Novel use of clinical drugs: Deubiquitinase inhibitor auranofin and disulfiram show synergistic anti-tumor effects in vitro and in vivo

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The deubiquitinases (DUBs) are emerging targets for cancer therapy. An increasing number of DUB inhibitors were discovered to be potential anti-tumor agents. We recently identified that auranofin (Aur), a gold-containing compound used clinically to treat rheumatoid arthritis, is an inhibitor of proteasome-associated DUBs. Besides its inherent anti-arthritis effect, Aur has been shown to exhibit a predominant anti-tumor property in various cancer phenotypes. Hence, we were prompted to enhance the anti-cancer capability of this promising drug. Disulfiram (DSF) is currently being used clinically for the treatment of alcoholism. Recent studies suggested that DSF could potentiate the effect of some other chemotherapeutic agents. In a recent study, we unraveled that Aur and DSF in combination potently induced apoptosis of hepatoma cells both in vitro and in vivo, and the synergistic cytotoxicity is associated with endoplasmic reticulum (ER) stress, loss of MMP, and caspase activation. Hence, we have identified a synergism model between two clinical drugs DUB inhibitor Aur and DSF in the induction of apoptosis as a potentially novel anticancer strategy for clinical use in the future.

Keywords: auranofin; deubiquitinase inhibitor; disulfiram; anticancer strategy


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Cancer cells depend on the ubiquitin proteasome system (UPS) more than normal cells for their robust protein synthesis and degradation. Numerous studies have shown that proteasome inhibition exhibits favorable anti-tumor effects in varying degrees. The discovery and application of the 20S proteasome peptidase inhibitor, bortezomib, brought a new hope for the treatment of multiple myeloma. However, the administration of bortezomib is limited by its narrow effectiveness and drug resistance, which prompts us to...
extend our attention from 20S proteasome peptidases to 19S proteasome-associated deubiquitinases (DUBs).

DUBs play a pivotal role in regulating the activity, location and degradation of proteins in eucaryotic cells [4-6] and thereby are involved in regulating multiple cellular processes, including cell cycle control [7, 8], DNA damage response [9-11], histone modification [12] and various cellular signaling pathways [13]. The principal function of DUBs is to remove mono-ubiquitin (Ub) or poly-ubiquitin chain from target proteins. It is estimated that the human genome encodes approximately 100 putative DUBs, which are subdivided into six families according to their sequence and structural difference [14-16]. Five families belong to cysteine proteases; they are ubiquitin C-terminal hydrolase (UCH), ubiquitin specific protease (USP), ovarian tumor domain protease (OTU), Josephin domain protease (MJD), and monocyte chemotactic protein-induced protein family (MCPIP). The other family of DUBs belongs to the JAB1/MPN/Mov34 metalloenzyme (JAMM) domain family of Zn$^{2+}$-dependent metalloproteases. Among these DUBs, POH1, UCHL5 and USP14 are associated with the 19S proteasome, which are often overexpressed in several carcinoma cells and supposed to be novel therapeutic targets [17-20].

Recently, an increasing number of DUB inhibitors were discovered and sought to be potential anticancer compounds. However, most of the DUB inhibitors stay in the experimental phase, and meanwhile, their real clinical anti-tumor effects and toxic side effects remain unknown. Therefore, we screened a series of clinically used agents that might inhibit 19S proteasome-associated DUBs and exhibit antitumor effects with little side toxicity, hoping that this type of compounds would be easier to be converted and applied in the clinic. Auranofin (Aur) is a gold (I)-containing compound, clinically used to treat rheumatic arthritis for more than 30 years. Recent studies have reported that Aur has potent antitumor effects beyond its inherent anti-inflammatory activity, and it has been approved by FDA for Phase II clinical trial in cancer therapy. To date, several acceptable insights into the anti-cancer effects of Aur were proposed, including inhibition of thioredoxin reductase (TrxR), reactive oxygen species (ROS) over generation [21, 22], endoplasmic reticulum (ER) stress response [23], and caspase activation [21-23]. However, we have recently discovered that Aur is an inhibitor of 19S proteasome-associated DUBs (UCHL5 and USP14), and Aur induced apoptosis is associated with accumulation of ubiquitinated proteins (Ub-prs), ER stress, loss of MMP, and caspase activation. In addition, our previous data demonstrate that Aur can stimulate cellular ROS generation but this is not required for Aur to induce apoptosis [24-26].

Given the great promise that Aur had shown, we further searched for other agents to potentiate the antitumor activity of Aur and lower its potential toxic side effects on normal cells. And one of them, disulfiram (DSF), which is currently being used clinically for the treatment of alcoholism by irreversibly inhibiting aldehyde dehydrogenase, stood out as the top candidate. It was well demonstrated that DSF possesses an anticancer activity in various cancer cells. DSF as a cooper-binding agent, induces apoptosis in breast cancer via proteasome inhibition [27]. In addition, DSF complexed with copper, induced ROS-dependent apoptosis of prostate cancer cells [28]. More importantly, DSF and its metabolites can be used as chemosensitizer of some anti-cancer agents [29]. Comparing to other chemosensitizers, as shown in our report, DSF itself does not cause cytotoxicity and apoptosis in normal cells at a definite dose, while it dramatically exacerbated Aur-induced apoptosis in human hepatoma cancer cells or xenograft models.

In the study highlighted here, we have shown that the combination of Aur and DSF synergistically enhanced cytotoxic effect and cell apoptosis in both cultured hepatoma cancer cells and xenograft models. Our data also support the notion that the synergistic antitumor effects of Aur and DSF in combination are through enhancement of DUB inhibition, induction of ER stress, suppression of MMP, and caspase activation. By using a CUSP9 or modified CUSP9 treatment protocol, a strong clinical effects of disulfiram plus auranofin in glioblastoma has been reported [30, 31, 32]. Our studies have provided compelling in vitro and in vivo evidence for a practical tactics to treat hepatoma cancers by combining two clinically-in-use drugs (a DUB inhibitor Aur and an aldehyde dehydrogenase inhibitor DSF). Its clinical efficacy is highly anticipated.

Conflicting interests
The authors have declared that no conflict of interests exist.

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Author contributions

H.H. W.X. and L.J. wrote the manuscript; L.Y., L.N. and C.J. analysed the data.

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