

1 This study aimed to clarify the anticancer mechanism of JC724 through network pharmacology analysis, and reveal the meaning of heat toxins in heat-clearing and detoxifying therapy. Machine learning, IAs, and networks are valid and important tools given our big data era, including in biological sciences. This study engages these tools in a complex disease. The manuscript is overall well written. A minor editing is required before publication. Thank you.

Thank you very much for your suggestions. We have edited the article to make it better. Thank you again for your approval.

2 The authors clarified the components of JC724 and investigated the mechanism of the anticancer effects of JC724 on HCT116. After a minor revision, it can be accepted for publication. Minor comments: 1. Please explain why the author chose HCT116 in this research. Many colorectal cancer cell lines have been used in basic research, and each cell line has different characteristics. 2. It is unclear whether the anticancer effect of JC724 on the NRF2 and IL-17 is specific for colorectal cancer, because the authors investigated the mechanism using only HCT116. Please discuss about this.

Thank you for your suggestions. Your questions are very professional and we will answer them one by one as followed.

1. **HCT116 cell is a poorly differentiated adenocarcinoma cell line established by Brattain et al. in 1979 from the cancer tissue of a male colon cancer patient. It is widely used in the research of biological characteristics of malignant tumor cells, mechanism of anti-tumor drug, anti-cancer drug screening and other fields [1-2]. In addition, many studies on the anti-colorectal cancer effect of traditional Chinese medicine have found that HCT116 cell has better effects than other cell lines [3-8]. It is an excellent experimental model in vitro, which has significant effects in terms of cytotoxicity assay, proliferation experiment, cell apoptosis assay, cell invasion and migration experiment, signaling pathway validation, etc. And we found that HCT116 cells were more stable and effective in the anti-cancer effect study in the previous study of JC724 drug [9]. Therefore, we chose HCT116 in this research. Thank you for your advice, we supplemented this explanation in the 2.3 part of the article.**
2. **The CRC-related targets used in this mechanism research were not only from the transcriptome of HCT116 cells, but also integrated with four authoritative databases (Drugbank, TTD, DisGeNET, and GeneCards), so the targets covered a wide range of CRC and can more comprehensively explain the anti-cancer mechanism. Through Venn diagram to screen the JC724-CRC intersection targets, we found that JC724 can act on core genes such as NRF2 both from the transcriptomics of HCT116 cells and the CRC databases. Therefore, the mechanism research was not only using HCT116, but also included the application of CRC databases.**

In addition, JC724 was created by adding Baihuasheshecao (*Hedyotis diffusa*) and Banzhilian (*Scutellaria barbata*) to Huangqin decoction. *Hedyotis diffusa* and *Scutellaria barbata* are commonly used anticancer drugs in TCM clinic. Huangqin decoction is mainly used for the treatment of gastrointestinal diseases in clinic, such as ulcerative

colitis and colorectal cancer, and there is no report on the treatment of other cancers. Therefore, we believe that JC724 mainly treats colorectal cancer and has its efficacy in our previous clinical studies. Recently, many studies showed the anti-colorectal cancer mechanism of these Chinese herbal ingredients on the NRF2 and IL-17 pathways [10-13], which proved the anti-colorectal cancer effect of JC724 on the NRF2 and IL-17. However, some studies have proved that *hedyotis diffusa* and *scutellaria barbata* can treat other diseases and cancers through NRF2 and IL-17 pathways[14-16]. Moreover, the active ingredients of traditional Chinese medicine are complex. The active ingredients in JC724 may also have therapeutic effects on other cancers through NRF2 and IL-17. Therefore, I speculated that JC724 has no specific anticancer effect on colorectal cancer. Thank you for your advice, we added the explanation in the conclusion part.

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