Combination of WIN55,212-2 and auranofin synergistically suppresses human hepatocellular carcinoma HepG2 cell proliferation and tumorigenesis and induces cellular senescence and apoptosis via Wnt/ β-catenin signaling

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Abstract

WIN55,212-2(WIN) is a cannabinoid receptor agonist. We previously found that WIN may induce cell cycle arrest and inhibit the proliferation and migration of hepatocellular carcinoma (HCC) BEL7402 cells. Auranofin (AUR) is an FDA-approved drug against rheumatoid arthritis that is currently enrolled in clinical trials as an anti-tumor agent. However, the precise functions of WIN and/or AUR on HCC carcinogenesis remain unexplored. In the present study, we aimed to study the synergistic antitumor effects and the associated underlying mechanisms of AUR/WIN combination therapy on HCC. We showed that WIN/AUR cotreatment synergistically suppressed HepG2 cell proliferation, migration and invasion. Western blot assay found that WIN/AUR cotreatment synergistically downregulated the expression of Bcl-2, cyclin D1, β-catenin, c-myc, and MMP-9 and increased the expression of Bip and ATF4, further activating Caspase3, Caspase 8, Caspase 9 and PARP cleavage. Cotreatment of AUR/WIN also arrested cell cycle at the G1 phase and induced senescence and apoptosis, evidenced by an increase in the number of SA-β-gal $^{+}$ senescent cells and loss of mitochondrial membrane potential (MMP) in AUR/WIN-cotreated cells. Luciferase reporter assay found that the activation of Wnt/β-catenin signaling was significantly suppressed upon WIN/AUR cotreatment in comparison with other groups. Moreover, WIN/AUR cotreatment synergistically inhibited tumor growth in subcutaneous xenograft mice models. In conclusion, our results demonstrated WIN/AUR-mediated apoptosis and suppressive effects on cell growth, migration and invasion were associated with Bcl-2 downregulation, endoplasmic reticulum (ER) stress and caspase activation, leading to downregulation of Wnt/β-catenin signaling. Therefore, WIN/AUR cotreatment may present promising combination therapy for the treatment of HCC.

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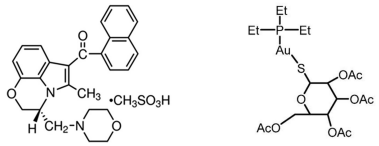
A



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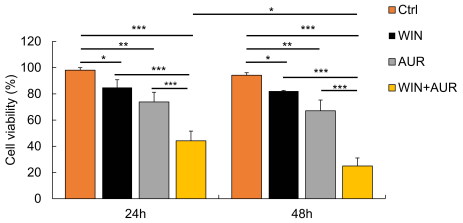
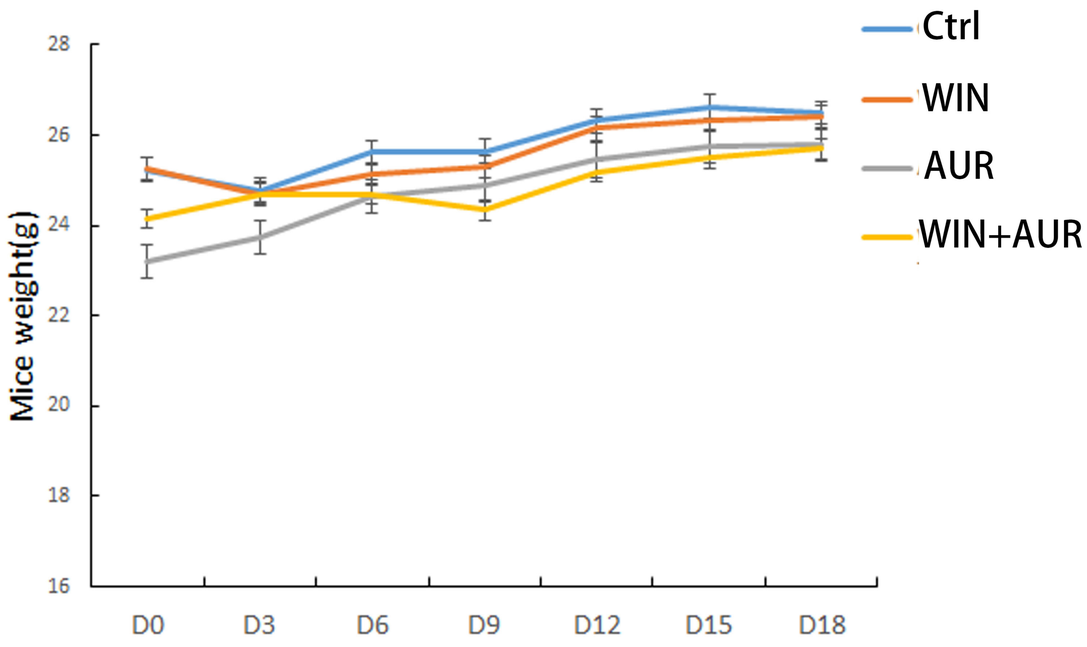
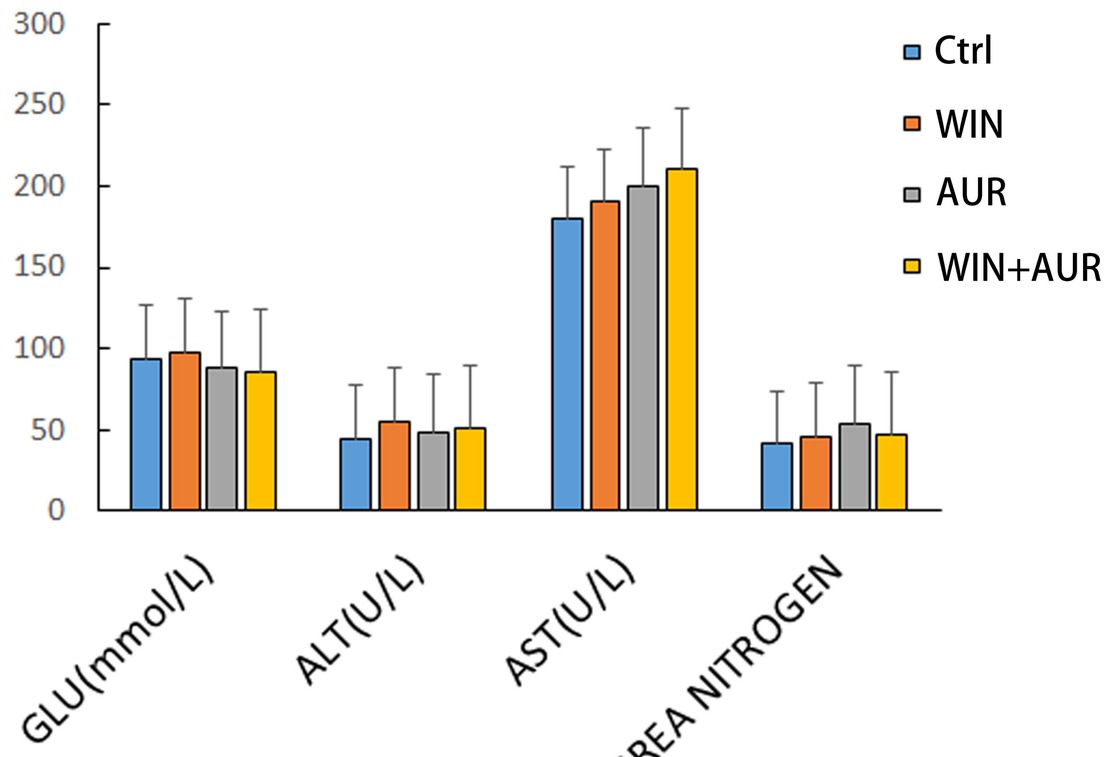


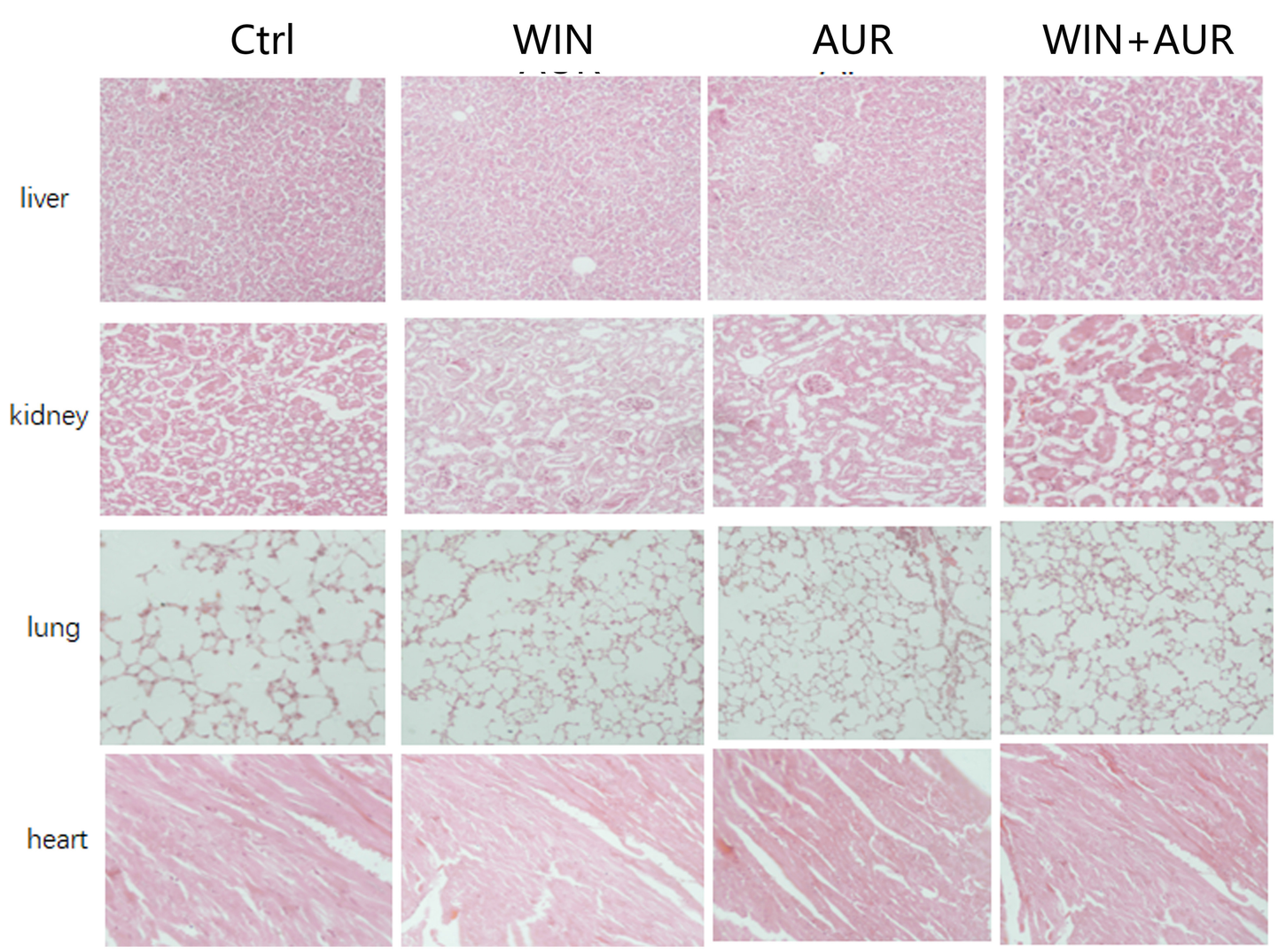
A.


Ctrl
WIN
AUR
WIN+AUR
Mice weight(g)
28
26
24
22
20
18
16
D0
D3
D6
D9
D12
D15
D18

B.


Ctrl
WIN
AUR
WIN+AUR
300
250
200
150
100
50
0
GLU(mmol/L)
ALT(U/L)
AST(U/L)
UREA NITROGEN

C.


Ctrl
WIN
AUR
WIN+AUR
liver
kidney
lung
heart