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Short communication

Shenqi Fuzheng injection alleviates chemotherapy-induced cachexia by restoring glucocorticoid signaling in hypothalamus

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Chemotherapy-induced cachexia (CIC) is a debilitating condition characterized by weight loss, muscle atrophy, and anorexia [1]. While peripheral mechanisms of cachexia have been extensively studied, the involvement of the central nervous system (CNS) in CIC is often overlooked. Chemotherapeutic drugs cause stress responses and inflammation, which may impact the hypothalamus and disrupt systemic energy and neuroendocrine functions. Understanding hypothalamic roles in regulating these processes can provide insights into CIC's mechanisms and aid in developing novel therapies. Shenqi Fuzheng injection (SQ), a Chinese proprietary medicine, has shown therapeutic effects in alleviating chemotherapy-related cytotoxicity and neurological symptoms [2]. SQ's chemical composition was investigated in-depth in our previous study (Fig. S1). However, its effects and mechanisms in alleviating CIC, particularly through hypothalamic modulation, are not fully understood. This study demonstrated SQ's protective effects on hypothalamic dysregulation and its potential in improving CIC-associated muscle atrophy through hypothalamic glucocorticoid receptor signaling pathway.

Here, we established a CIC mouse model based on CapeOX regimen, a first-line treatment consisting of oxaliplatin and capecitabine for colorectal cancer [3], to evaluate SQ's effects on CIC with indomethacin as the positive control. The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China (Approval No.: 2021-04-WWY-05). Animals were handled with care under the committee's guidelines. All materials and methods used in this study are shown in the Supplementary data. We found that SQ treatment improved weight loss, reduced food intake, and decreased muscle and organ weights in the CIC model (Figs. S2A–D and Tables S1 and S2). SQ attenuated muscle fiber atrophy in the CIC (Figs. S2E and F). Furthermore, our data revealed that SQ treatment effectively reduced the elevated levels of pro-inflammatory cytokine interleukin 6 (IL-6) (Fig. S2G) and glucocorticoids (Fig. 1A and S3) in the CIC model. These

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findings indicate that SQ exerts protective effects in CIC by suppressing abnormal glucocorticoid levels. Considering glucocorticoids expression is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for a cascade of hormone signals that begins in the brain, our data lead us to investigate SQ's role in modulating hypothalamic functions.

To investigate our hypothesis, we performed immunofluorescence staining in the CIC model, and the results revealed an increase in the number and fluorescence intensity of c-Fos-positive neurons (Fig. 1B), indicating neuronal activation in the hypothalamus. SQ treatment significantly reduced these indicators, suggesting its inhibitory effect on hypothalamic neuronal activation. Dysregulated expression of appetite-regulating neuropeptide genes, including *Npy*, *Agrp*, and *Pomc*, was detected in the CIC group, and SQ intervention partially restored the dysregulated expression of these neuropeptides (Fig. S4A and Table S3). Liquid chromatography-mass spectrometry (LC-MS) analysis showed significant changes in neurotransmitter levels in the hypothalamus of the model group, with increased L-aspartate, L-glutamate, and L-glycine levels, and decreased levels of gamma aminobutyric acid (GABA), acetylcholine, and serotonin [4], and SQ intervention effectively restored aberrant changes in neurotransmitter levels (Figs. 1C and S5). Additionally, synapse loss, indicated by decreases in synaptophysin- and postsynaptic density protein-95 (PSD-95)-positive fluorescent puncta, was observed in hypothalamus of the model group, while partial recovery was observed in the IND and SQ groups (Figs. 1D and S4B). These findings demonstrate that SQ treatment can effectively prevent the hypothalamic abnormal neuronal activation, neurotransmitter dysregulation, and synapse loss in CIC.

Hypothalamic inflammation, characterized by glial activation, disrupts local neuronal function. To investigate whether hypothalamic immune activation occurs in CIC and SQ's effects, we performed immunofluorescence staining of microglia and astrocytes. While no significant changes were observed in astrocyte activation (Fig. S4C), we found an increase in the number of activated microglia with morphological changes in the hypothalamic nuclei (Figs. 1E and S4D). Treatment with SQ inhibited microglial

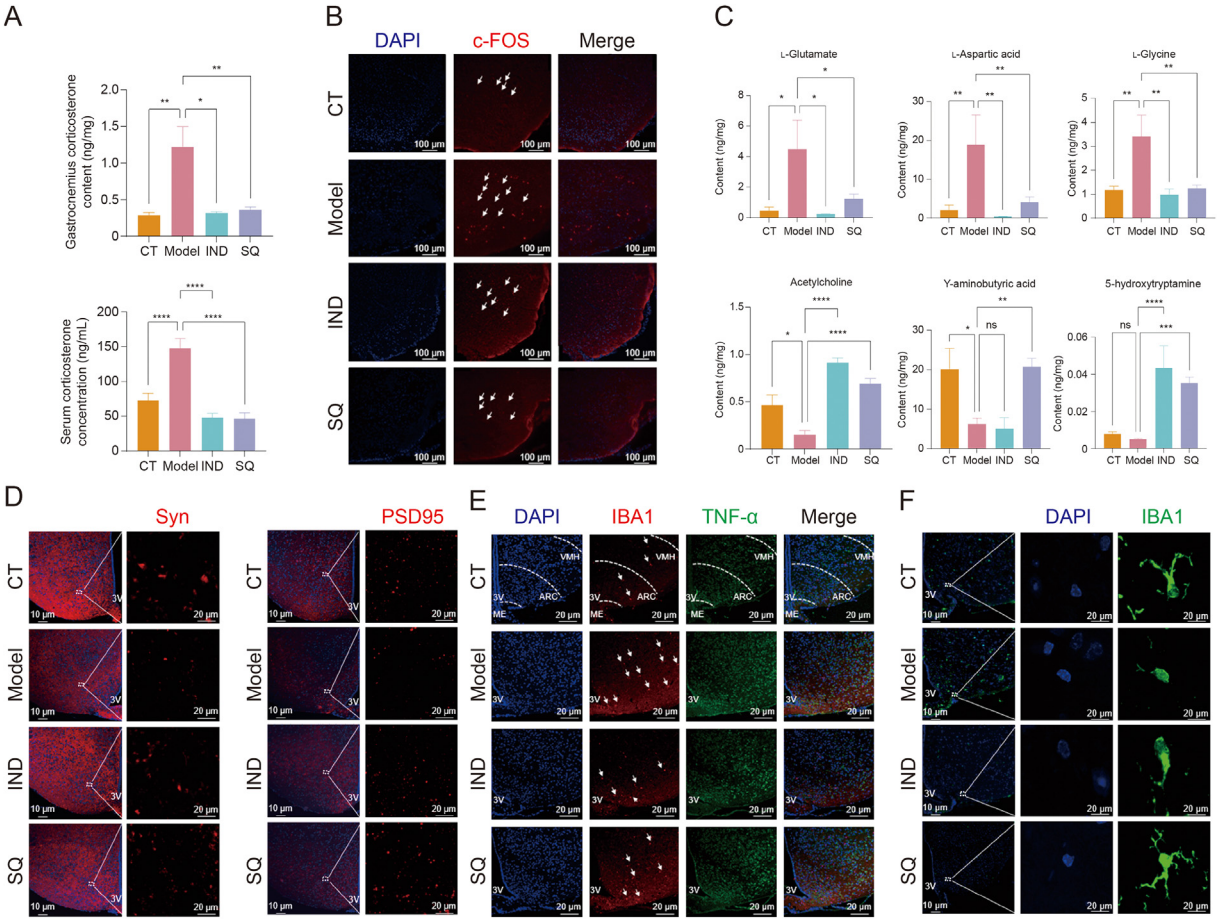

Fig. 1. Pharmacological effects of Shenqi Fuzheng injection (SQ) on chemotherapy-induced cachexia (CIC) at both peripheral and central levels. (A) Gastrocnemius corticosterone level in gastrocnemius and serum was determined using gas chromatography-mass spectrometry (GC-MS) ($n = 7–10$). (B) Representative images of immunofluorescence staining of c-Fos in the hypothalamus. White arrows indicate c-Fos-positive neurons. (C) The contents of L-glutamate, L-aspartate, L-glycine, acetylcholine, γ-aminobutyric acid, and 5-hydroxytryptamine in the hypothalamus of each group of mice were measured using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) ($n = 6$/group). (D) Representative images of immunofluorescent puncta of synaptophysin (red, left) and postsynaptic density protein-95 (red, right) in the hypothalamus. (E) Representative images of immunofluorescence staining of microglia with ionized calcium-binding adaptor molecule 1 (IBA1) (red) and tumor necrosis factor alpha (TNF-α) (green) in the hypothalamus. (F) Representative immunofluorescent images of hypothalamic IBA1-positive microglia at high magnification to demonstrate their morphological differences among various treatment groups. The data were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$, compared with the model group; ns: no significance. CT: control; IND: indomethacin; DAPI: 4′,6-diamidino-2-phenylindole; VMH: ventromedial hypothalamus; ARC: arcuate nucleus; ME: median eminence.

activation (Figs. 1F and S4E) and reduced the expression of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) (Figs. 1E and S4F). These findings indicate that SQ alleviates CNS inflammation by inhibiting microglial activation and reducing TNF-α level.

To further investigate SQ's molecular mechanisms on the CIC model at the central level, RNA-sequencing analysis of the hypothalamus revealed significant changes in gene expression in the CIC model compared to the control group, and SQ treatment led to distinct gene expression patterns compared to the model group (Fig. S6A). We identified 946 common differentially expressed genes (DEGs) between the two datasets (Fig. S6B). Gene ontology (GO) analysis highlighted SQ's involvement in regulating neurotransmitter receptor activity and synaptic membrane (Fig. 2A and Table S4). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated SQ's influence on neurotransmitter-related pathways (Figs. S6C and D and Table S5). Further analysis of the DEGs identified key genes involved in neurotransmitter and neuropeptide signaling. The model group exhibited upregulation of neuropeptide genes and immune response-related genes, which were restored after SQ treatment (Figs. S6E and F). These findings provide molecular evidence of dysregulated gene expression in the hypothalamus in CIC and SQ's protective effects at the CNS level.

SQ demonstrated a significant inhibitory effect on peripheral corticosterone elevation (Fig. 1A). Therefore, to investigate SQ's central effects of glucocorticoid signaling on CIC, we retrieved the altered glucocorticoid-responsive genes, such as *Fkbp5*, *Nr3c1*, and *Crh*. SQ treatment mitigated dysregulated gene expressions in the hypothalamus of CIC (Figs. 2B and S7A and Table S3), suggesting its regulatory role in the central glucocorticoid signaling pathway.

In the CIC model, the effects of glucocorticoids and

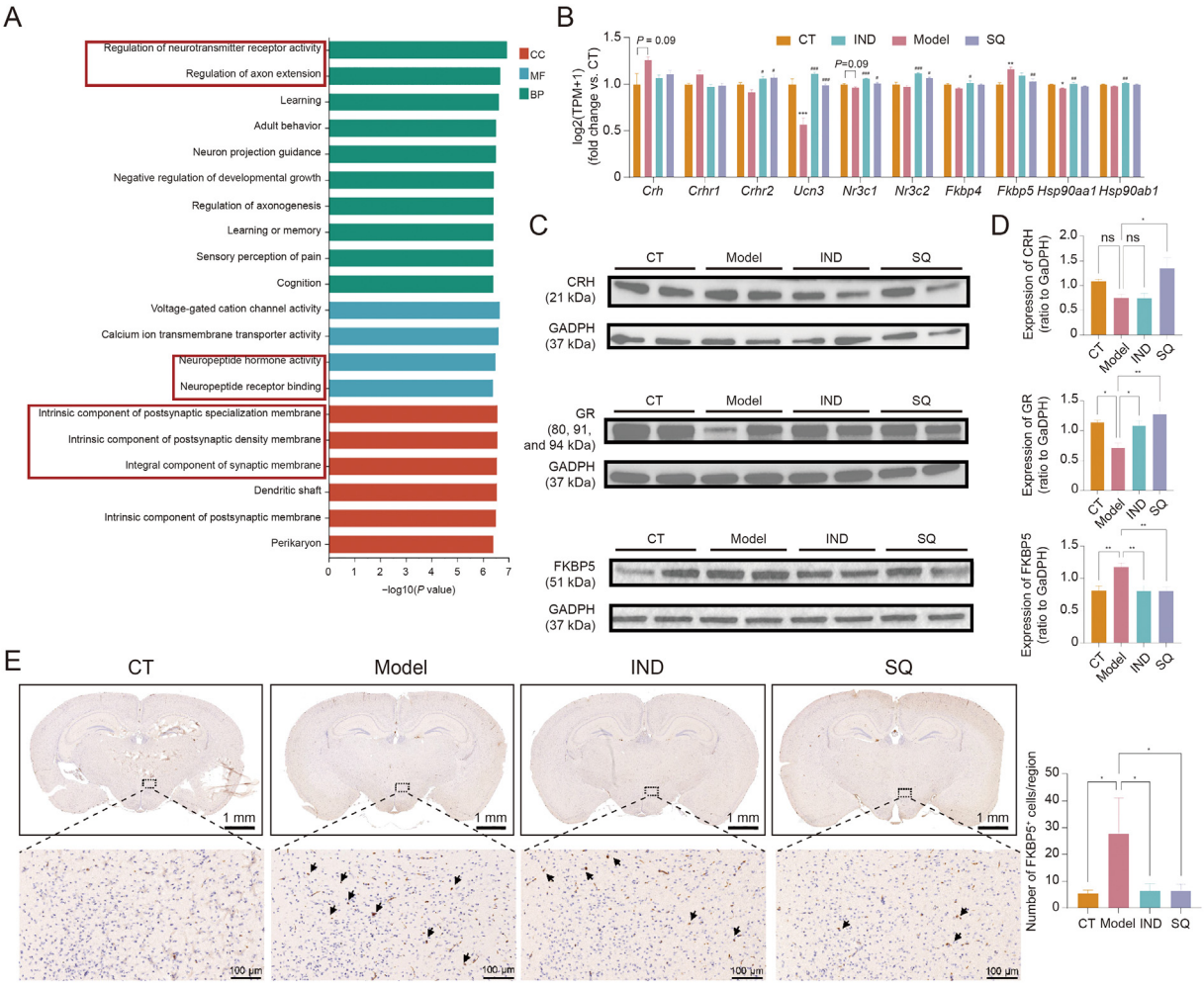

Fig. 2. Underlying molecular mechanisms of Shenqi Fuzheng injection (SQ) on chemotherapy-induced cachexia (CIC) through hypothalamic glucocorticoid signaling pathway revealed by transcriptomic and molecular analyses. (A) Gene Ontology (GO) enrichment analysis of the 946 common differentially expressed genes (DEGs) in three main categories (biological process (BP), cellular component (CC), and molecular function (MF)) with $P < 0.05$. The *x*-axis indicates the number of genes in each category. Top GO terms revealed that SQ might exert its effects on the central nervous system by influencing "regulating neurotransmitter receptor activity", "neuropeptide hormone activity", and "integral component of synaptic membrane". (B) Gene expression of glucocorticoid (GC)-responsive genes was retrieved and analyzed in the RNA-sequencing data ($n = 3$/group). (C) Representative Western blot images of different proteins in the hypothalamus (corticotropin-releasing hormone (CRH), glucocorticoid receptor (GR), and FKBP prolyl isomerase 5 (FKBP5)). (D) Relative density analysis of proteins in Fig. 2C with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the loading control ($n = 4$/group). (E) Representative images and quantification of immunohistochemical staining of FKBP5 positive neurons in the paraventricular (PVN) of the hypothalamus ($n = 3$/group). The data were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA). $^{*}P < 0.05$, $^{*}P < 0.01$, and $^{***}P < 0.001$, compared with the control (CT) group; $^{\#}P < 0.01$, $^{\#\#}P < 0.001$, and $^{\#\#\#}P < 0.0001$, compared with the model group; IND: indomethacin; TPM: transcripts per million.

glucocorticoid receptor (GR) signaling on muscle atrophy are well-established [5]. However, their regulatory effects at the central level have not been fully investigated. To explore the mechanisms by which SQ regulates hypothalamic glucocorticoid signaling pathway in the CIC model, key proteins involved in GR sensitivity were examined. Interestingly, no significant changes in *Crh*, *Nr3c1*, and *Fkbp5* genes were detected at the transcriptional level (Figs. S7B–D). However, immunoblotting analysis revealed decreased GR levels and increased FKBP prolyl isomerase 5 (FKBP5) levels in hypothalamus of the CIC group (Figs. 2C and D). Following SQ treatment, GR levels were upregulated, and the elevation of FKBP5 was inhibited (Figs. 2C and D). Immunohistochemistry in paraventricular nuclei (PVN), a key brain region in regulating the HPA axis, showed an increase in FKBP5-positive cells in the CIC group, which was suppressed by SQ treatment (Fig. 2E). These findings suggest that SQ treatment restores GR protein levels and inhibits FKBP5 elevation, thereby restoring the sensitivity of hypothalamic glucocorticoid signaling.

This study integrated various analytical techniques to investigate SQ's novel function in modulating hypothalamic glucocorticoid signaling pathway, which thereby maintained neuronal excitability and resident immune responses to alleviate hypothalamic dysfunctions, and ultimately attenuating muscle atrophy in CIC. Furthermore, SQ regulated GR and FKBP5 levels in PVN, which may improve GR nuclear translocation and transcriptional activity to restore negative feedbacks of HPA axis to glucocorticoid, breaking the cycle between sustained elevated glucocorticoid and muscle atrophy. In summary, our findings advance mainstream studies on CIC from peripheral organs to the CNS, provide insights into SQ's modulatory roles in the CNS, and offer a new perspective for the treatment and drug development for CIC.

While our study highlights SQ's effectiveness in CIC, further research is needed to elucidate SQ's active constituents within the mixture and its specific targeted receptors in hypothalamic cell subpopulations to strengthen SQ's clinical application as a CIC therapeutic.

CRediT author statement

Entezar Berik: Conceptualization, Investigation, Formal analysis, Methodology, Writing - Original draft preparation; **Zijia Zhang:** Conceptualization, Investigation, Data curation, Methodology, Writing - Original draft preparation, Reviewing and Editing; **Wei Li:** Methodology, Investigation, Formal analysis, Validation, Software, Visualization; **Yawen Liu:** Formal analysis, Investigation, Resources; **Sihan Chen:** Formal analysis, Investigation, Visualization; **Wenyong Wu:** Formal analysis, Software; **Zhaojun Wang** and **Xinqin Kong:** Validation, Resources; **Wenqiang Pei:** Formal analysis, Validation; **Huiqing Dong:** Investigation, Visualization; **Huali Long:** Project administration, Resources; **Min Lei:** Supervision; **Jennifer Yiyang Wang:** Project administration, Validation; **Liangfeng Liu:** Resources; **Jinjun Hou:** Supervision, Data curation; **Jing Feng:** Conceptualization; Supervision; Writing - Reviewing and Editing; **Zhaoxia Li:** Project administration, Supervision; **Wanying Wu:** Funding acquisition, Project administration, Resources, Supervision; **De-an Guo:** Funding acquisition, Project administration.

Declaration of competing interests

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.08.015.

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