Oxaliplatin-resistance in colorectal cancer cells is mediated by NF-κB/ABCG2 signaling pathway.

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Abstract

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in both males and females all over the world; however, its incidence is rising in Asian during the past few decades. Oxaliplatin (OXA) , a platimum based agent is used as a chemotherapy to treat CRC patients. it inhibit DNA replication and transcription by forming inter- and intra-strand DNA crosslinks. Nevertheless**,** drug resistance remains a major clinical challenge for cancer treatment. The mechanisms involved in oxaliplatin resistance are still poorly understood. LoVo cells were treated in a gradually increasing concentration of Oxaliplatin to create Oxaliplatin resistant cancer cells. Chemoresistance is one of critical factors that facilitate migration and metastasis, we firstly observed the migration ability is decreased in OXA-R cells compare d to parental cells by western blot and by wound-healing assay.

ATP-binding cassette sub-family G member 2 (ABCG2) is important for mediating cellular resistance by efflux chemotherapeutic drugs from cells. ABCG2 work as a surviving factor in plasma cells under ER stress. We found that the expression of ABCG2 is higher in OXA- R cells than parental LoVo cells by western blot and RT-PCR. Moreover, the expression of ER stress marker were low than parental LoVo cells. OXA-R cells promote pro-survival capability via EGFR/PI3K/Akt/NF-κB signaling pathway and increased cell cycle proteins - cyclin D and cyclin B. NF-κB has been shown to regulate cell survival, migration ,metastasis and chemoresistance in cancer cells. To understand whether ABCG2 can determined chemoresistance in OXA-R cells, we estimated the survival rate by treating them with Verapamil (an ABCG2 inhibitor). Compared to parental LoVo cells, the cell viability, Cell migration and Invasion were decreased in Oxaliplatin Resistant cell. Besides that we found , Lupeol could sensitize Oxaliplatin resistant cell to Oxaliplatin.

Results

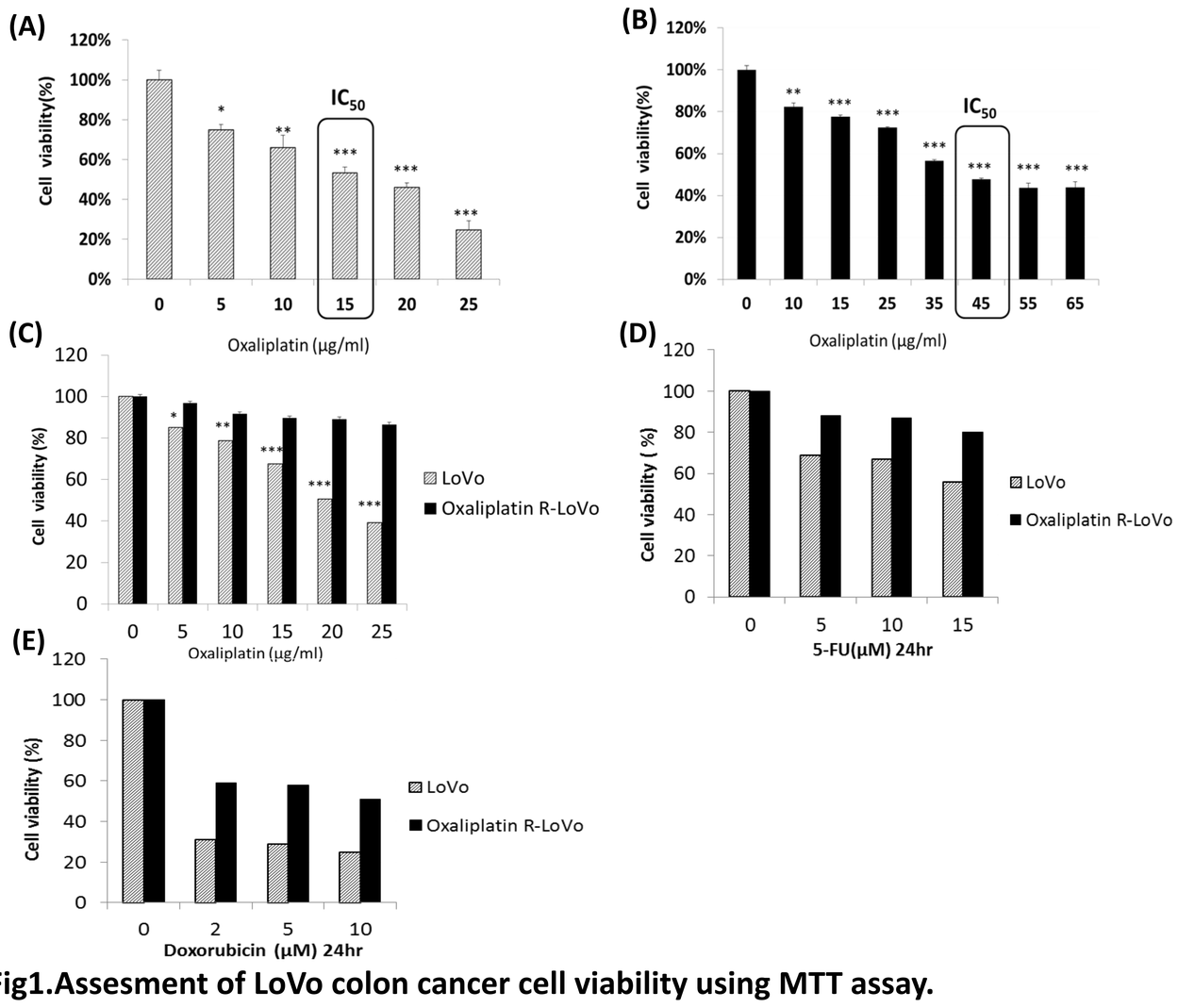

Fig1.Assesment of LoVo colon cancer cell viability using MTT assay.
(A)IC_{50} was determined in Oxaliplatin treated LoVo cancer cells in a dose dependent manner. Cells were challenged with Oxaliplatin IC_{50}, the resistance was increased(B).(C) Both WT and Oxaliplatin –R cells were treated with varying concentration of Oxali for 24 h and then analyzed for cell viability. Treat 5-FU (D)and Doxorubicin (E) in 24 hour for Detection of Multidrug Resistance in Oxaliplatin-R cells. *,p<0.05.,p<0.005.***,p<0.001.**

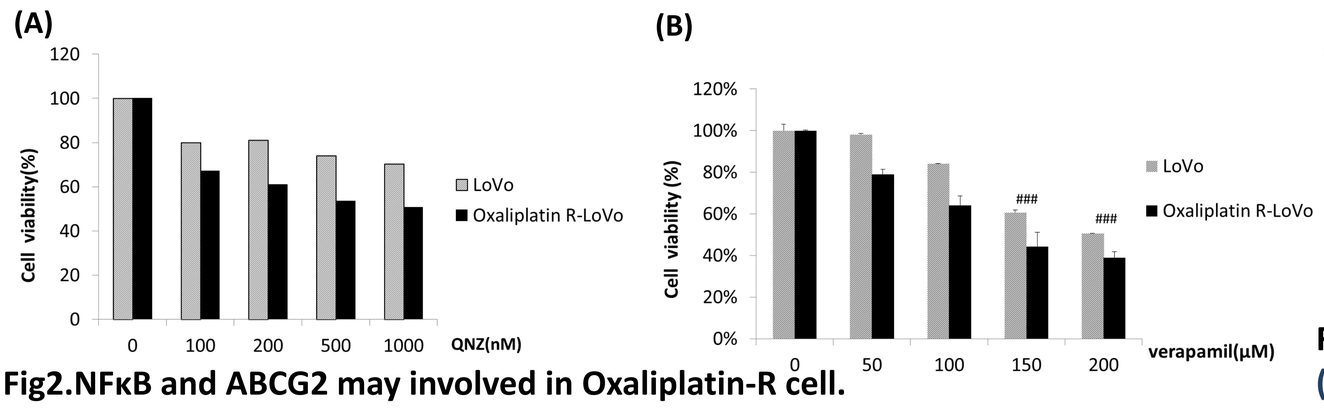

Fig2.NFκB and ABCG2 may involved in Oxaliplatin-R cell.
Both the Wt and Resitant cells were treated with QNZ(A)and Verapamil(B) in dose-dependent manner and then cell viability was analysed by MTT assay.
,p<0.05.**,p<0.005.,p<0.001.**

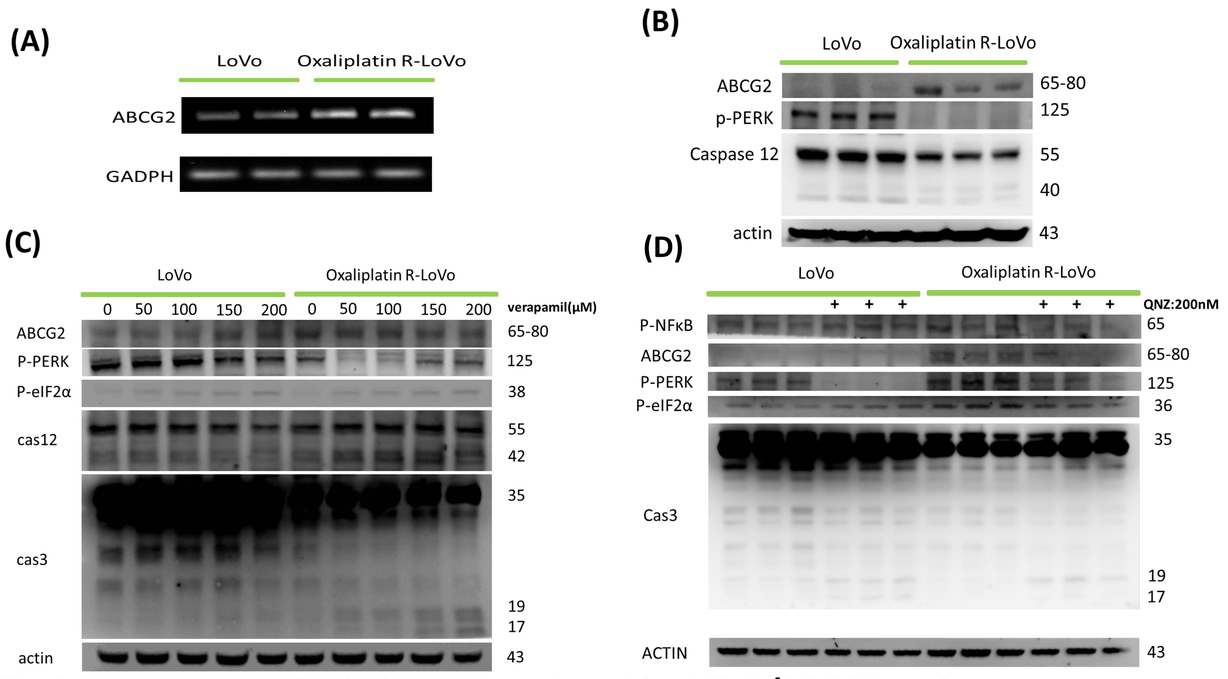

Fig.3.Resistance to Oxaliplatin is mediated by NFκB/ABCG2 pathway.
(A) ABCG2 mRNA expression was analyzed in both in WT and Oxaliplatin Resistant cells (B) Protein level of ABCG2 , p-ERK and Caspase 12 was analysed by western blotting . (C) Cells were treated with various concentration of (C)Verapamil and (D) QNZ and analyzed for ABCG2 , p-NF-kB,p-PERK, peIF2α , capase-12,3 expression.

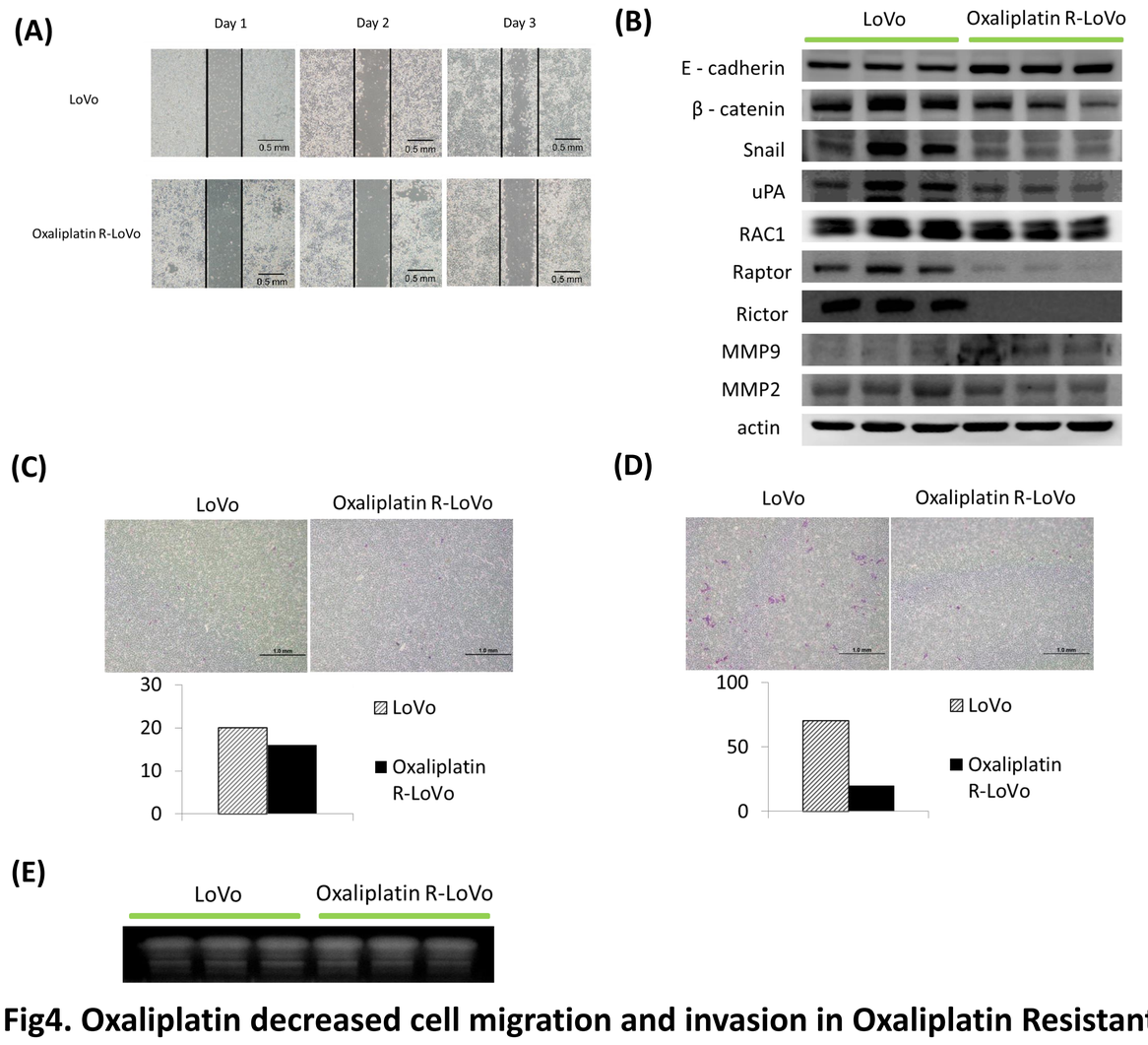

Fig4. Oxaliplatin decreased cell migration and invasion in Oxaliplatin Resistant cell.
(A) Wound healing assay showed that the migration ability was lower in parental cell. (B)Migration markers were decreased in oxaliplatin resistant cells, except for E-cadherin. (C) &(D) Transwell assay showed that invasion ability was decreased in Oxaliplatin resistant cell. (E) MMP 2 and MMP 9 expression was analyzed by Zelatin zymography

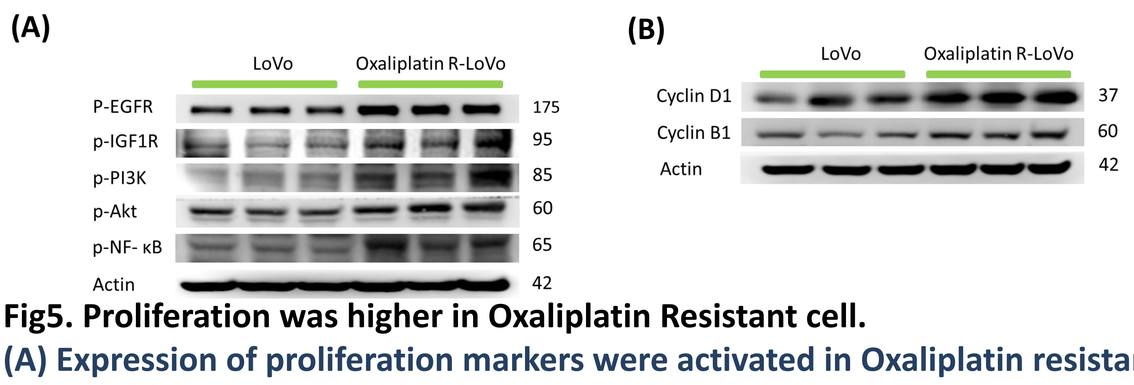

Fig5. Proliferation was higher in Oxaliplatin Resistant cell.
(A) Expression of proliferation markers were activated in Oxaliplatin resistant cells. (B) Oxaliplatin resistance cells showed higher CyclinD1 and Cyclin B1 expression than WT cells. β-actin was used as an loading control.

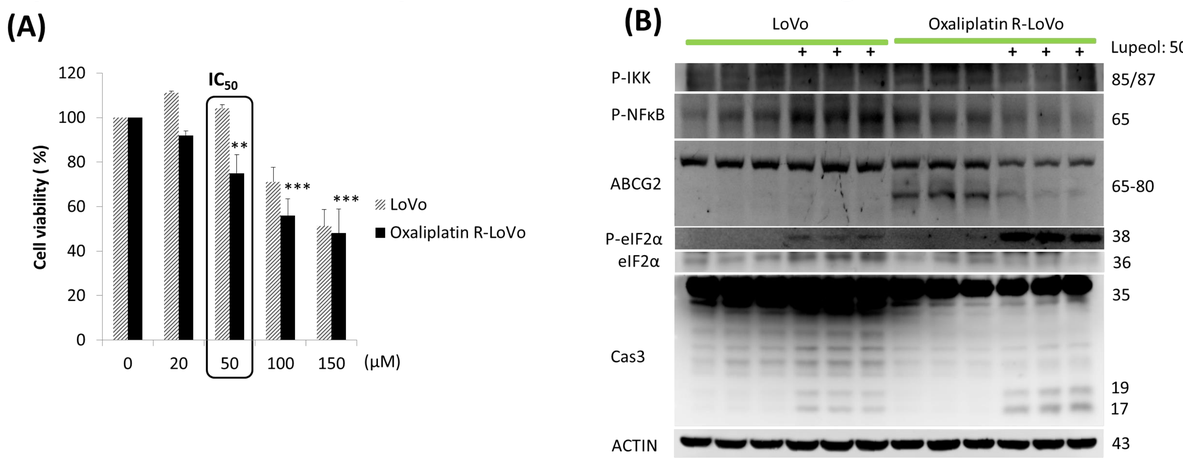

Fig6. Lupeol sensitizes Oxaliplatin resistant cell to chemotherapy through NFκB/ABCG2 signaling pathway.
(A)Cell viability was significantly decreased after treatment of Lupeol in dose-dependent manner in Oxaliplatin Resistant cell.
(B)After treatment of Lupeol, phosphorylation of IKK /NFκB and expression of ABCG2 were decreased, which lead to eIF2α phosphorylation to activate caspase3.

Conclusion

From this study, it was found that Oxaliplatin resistance in LoVo colon cancer cells is mediated by elevation of ABCG2/NF-κB signaling pathway. However, treatment with Lupeol sensitizes Oxaliplatin resistant cells.