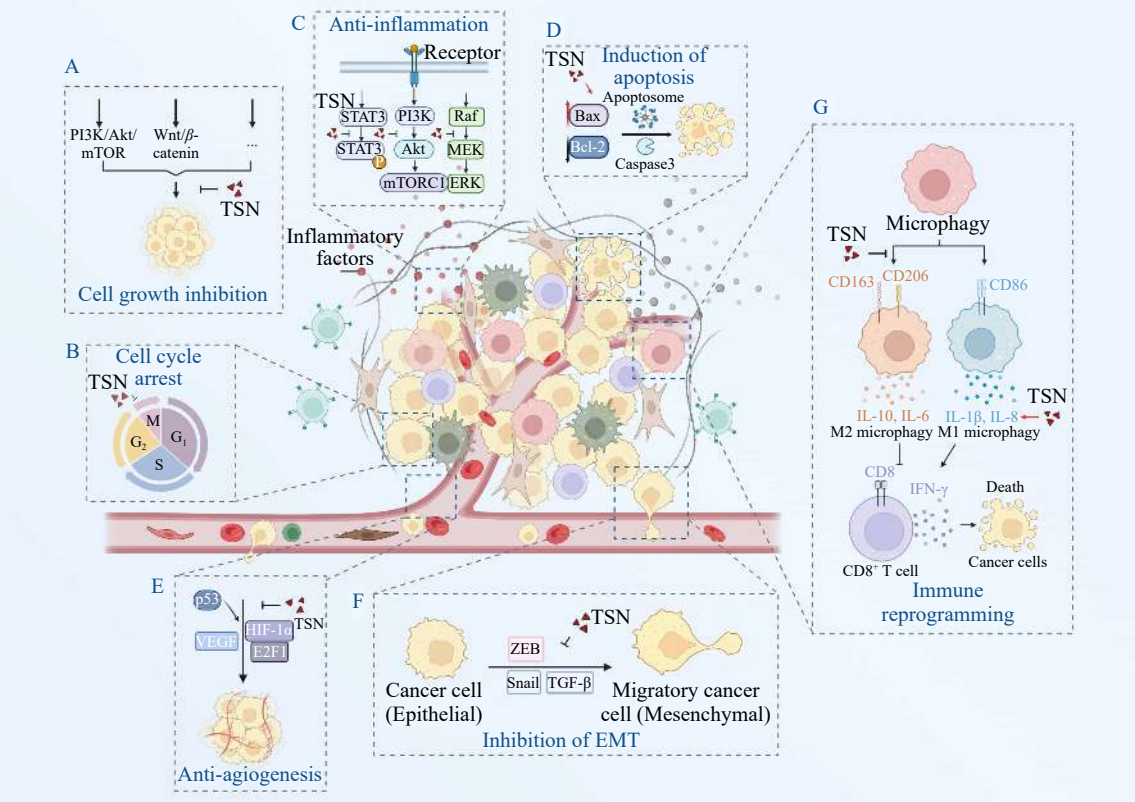

Fig. 2 Antitumor mechanism of TSN. TSN exerts anticancer effects *via* the following biological processes: (A) Cell growth inhibition; (B) Cell cycle arresting; (C) Inhibition of the release of inflammatory factors; (D) Apoptosis induction; (E) Anti-angiogenesis; (F) Inhibition of EMT; (G) Immune reprogramming.

cells, TSN also leads to cellular proliferation inhibition by blocking the Wnt/*β*-catenin and AKT/GSK-3β/*β*-catenin pathways [14, 21]. The p27kip1 protein, a crucial cell cycle-dependent kinase inhibitor, is implicated in the negative regulation of the cell cycle, emphasizing its role in cell proliferation, differentiation, and apoptosis [25]. Recent studies have demonstrated that TSN can upregulate the expression of the oncogenic factor cyclic RNA YAP1 (circYAP1) in GC MKN-45 and HGC-27 cells. This upregulation occurs through the sequestration of miR-367-5p, leading to increased expression of p27kip1, which results in cell cycle arrest and subsequent inhibition of cell proliferation [26].

TSN induces programmed cell death in cancer

Programmed cell death (PCD) is a conserved cellular suicide mechanism controlled by multiple signaling pathways and plays a crucial role in numerous biological processes, including apoptosis, necroptosis, pyroptosis, ferroptosis, and necroptosis [27-30]. Apoptosis can be activated through three primary mechanisms: the mitochondrial, death receptor, and endoplasmic reticulum pathways [31]. Notably, TSN primarily promotes apoptosis *via* the mitochondrial and death receptor pathways, with the mitochondrial pathway being more prominent in various malignancies [32]. The release of apoptotic factors in the mitochondrial pathway is primarily regulated by the B-cell lymphoma 2 (Bcl-2) family proteins, which consist of anti-apoptotic and pro-apoptotic members that either preserve or disrupt mitochondrial integrity [33-35]. The regulation of the balance between these two groups of Bcl-2 proteins largely determines cell survival or death [36]. In GBM cells U87 and C6, TSN regulates the expression of Bcl-2 family proteins to induce apoptosis [37]. In human GC cell MKN-45, TSN-mediated apoptosis is triggered by the downregulation of Bcl-2 *via* miR-23a-3p [7]. Moreover, TSN decreases mitochondrial membrane potential and upregulates Bcl-2-related X protein (Bax), cytochrome c (Cyt c), and apoptotic protease activating factor-1 (APAF-1) gene expression while enhancing the activity of caspase-3 and caspase-9 in human GC MGC-803 cells [38]. In human hepatocellular carcinoma (HCC) cells, TSN induces apoptosis by upregulating Bax and downregulating Bcl-2 expression [39]. Furthermore, TSN also promotes cancer cell apoptosis by activating the ubiquitin E3 ligase pathway to degrade the anti-apoptotic proteins in the BCL-2 family [40]. Molecular mechanistic studies have linked TSN to the reduced expression of survivins, poly (ADP-ribose) polymerase 1 (PARP-1), Bcl-2, B-cell lymphoma-extra-large (Bcl-xl), caspase-3, caspase-9, matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9), and increased expression of cleaved PARP-1, Bax, cleaved caspase-3, and cleaved caspase-9. In OC cells, TSN triggers apoptosis through the caspase-dependent mitochondrial pathway, resulting in mitochondrial membrane potential reduction and cell cycle arrest in the S-phase and G_2/M-phase [9].

TSN regulates the immunosuppressive microenvironment of tumor cells

In recent years, the focus of cancer-related research has shifted from cancer cells to the tumor microenvironment (TME) and the critical interactions between immune cells and tumor cells. Notably, tumor immunotherapy has become a pillar of cancer treatment [41]. T cells generate antitumor immune responses by recognizing antigens on tumors, and immunotherapy that activates or uses T cells through immune checkpoint blockade or overt T cell transfer has produced extraordinary efficacy in cancer treatment [42]. Studies have shown that TSN in macrophages (Mϕs) treated with interleukin-4 (IL-4) and IL-6, two cytokines known to induce immunosuppressive polarization of macrophages, almost completely abrogated the expression of CD206 and IL-10, the immunosuppressive surface markers of polarized M2-like macrophages. Consequently, TSN rescued T-cell proliferation, blocked macrophage-mediated immunosuppression, and activated T cells to achieve antitumor effects [43]. Furthermore, PCD can also modulate tumor immunity. As a non-immunogenic process, PCD generally maintains the integrity of the cell membrane without leakage of cellular contents, leading to "silent" clearance by phagocytes without triggering further inflammation [44]. Although apoptosis is typically non-immunogenic, studies have demonstrated that under certain conditions, such as cysteine deficiency, apoptosis can trigger adaptive antitumor or anti-viral immune responses through activation of NF-*κ*B signaling and the cGAS/STING pathway [45–47]. Therefore, although not specifically studied, TSN may modulate tumor immunity by mediating apoptosis, warranting further in-depth investigation.

TSN suppresses the migration and invasion of cancer cells

Cancer metastasis, a primary contributor to cancer-related mortality, results from a complex series of biological processes, including the ability of cancer cells to spread from the primary tumor site to adjacent tissues, disseminate through blood and lymphatic vessels, and proliferate in distant organs [48]. This intricate, multistage process is characterized by cell migration and invasion, which are hallmarks of malignant tumors [49]. The epithelial-mesenchymal transition (EMT) plays a pivotal role in this process, whereby epithelial cells lose their epithelial characteristics and acquire mesenchymal properties, enabling them to adopt a migratory phenotype. Notably, transforming growth factor-β1 (TGF-β1) is a key mediator of EMT, inducing cellular spindle alterations and modulating the expression of several markers, including the upregulation of Neural-cadherin/Cadherin-2 (N-cadherin), vimentin, and extracellular regulated protein kinases (p-ERK1/2), as well as the downregulation of E-cadherin, ultimately enhancing migratory and invasive capacities. Remarkably, these effects are substantially reversed by TSN at low concentrations [50]. TSN has been shown to inhibit cell growth, migration, invasion, and TGF-β1-induced EMT in SGC-7901 cells, accompanied by reduced cell-cycle arrest and increased cell apoptosis [51]. Interestingly, TSN exhibits the capacity to inhibit cancer cell migration and invasion through the modulation of several signaling pathways. TSN exerts notable anti-metastatic effects on HCC cells through a WW domain-containing oxidoreductase (WWOX)-dependent mechanism [52]. This process involves the inhibition of the Wnt/*β*-catenin signaling pathway and alters the interaction with WWOX. In-depth studies reveal that TSN impedes the progression of gastric cancer cells both *in vitro* and *in vivo* by modulating the high-mobility group box protein 2 (HMGB2)-miR-874/*β*-catenin pathway [53]. In the human ovarian cancer cell line SKOV3, TSN inhibits the LIM domain kinase 1 (LIMK-1)/cofilin signaling pathway by affecting the expression of LIMK-1, thereby inhibiting cancer cell migration [8, 9]. TSN also curbs the growth and liver metastasis of *in situ* transplanted SGC-7901 cells *in vivo via* the miR-200a-mediated *β*-catenin pathway [51]. Additionally, TGF-β activates transcription programs during TGF-β-induced EMT [54], including Snail, zinc finger E-box binding homeobox (ZEB), and basic helixloophelix protein (Bhlh) [55]. These factors promote EMT by downregulating the expression of epithelial cell markers, such as E-cadherin, thereby enhancing cell migratory and invasive capacities [56]. TSN has been confirmed to affect the expression of these transcription factors by regulating upstream signaling pathways. In a previous study, TSN was shown to inhibit migration and invasion of human ovarian cancer cells by suppressing EMT, mediated through the nuclear factor kappa-light-chain-enhancer of activated B cells/snail family transcriptional repressor 1 (NF*κ*B/Snail) signaling pathway [57]. Another study observed that TSN significantly inhibits TGF-β1-induced EMT and migratory invasion in lung cancer cells *via* the ERK/Snail pathway [58]. In summary, TSN exhibits significant potential in inhibiting cancer metastasis.

TSN is involved in the regulation of cancer-related inflammation

Tumor-promoting inflammation, another hallmark of cancer [23], facilitates cancer initiation, promotion, progression, and metastasis by triggering the release of various growth factors, cytokines, and chemokines, resulting in reduced survival time among cancer patients [59]. Cytokines such as tumor necrosis factor-α (TNF-α), IL-6, IL-8, and transforming growth factor-β (TGF-β) play a crucial role in this process [60], making their associated molecules and pathways viable targets for cancer prevention and treatment [61]. TSN can target various molecular pathways, including the STAT3, PI3K/Akt/mTOR, MAPK, and androgen receptor (AR) pathways, to decrease the release of inflammatory cytokines, subsequently affecting cell proliferation, apoptosis, and metabolism in prostate cancer [62]. Furthermore, TSN exposure significantly alters mRNA expression levels in genes related to inflammation, autophagy, apoptosis, and transporter protein pathways, ultimately influencing tumor growth [63].

TSN possesses anti-angiogenesis function

Angiogenesis, the dynamic process of generating new blood vessels from pre-existing ones, is crucial for cancer de-

velopment and metastasis. The increased permeability of the tumor vasculature alters blood flow, facilitating tumor cell diffusion into the interstitial space and creating a hypoxic tumor microenvironment. These conditions promote neovascularization and cancer progression [64, 65]. Vascular endothelial growth factor (VEGF), the primary pro-angiogenic factor, exhibits a potent mitogenic effect and stimulates angiogenesis [66]. Research has shown that p53, a tumor suppressor, plays a role in regulating VEGF expression [67]. During the early stages of hypoxia, p53 binds to the functional site in the VEGF promoter and induces VEGF transcription *via* the p21/Rb pathway. However, during prolonged hypoxia, p53 downregulates VEGF expression, consequently inhibiting angiogenesis in cancer cells and exerting a suppressive effect on tumor growth and progression [68]. A growing body of evidence suggests that TSN may inhibit the pro-angiogenic effects of VEGF by upregulating p53 protein expression, ultimately suppressing tumor cell growth and proliferation [4, 69, 70].

TSN in the combination therapy

Given the genetic complexity of most malignancies and the limited efficacy of single targeting, medication combinations with distinct mechanisms are required for sustained growth control [71]. Numerous preclinical studies suggest that TSN may enhance anticancer effects when combined with other medications. For instance, the synergy between TSN and paclitaxel (PTX) significantly inhibits the proliferation of triple-negative breast cancer (TNBC) cells [16]. The TSN-PTX combination has demonstrated superiority over PTX alone by downregulating the PTX-induced overexpression of Adenosine A2A receptor (ADORA2A), an adenosine receptor involved in the regulation of EMT, suggesting that the TSN-PTX combination may be an effective adjuvant chemotherapy strategy for patients with TNBC, particularly those with metastatic disease [16]. Additionally, in a mouse model of homozygous glioblastoma multiforme (GBM), immune checkpoint blockade combined with TSN treatment induces tumor regression. TSN also sensitizes GBM to epidermal growth factor receptor (EGFR) and chimeric antigen receptor (CAR) T-cell therapy and reprograms macrophages to enhance antitumor immunity [43]. Furthermore, TSN upregulates the expression of death receptor 5 (DR5) in HCC cells and potentiates the antitumor activity of TNF-related apoptosis-inducing ligand (TRAIL) [72]. It synergizes with γδ T cells, enhancing their anti-colorectal cancer effect by inhibiting the expression of MCL-162 [73, 74].

Considering that TSN-induced liver dysfunction and DNA damage are closely associated with reactive oxygen species (ROS) generated in mitochondria, we hypothesized that Nrf2-mediated antioxidant activation could play a protective role in hepatocytes [75]. Various natural active ingredients, such as *Lycium barbarum* polysaccharides, *Bambusae caulis* in Liquamen extracted from fresh bamboo stems, and guava leaf polysaccharides, have been reported to inhibit the production of ROS induced by hydrogen peroxide and effectively treat oxidative stress-induced liver cell damage [76–79]. When combined with these compounds, TSN is anticipated to enhance antitumor efficacy and potentially mitigate liver damage to a certain degree, underscoring the potential of TSN in combination therapy, which warrants further investigation.

Application limitations of TSN

As a principal ingredient in antiparasitic drugs and pesticides, TSN's toxicological effects are as significant as its antitumor properties (Supplementary table 2). Chinese medical practitioners historically used Fructus Meliae Toosenda (FMT), the source of TSN, with caution, as reflected in extensive documentation in classical medical texts. Studies have revealed that after extraction of FMT using ethyl acetate, TSN was found in the extracted solution [75]. TAN *et al.* compared the acute toxicity of raw and processed FMT, and the median lethal dose (LD_{50}) for 70% ethanol extracts of raw and fried yellow FMT were determined to be 80.92 and 67.75 $g \cdot kg^{-1}$ of raw medicine, respectively [80]. The toxic effects mediated by TSN mainly focus on hepatotoxicity, while also encompassing certain reproductive toxicity [81]. This section aims to delve into the toxic mechanisms underlying TSN. However, there have been few clinical studies on the toxicity of TSN, with only partial reports on the clinical toxicity of its source material, Fructus Toosendan. By 1999, a total of 28 cases of neem poisoning with 10 deaths were recorded in China [82]. Clinical data showed that most patients with excessive raw Fructus Toosendan poisoning experienced gastrointestinal irritation 1−2 h after ingestion, with some patients even developing acute toxic hepatitis [83]. Another study analyzed 92 cases of pharmacological liver injury caused by traditional Chinese medicines over a 5-year period, most of which contained Fructus Toosendan [84].

Hepatotoxicity

Drug-induced liver damage (DILI) is the primary reason for drug recalls and accounts for a significant portion of adverse drug responses [85]. In zebrafish models treated with TSN, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were elevated, accompanied by severe cytoplasmic vacuolation and nuclear shrinkage of hepatocytes [63]. The gene expression profile further demonstrated that autophagy and apoptosis pathways were involved in hepatotoxicity [63]. Furthermore, FMT treatment reduced the level of miR-370-3p in circulating exosomes, leading to the activation of the p53 pathway. Elevated phosphorylated p53 (p-p53) protein decreases the mitochondrial membrane potential, ultimately inducing apoptosis in liver cells [75]. Additionally, TSN upregulated the expression of activation transcription factor 3 (ATF3) *via* the protein kinase R-like endoplasmic reticulum kinase (PERK)-eukaryotic initiation factor 2α subunit (eIF2α)-activation transcription factor 4 (ATF4) signaling pathway, thereby increasing transferrin receptor 1 (TFRC) expression and inducing iron accumulation (Fig. 3). Excessive iron accumulation leads to ferroptosis in hepatocytes [86].

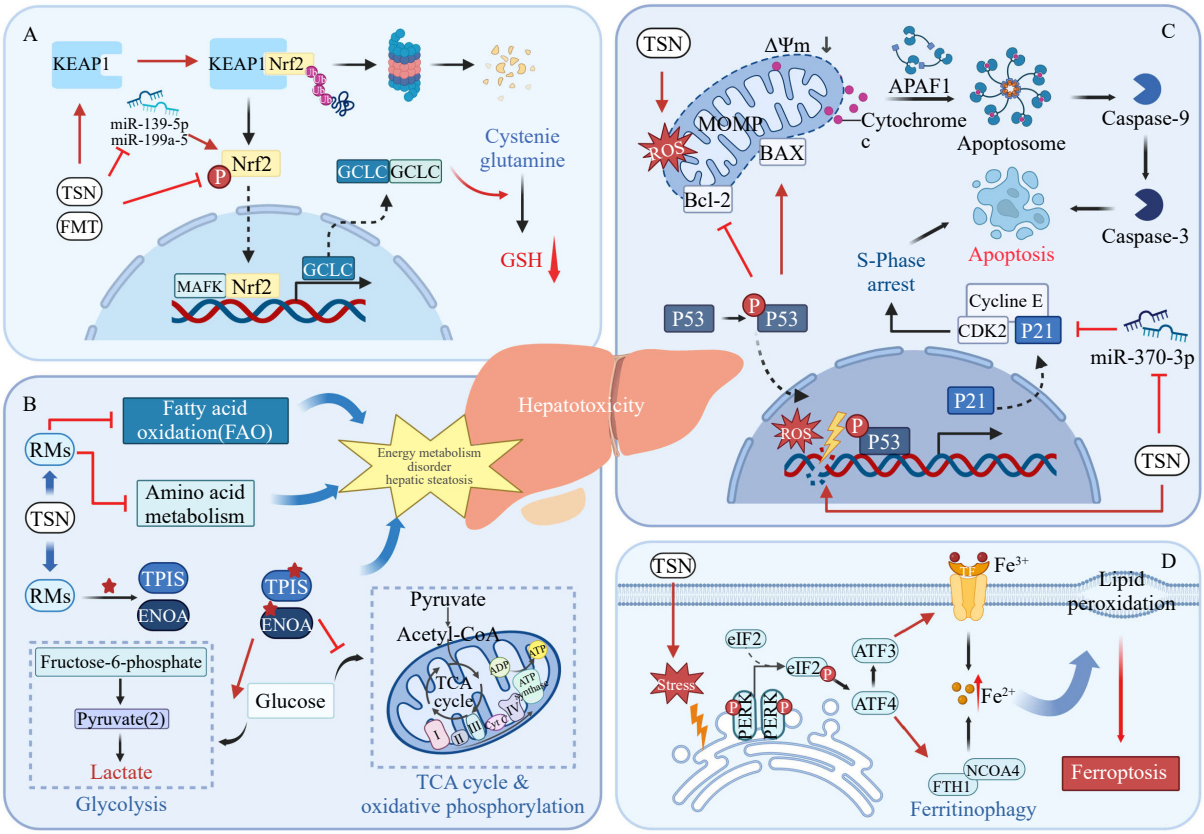

Fig. 3 Mechanisms of hepatotoxicity induced by FMT/TSN. (A) TSN induces hepatotoxicity by leading to oxidative stress *via* decreasing Nrf2 expression through multiple pathways. (B) TSN metabolites inhibit the TCA cycle and oxidative phosphorylation, stimulating glycolysis and regulating fatty acid metabolism and amino acid metabolism. (C) TSN induces mitochondrial dysfunction, leading to cell cycle arrest. (D) TSN triggers endoplasmic reticulum (ER) stress and activates the PERK/eIF2/ATF4/ATF3 pathway, inducing excessive iron accumulation and ferroptosis.

Research has demonstrated that the hepatotoxicity of TSN is partially attributed to the suppression of the glycolytic enzyme triosephosphate isomerase 1 (TPIS) and the upregulation of *α*-enolase (ENOA), concomitant with the inhibition of oxidative phosphorylation and the stimulation of glycolysis [87] (Fig. 3). Furthermore, serum fatty acid levels in mice with liver damage induced by FMT exhibited significant deviations from normal levels [88], indicating a strong correlation between altered fatty acid metabolism and FMT-induced hepatotoxicity. FMT also perturbs normal hepatic and renal metabolism, contributing to toxic accumulation by reducing serum creatinine levels, elevating triglyceride levels, and adsorbing creatinine in the gastrointestinal tract, thereby increasing excretion [89]. Additionally, FMT influences purine and pyrimidine metabolism, potentially increasing the risk of hepatic and renal toxicity through the upregulation of hypoxanthine nucleosides and the elevated excretion of pyrimidine metabolites in the urine.

Studies have demonstrated that TSN profoundly inhibits cytochrome P4503A (CYP3A), an essential enzyme in TSN metabolism, as indicated by a notable decrease in hepatic CYP3A activity. The proposed mechanism by which TSN modifies CYP3A-related hepatotoxicity is as follows: 1) Decreased TSN metabolism due to inhibited CYP3A activity, leading to elevated blood concentrations of aconitine and increased bioavailability, potentially resulting in toxic reactions; 2) TSN may compete with other CYP3A substrates or inhibitors for binding, reducing its clearance rate and increasing bioavailability, thereby enhancing therapeutic effects or potentially causing adverse reactions [90] (Fig. 3).

Reproductive toxicity

Recent investigations have linked TSN to reproductive toxicity. ZHANG *et al*. administered TSN at graduated doses into pregnant mice to assess the abortifacient impact and underlying mechanisms of TSN. A dosage of less than 1/10 LD_{50} (13.8 $mg \cdot kg^{-1}$) could cause abnormalities in the embryo of mice. The findings demonstrated that TSN raises the levels of miscarriage-associated cytokines TNF-α and IFN-γ in both uterine tissues and blood. Another study also revealed that TSN-mediated reproductive toxicity is related to the upregulation of interferon-γ (IFN-γ) and TNF-α [91]. These results indicate that TSN-induced reproductive toxicity significantly relies on the regulation of Th1 cytokines.

Pharmacokinetic and Safety Assessment of TSN

Natural active ingredients often possess both toxicological and therapeutic properties, necessitating the investigation of their *in vivo* absorption, distribution, metabolism, and ex-

cretion processes to evaluate their clinical safety. However, there is a paucity of information regarding the pharmacokinetic characteristics of TSN, and few analytical methods for its determination and/or quantification have been reported (Supplementary Table 3). In 2012, the first UHPLC-MS/MS was developed for the pharmacokinetic determination of TSN in rat plasma samples. The results indicated that TSN exhibited a rapid absorption rate, with a Tmax (h) value of 0.63 h following oral administration. Regarding Vd levels, oral administration (444 380.3 ± 204 747.7 mL·kg^{-1}) demonstrated a wider distribution compared to intravenous administration (32 062.4 ± 18 562.8 mL·kg^{-1}). In addition, there was a significant difference in clearance rates between the two modes of administration, with the results suggesting a faster elimination rate for TSN administered orally [92].

Furthermore, a research team used a simple liquid-liquid extraction method to extract TSN from rat plasma, and the sample solution was finally injected into a LC-MS/MS system for analysis. The results showed the high stability of TSN in rat plasma. After oral administration of 10 mg·kg^{-1} TSN to rats, TSN was rapidly absorbed, attaining peak plasma concentration after approximately 1.67 hours and exhibiting a half-life of around 5.5 hours [93]. Additionally, TSN was incubated with human liver microsomes, and its metabolites were identified using UHPLC-quadrupole time-of-flight MS. Based on the MS spectral information, six metabolites (M1-M6) were initially identified (Fig. 4). Among them, M1, M2, and M3 were produced by TSN oxidation, M6 by TSN dehydrogenation, and M4 and M5 by oxidative dehydrogenation. Notably, the stability of the metabolites exhibited temporal variation, with M1-M5 demonstrating robust stability lasting for 120 min. In contrast, M6 reached its maximum at 20 min and then gradually declined. These findings provide a solid foundation for elucidating the metabolic fate of TSN [94]. In addition, the co-administration of TSN and trans-anethole reduces the absorption and bioavailability of TSN and accelerates the elimination process of TSN, thereby reducing the risk of toxicity accumulation [95].

Drug Design and Structural Modification of TSN

TSN exhibits potent anticancer activity in preclinical trials, rendering it a promising potential drug for cancer treatment. However, its further clinical use is limited by severe side effects. Therefore, improving TSN's targeting efficacy is crucial to enhance its anticancer efficacy and mitigate adverse effects on healthy tissues. Proteolysis-targeting chimeras (PROTAC) degrades proteins involved in cancer growth, yielding significant outcomes across various cancer types. Furthermore, PROTAC's catalytic nature allows for significantly lower dosages to achieve equivalent pharmacological effects compared to small-molecule inhibitors, reducing off-target toxicity [96]. Similarly, nanoparticles (NPs) are widely employed as delivery systems for cancer drugs, enhancing the bioavailability and promoting drug accumulation in tumors. By leveraging these advantageous properties, NP-mediated targeted drug delivery systems (DDS) improve the efficacy of cancer treatments while minimizing side effects [97]. This section highlights the immense potential of PROTAC and nano-delivery systems in enhancing the safety and effectiveness of TSN in clinical applications by focusing on their roles in augmenting anticancer benefits and reducing toxic effects (Fig. 4).

PROTAC

PROTAC technology directly targets proteins and catalyzes their degradation through a novel event-driven mode of action, presenting significant clinical application prospects for various diseases [98]. This emerging technology induces polyubiquitination of target proteins, leading to their degradation *via* the proteasome pathway. PROTACs consist of three essential elements: a ligand targeting the protein of interest (POI), an E3 ubiquitin ligase ligand that facilitates protein tagging, and a strategically designed linker connecting these ligands to form a trivalent architecture [99]. PROTAC molecules exhibit good tissue distribution and the ability to target intracellular proteins [100]. They are also used in attenuated vaccine preparation and as effective anticancer agents [101, 102]. Since 2001, numerous PROTACs have been described, greatly expanding our knowledge and capacities in targeted protein degradation (TPD) [103–106]. In a preclinical study, JIN *et al.* explored the potential of STAT3, a key protein in epithelial cancers, as a target for PROTAC technology [107, 108]. Using the PROTAC strategy, they ingeniously utilized TSN as a prototype compound to craft a series of

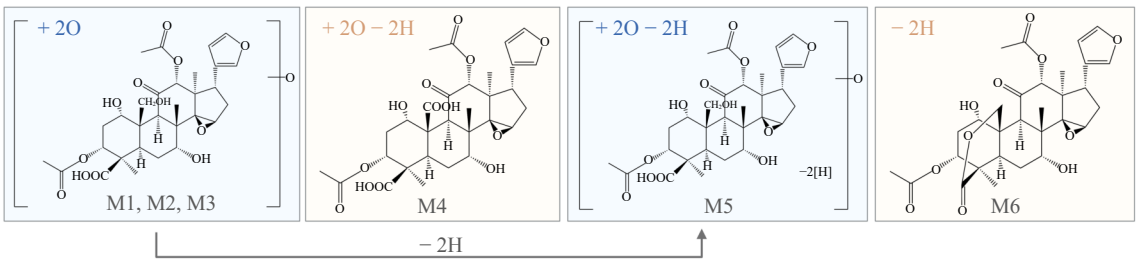

Fig. 4 Six metabolites of TSN: M1-M6. Compared to TSN, M1 and M2 have two more oxygen atoms. M3 is an isomer of M1 and M2, and the difference may lie in the position of hydroxylation on the methyl, methylene, or methylene group. M4 and M5 have two additional oxygen atoms and two additional hydrogen atoms compared to TSN. M5 is a dehydrogenation product formed from M1-3, and M6 is formed by dehydrogenation of TSN.

STAT3-targeting PROTAC derivatives. Among them, the lead compound, TSM-1, exhibited remarkable efficacy. TSM-1 quickly and effectively promoted the creation of ternary complexes, an essential stage in the PROTAC process, resulting in the targeted destruction of STAT3 proteins both *in vitro* and *in vivo* with good safety. The study shed light on the possibility of using TSN-based heterofunctional PROTAC molecules, such as TSM-1, as a strong, reversible, and efficient way to degrade STAT3 proteins in the treatment of malignant epithelial-derived cancers [109–111]. Leveraging the development of PROTAC technology and TSN's properties, effective cancer therapies can be developed while reducing the natural toxicity of TSN [99]. Notably, PROTAC molecules demonstrate increased therapeutic efficacy and a decreased propensity for drug resistance, which poses a significant obstacle to traditional cancer treatments [112–114].

Drug Nano-delivery Systems

Drug nano-delivery systems, primarily utilized for therapeutic applications and medical research, employ nanoscale particles as carriers for the precise administration of organic small molecules or biological macromolecules into specific cells and tissues [115, 116]. Advancements in materials science and nanotechnology have further diversified these NPs, which encompass organic, inorganic, and polymeric materials [117]. Progress in nanotechnology has facilitated the design of drugs and drug carriers capable of traversing various biological barriers and targeting specific disease sites. By accurately delivering therapeutic drugs to the lesion, the NP-based targeted delivery system enables reduced systemic toxicity and enhanced anticancer efficacy. Compared to free curcumin (Cur), Cur-NPs exhibited superior effectiveness in inducing mitochondrial damage and suppressing tumor cell invasion and migration [118]. Furthermore, nano-delivery technology has been successfully employed to precisely deliver pentacyclic triterpene compounds, ginseng extracts, and polysaccharides, yielding promising results [119, 120]. These findings suggest that NPs may serve as a potential vector for the targeted delivery of TSN into cancerous cells, thereby mitigating side effects associated with the inherent toxicity of TSN. Moreover, nanotechnology for targeted cancer therapy presents prospective opportunities for the nano-delivery of TSN, subsequently enhancing its therapeutic index [121, 122]. The adoption of nanoparticle-based delivery systems may improve the therapeutic efficacy of TSN while reducing adverse effects. The rational utilization of these technologies may facilitate the clinical translation of TSN.

Liposomes

Liposomes have garnered significant attention as highly applicable nanosystems due to their properties, such as biocompatibility, biodegradability, and reduced toxicity [123, 124]. A growing body of evidence suggests that liposomes exhibit great potential in enhancing bioavailability and mitigating the toxicity of natural active compounds. There remains substantial room for improvement in the antitumor treatment of liposomal formulations of Chinese medicine injections, and continued progress is anticipated. Studies have demonstrated that the modification of PTX-coated liposomes with chitosan (CSO) exhibited promising applicability in drug design for the treatment of lung cancer [125]. In addition, unique liposomes based on ginsenoside Rg3 significantly enhanced the specificity of tumor tissue distribution, achieving a high tumor inhibition rate of 90.3% [126]. Liposomes have also been employed in combination with natural active ingredients, such as curcumin, to improve drug stability [127]. Although studies on the drug design of tanshinone IIA (TSN) with liposomes are currently lacking, analogies can be drawn from existing experimental studies targeting natural active compounds. Considering the antitumor mechanisms associated with TSN and the challenges of drug resistance and toxicity, the potential of liposome-encapsulated TSN-specific DDS is expected to be immense.

Micelles

The emergence and significant advancement of polymer-based drug delivery vehicles and systems in modern research have surpassed traditional drug approaches, providing nanostructures with ideal particle size, surface properties, permeation profiles, and flexibility [128]. Micelles typically have lower critical micelle concentrations, slower dissociation rates, and higher drug accumulation at the target due to their enhanced solubility, which improves intestinal permeability [129–131]. In a seminal study, considering that the mechanism of curcumin (Cur)-induced apoptosis of liver cancer cells was closely related to the inhibition of mitochondrial function, the authors designed a special mitochondrial-targeted delivery system based on triphenylphosphonium bromide-chitosan-g-poly-(*N*-3-benzyloxy-l-lysine) to promote cellular drug uptake and ultimately target mitochondria. The results demonstrated that Cur-nanocolloid significantly decreased the mitochondrial membrane potential, exhibiting superior performance compared to Cur alone, indicating its potential as an efficient DDS targeting mitochondria in HCC cells. Furthermore, *in vivo* pharmacokinetic experiments revealed that drug-loaded nanomicelles exhibited improved metabolic behavior compared to the free drug, substantially increasing the blood concentration of curcumin and prolonging the drug's half-life. Another study showed that PTX-polymerized micelles significantly reduced the incidence of brain and liver damage while enhancing the drug's anticancer ability [132–134].

Albumin

Albumin serves as a crucial source of nutrients for the human body and has been extensively investigated as a natural DDS due to its high biocompatibility, facile surface modification, and favorable biodegradability [135]. Notably, albumin can be intrinsically formulated within the hydrophobic interior of hydrophobic anticancer drugs, resulting in enhanced pharmacokinetic profiles and prolonged circulating half-life. Nanoparticle albumin-bound paclitaxel (nab-PTX) represents the pioneering nanotechnology-based drug developed for cancer therapy. Studies have demonstrated that

nab-PTX can be phagocytosed by neutrophils and delivered to the tumor site *in vivo*, thereby improving therapeutic efficacy and mitigating myelosuppression. Simultaneously, nab-PTX significantly potentiated the inhibitory effect of local radiotherapy on tumor growth and attenuated hematotoxicity [136]. Furthermore, nab-PTX exhibited enhanced solubility and reduced infusion-related toxicity compared to solvent-based paclitaxel, with an excellent safety profile [137]. Overall, nab-PTX has been used in pancreatic cancer [138, 139], cholangiocarcinoma [140], GC [141, 142], and CRC as a novel strategy with high safety and therapeutic efficacy [143-149]. The application of albumin to the antitumor study of TSN presents a promising prospect with broad potential.

Discussion

The dual nature of TSN, acting as both a promising therapeutic agent and a significant toxicological concern [150-161], presents unique challenges in the field of oncology. A thorough analysis of its therapeutic actions in various cancer types and a comprehensive assessment of its toxicological profile are required. The known side effects of TSN, particularly hepatotoxicity, severely limit its clinical use. Novel approaches to drug design, such as enclosing TSN in PROTACs (Proteolysis Targeting Chimeras) and nano-delivery systems [162], represent a tactical step toward reducing its toxicity and enhancing its anticancer effectiveness by maximizing targeted delivery while minimizing systemic toxicity, potentially paving new directions in cancer treatment. Future research could focus on refining TSN's delivery methods, potentially through structural modifications or advanced targeting strategies, to mitigate its adverse effects while maintaining its therapeutic efficacy. Despite the significant potential for cancer treatment, a careful evaluation of its possible adverse effects is necessary before clinical implementation. A comprehensive understanding of the molecular interactions and pathways of TSN will be crucial for developing safer and more effective cancer therapies. To date, T cell-based immunotherapies have demonstrated remarkable efficacy in a subset of human cancers [163], but their effectiveness has been hindered by intratumoral immunosuppression. Notably, metabolic reprogramming of cancer is considered a novel marker of tumorigenesis and antitumor immune response. Alterations in cancer cell metabolites can significantly influence the recognition and presentation of antigens by the immune system. Therefore, insight into the unique metabolic patterns of immune cells enables us to enhance the immune system's ability to monitor and suppress tumor progression. As TSN reverses macrophage-mediated tumor immunosuppression, enhances T cell infiltration and activation, and reduces exhaustion, the effect of TSN on the metabolic reprogramming of immune cells warrants further investigation.

Supplementary materials

Supporting materials of this paper can be requested by sending E-mail to the corresponding authors.

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