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Metabolomic Profiling Reveals the Mechanisms Underlying the Nephrotoxicity of Methotrexate in Children with Acute Lymphoblastic Leukemia

Yu Cheng[1], Yanan Chen[2], Mingming Zhao[1], Minglu Wang[1], Maobai Liu[3], and Limei Zhao[1]

[1]Shengjing Hospital of China Medical University
[2]Jiangsu Cancer Hospital
[3]Fujian Medical University Union Hospital

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Abstract

Background: Methotrexate is widely recommended as a first-line treatment for the intensive systemic and consolidation phases of childhood acute lymphoblastic leukemia. However, methotrexate-induced nephrotoxicity is a severe adverse reaction, of which the mechanism remains unclear. **Methods:** An untargeted metabolomics analysis of serum from childhood acute lymphoblastic leukemia with delayed methotrexate excretion with or without acute kidney injury was performed to identify altered metabolites and metabolic pathways. An independent external validation cohort and in *vitro* assays further confirmed the candidate metabolites and the mechanisms underlying the nephrotoxicity of methotrexate. **Results:** Four metabolites showed significant differences between normal excretion and delayed excretion, seven metabolites reflected the differences between groups with or without acute kidney injury, and six pathways were finally enriched. In particular, oxidized glutathione is confirmed as an candidate metabolite involved in the toxicity of methotrexate. Based on the depletion of glutathione mediated cell death, it was found that methotrexate overload significantly reduced cell viability, triggered reactive oxygen species and intracellular Fe^{2+} accumulation, and altered the expression of ferroptosis-related proteins in HK-2 cells. These methotrexate-induced changes were alleviated or reversed by the administration of a ferroptosis inhibitor, further suggesting that ferroptosis promoted methotrexate-induced cytotoxicity in HK-2 cells. **Conclusions:** Our findings revealed complex metabolomic profiles and provided novel insights into the mechanism by which ferroptosis contributes to the nephrotoxic effects of methotrexate.

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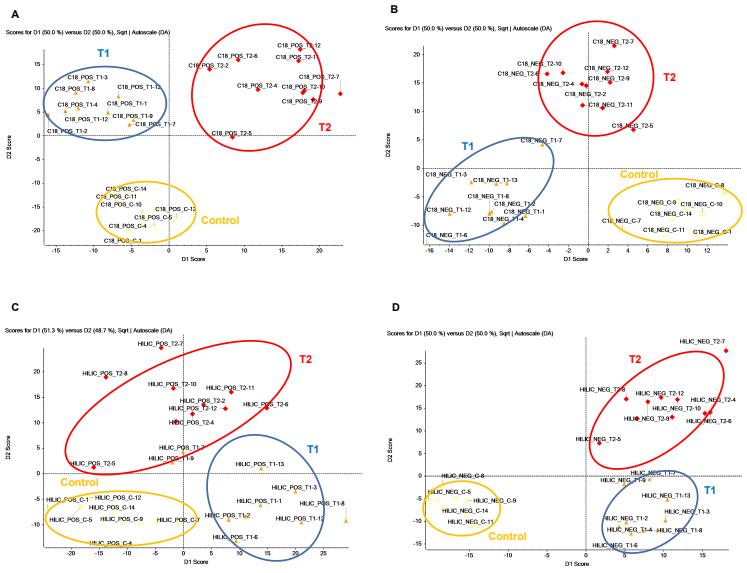

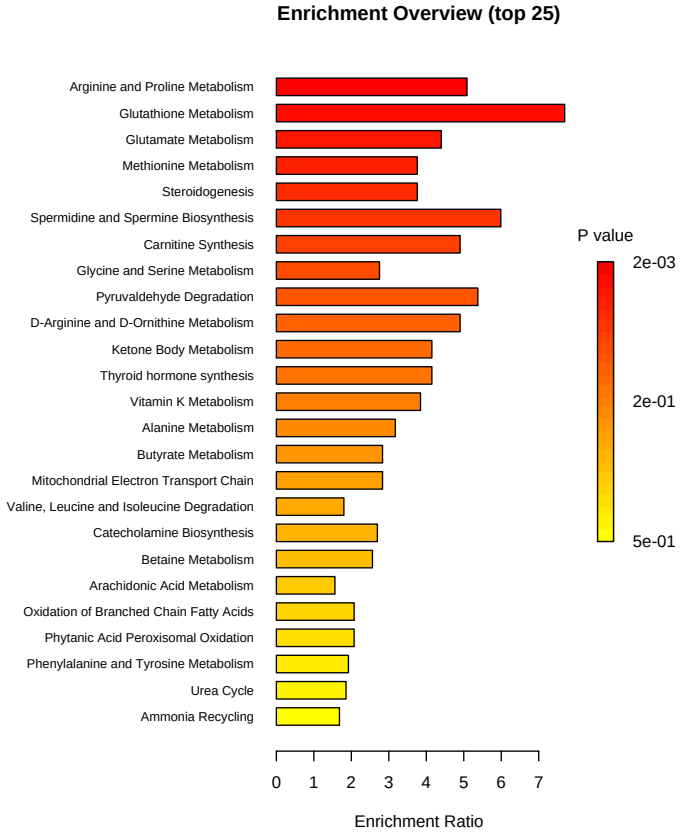

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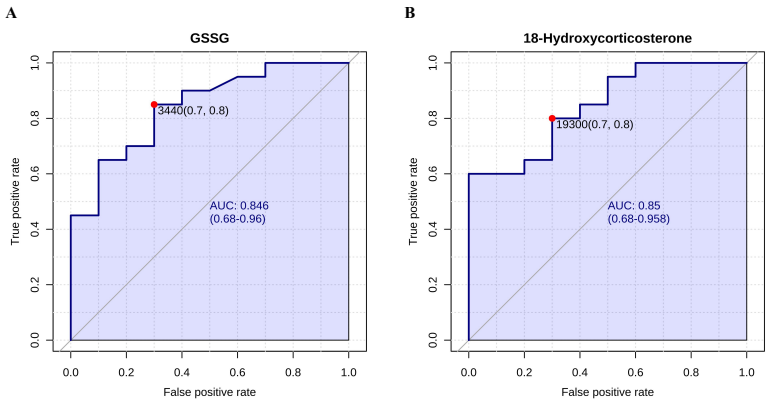

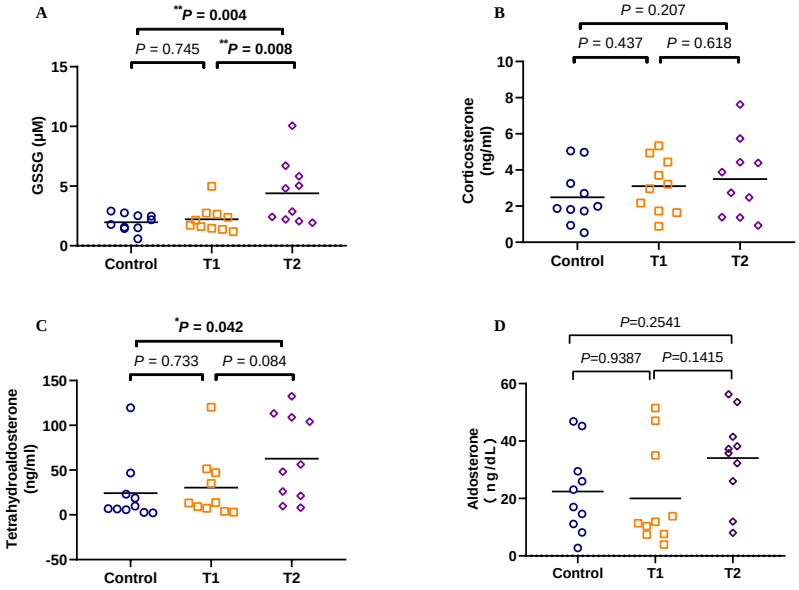

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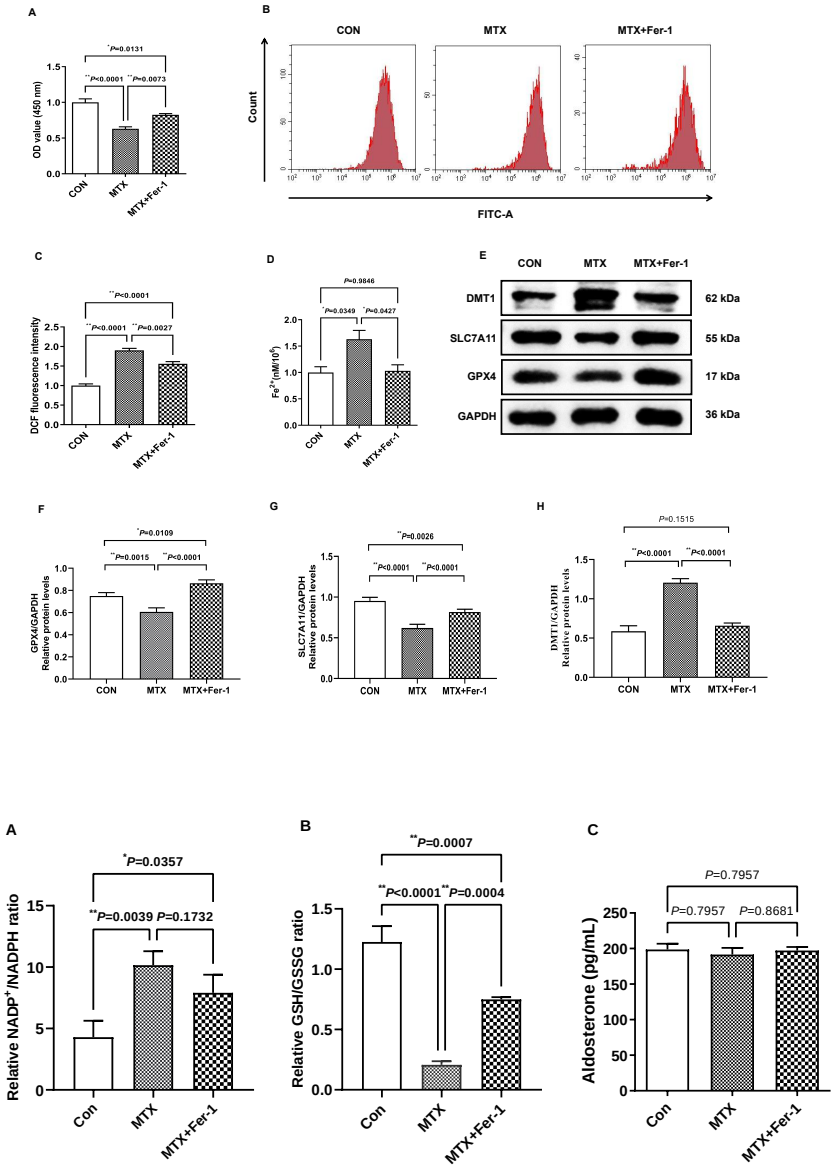

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