



Fuzheng YiLiu Decoction Suppresses Colorectal Cancer Growth by Inhibiting IGF2/IGF1R/PI3K/AKT Signaling

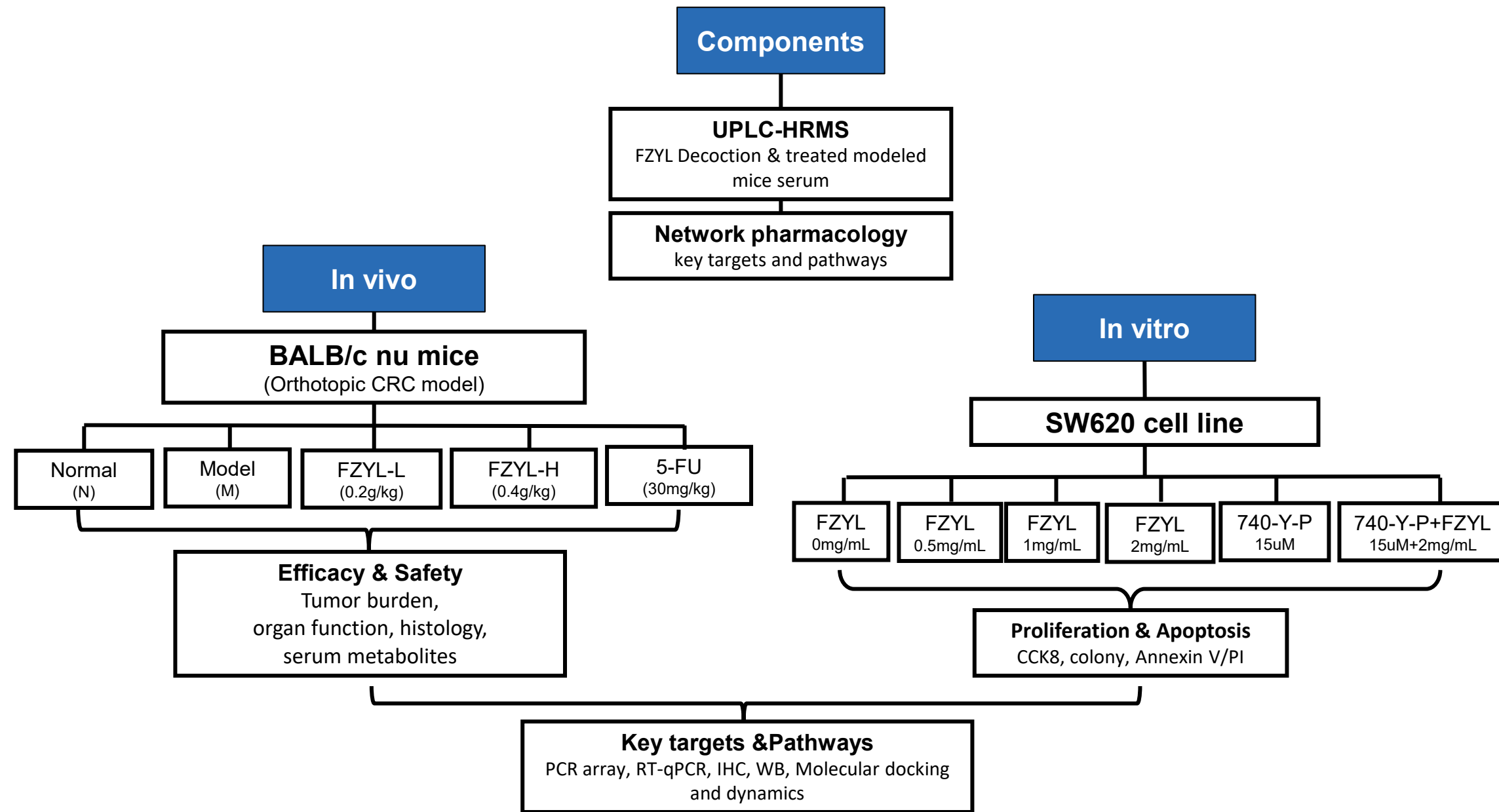
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Introduction

- ◆CRC remains a major global health burden with recurrence /metastasis and treatment toxicity.
- ◆FuZheng YiLiu (FZYL) decoction is widely used clinically for CRC, but mechanisms remain unclear. (FZYL can effectively relieve diarrhea, vomiting, hand-foot syndrome, pain and other discomfort caused by postoperative chemotherapy for bowel cancer, improve the completion rate of chemotherapy, improve the short-term and long-term quality of life and disease-free survival(Cai-yang et al., 2022). After treatment, serum CEA and AFP levels decreased (Ting-Ting et al., 2024). At the same time, it was also found that FZYL inhibited the proliferation of HT-29 cell line, suggesting that the mechanism may be related to the down-regulation of Bcl-2 and up-regulation of p53 expression, thus inhibiting cell proliferation.)
- ◆Chinese invention patents:
Patent No. 2025105560629.
- ◆Aim: evaluate efficacy/safety in an orthotopic model and dissect mechanisms via multi-omics and validation.
- ◆Keywords: CRC, TCM, Cell proliferation, Cell apoptosis, IGF2/IGF1R/PI3K/AKT pathway, 740-Y-P.

Methods



- BALB/c nu mice bearing orthotopic colon tumors were treated with FZYL.
- Tumor burden, organ function, histology, and serum metabolites were evaluated.
- UPLC-HRMS identified FZYL components.
- Network pharmacology predicted key targets and pathways.
- In vitro assays assessed proliferation and apoptosis.
- PCR array, RT-qPCR, IHC, and WB validated molecular mechanisms.
- Molecular docking and dynamics examined interactions between major FZYL compounds and IGF1R, PI3K, AKT1.

Results

◆ FZYL Inhibits Colorectal Tumor Growth and Intestinal Inflammation in Cecal Implantation-molded Nude Mice.

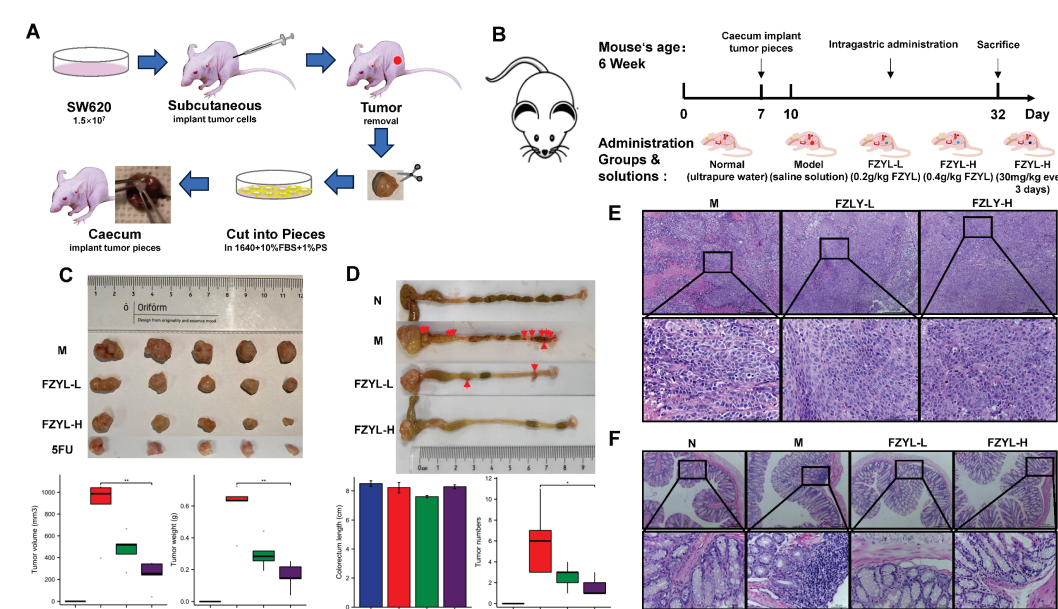


Figure 1. (A) Modeled protocol of BALB/c nu mice. (B)Treatments of in vivo experiences. (C)Volume of tumors in four groups nude mice. (D)Length of the colon and number of tumors in four groups of nude mice. (E)H&E staining of tumors in three groups nude mice. (F) H&E staining of colon in four groups nude mice. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

◆ FZYL is Safe and Relieves Liver Metastasis and Organ Damage in Nude Mice with Colorectal Cancer.

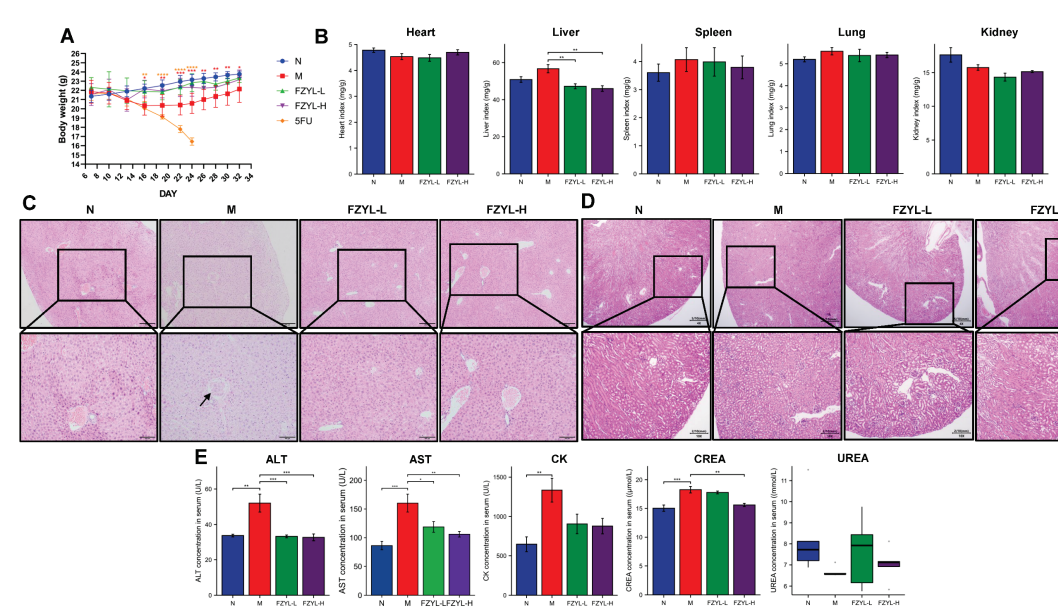


Figure 2. (A) Weight of nude mice. (B) Heart, liver, spleen, lung, and kidney index in nude mice. (C) H&E staining of liver in four groups nude mice. (D) H&E staining of kidney in four groups nude mice. (E) ALT, AST, CK, CREA, UREA concentration in nude mice's serum. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

◆ Chemical Composition of FZYL in Serum and Targets of FZYL in the Treatment of Colorectal Cancer through Network Pharmacology.

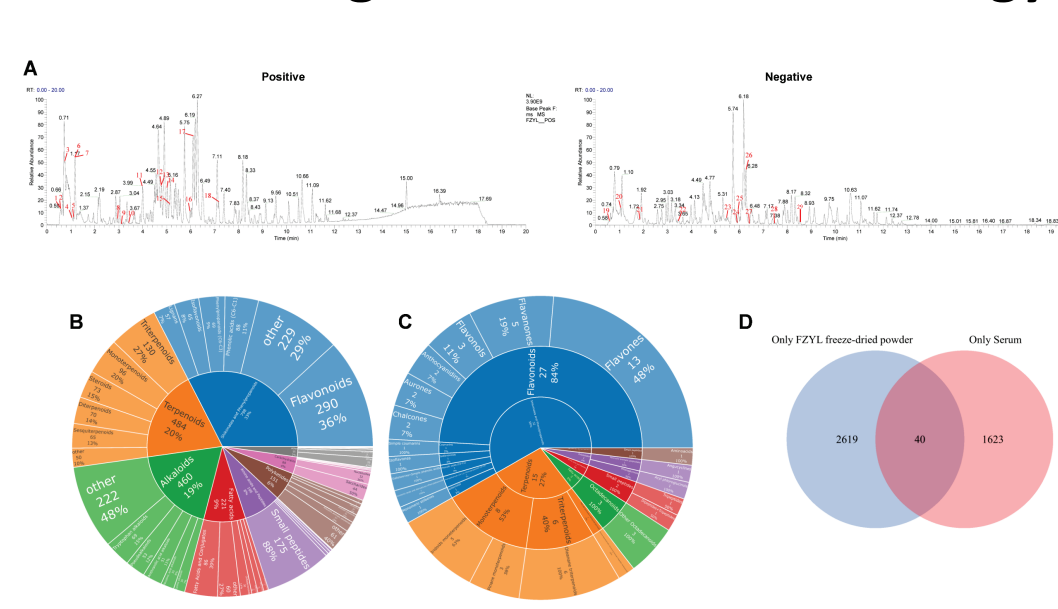


Figure 3. (A) BPC chromatograms of FZYL in positive ion mode and negative ion mode—annotated peaks. (B) Proportional distribution of identified compounds in FZYL across various chemical classifications. (C) Proportional distribution of identified compounds in FZYL based on their chemical classifications. (D) Venn diagram of the common components in FZYL freeze-dried powder and serum.

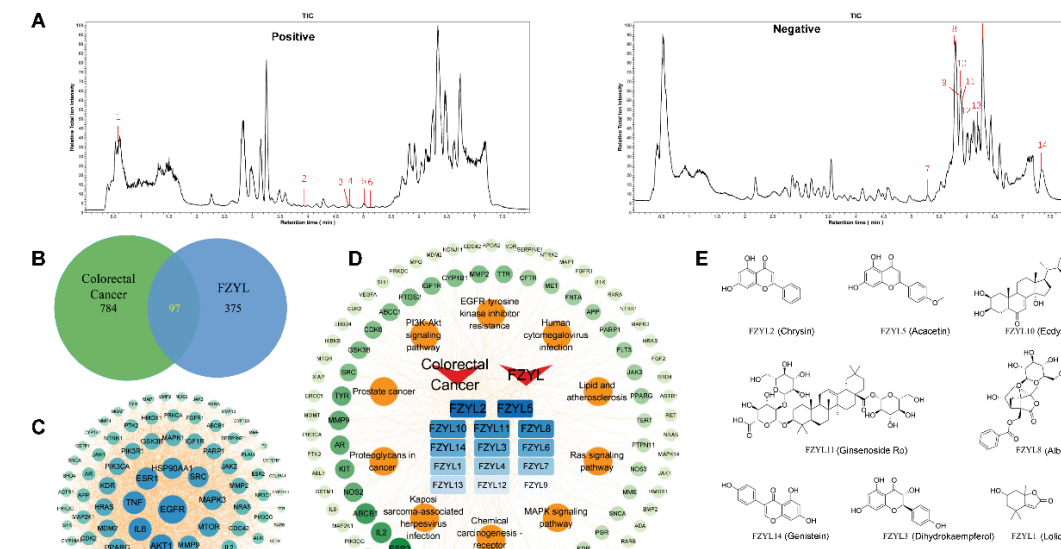


Figure4. (A) BPC chromatograms of FZYL Serum in positive ion mode and negative ion mode—annotated peaks. (B) Venn diagram of the intersecting targets between FZYL and colorectal cancer. (C) PPI (protein-protein interaction) network. (D) "FZYL-target-colorectal cancer-signaling pathway" network. (E) Molecular structures of key active compounds.

◆ FZYL May Treat Colorectal Cancer via IGF2/IGF1R/PI3K-AKT Pathway, as Demonstrated through In Vivo Experiments.

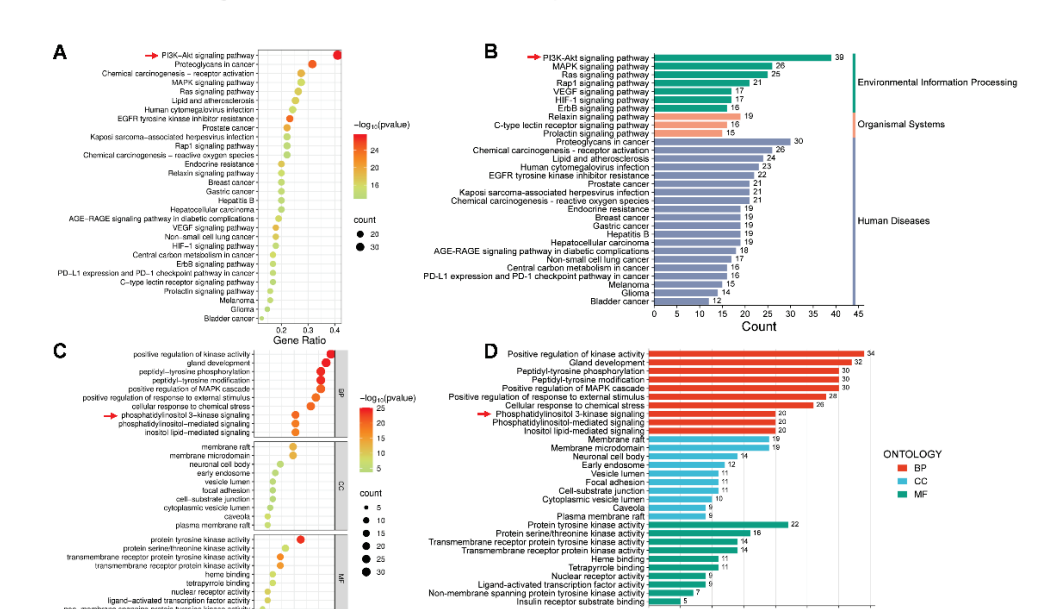


Figure 5. (A-B) KEGG bubble plot and bar chart of classification. (C-D) GO bubble plot and bar chart of classification.

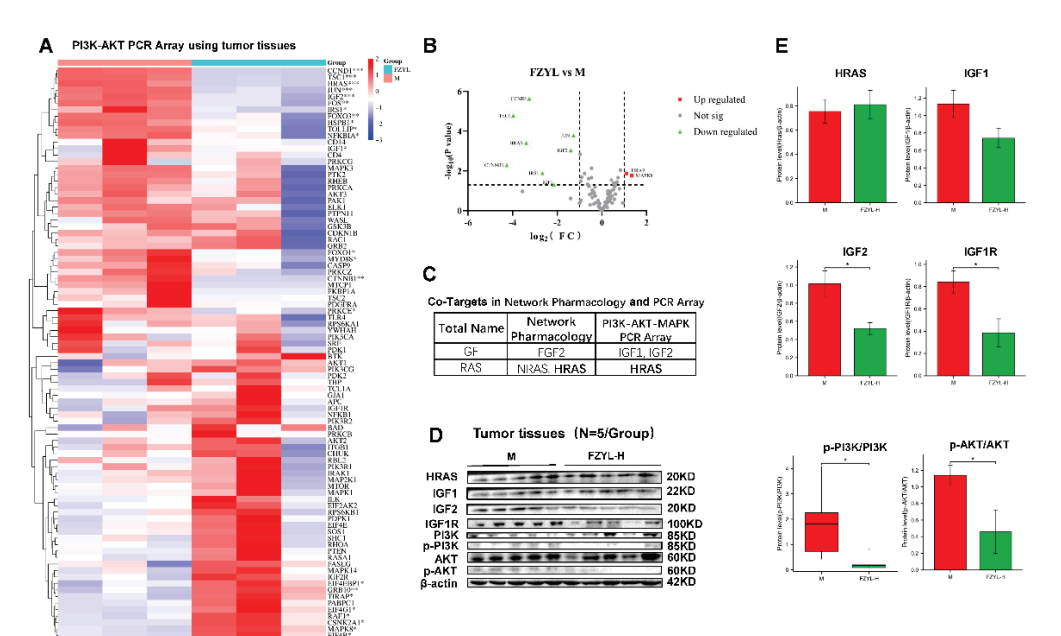


Figure 6. (A)gene expression heatmap of PI3K/AKT PCR Array using tumor tissues. (B) volcano plot of differentially expressed genes. [log2FC] > 1, P<0.05. (C) Co-Targets in Network Pharmacology and PCR Array. (D) Tumor tissues' Western Blot band image in M and FZYL-H group. (E) Protein levels in tumor tissue. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

◆ FZYL Inhibited Tumor Proliferation and Increased Apoptosis in Nude Mice with Colorectal Cancer.

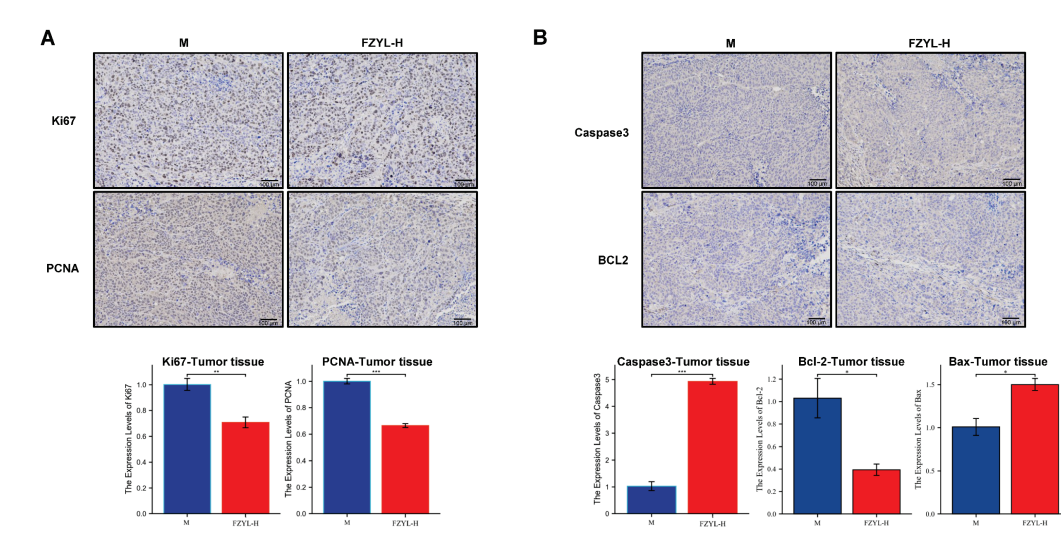


Figure 7. (A) Immunohistochemical staining of proliferation-related proteins and expression levels of proliferation-related genes in tumor sections of nude mice. (B) Immunohistochemical staining of apoptosis-related proteins and expression levels of apoptosis-related genes in tumor sections of nude mice. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

◆ FZYL Inhibited Tumor Cell Proliferation and Increased Cell Apoptosis in SW620 Cells.

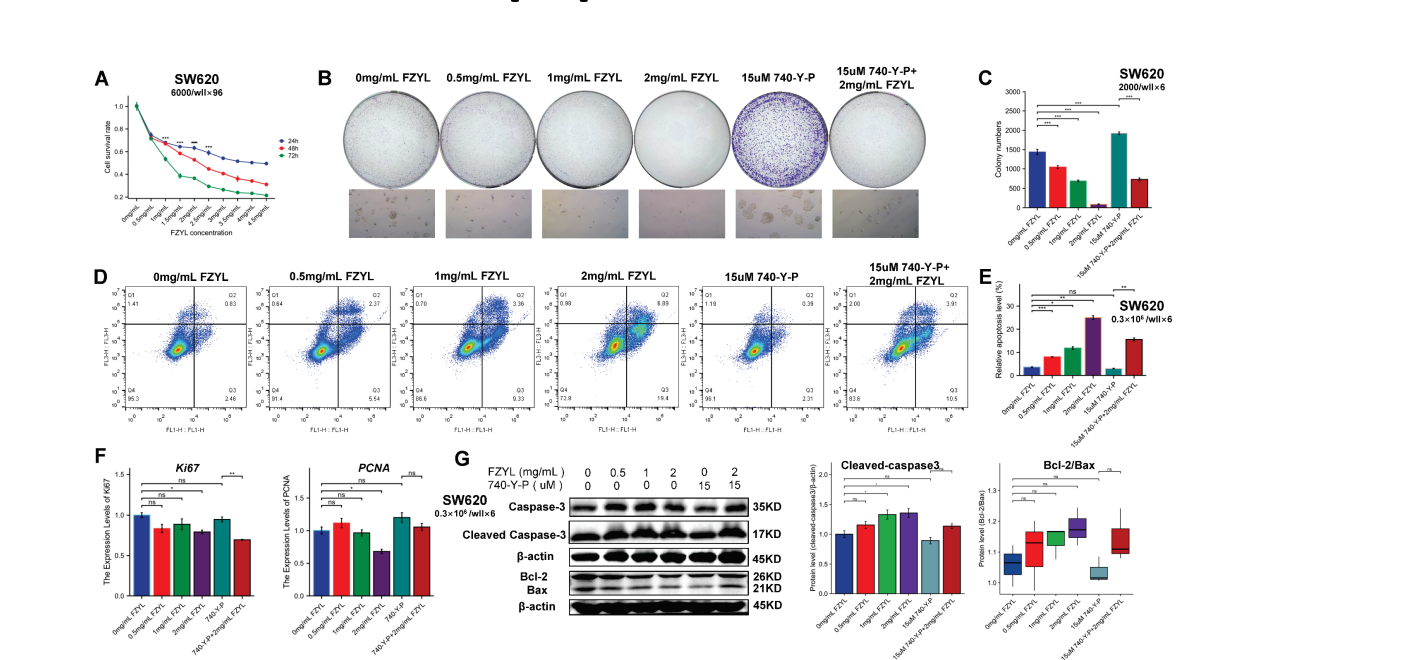


Figure 8. (A) Immunohistochemical staining of proliferation-related proteins and expression levels of proliferation-related genes in tumor sections of nude mice. (B) Immunohistochemical staining of apoptosis-related proteins and expression levels of apoptosis-related genes in tumor sections of nude mice. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

◆ FZYL May Treat Colorectal Cancer via the IGF2/IGF1R/PI3K/AKT Pathway Based on Cells Experiences, Molecular Docking and Molecular Dynamics.

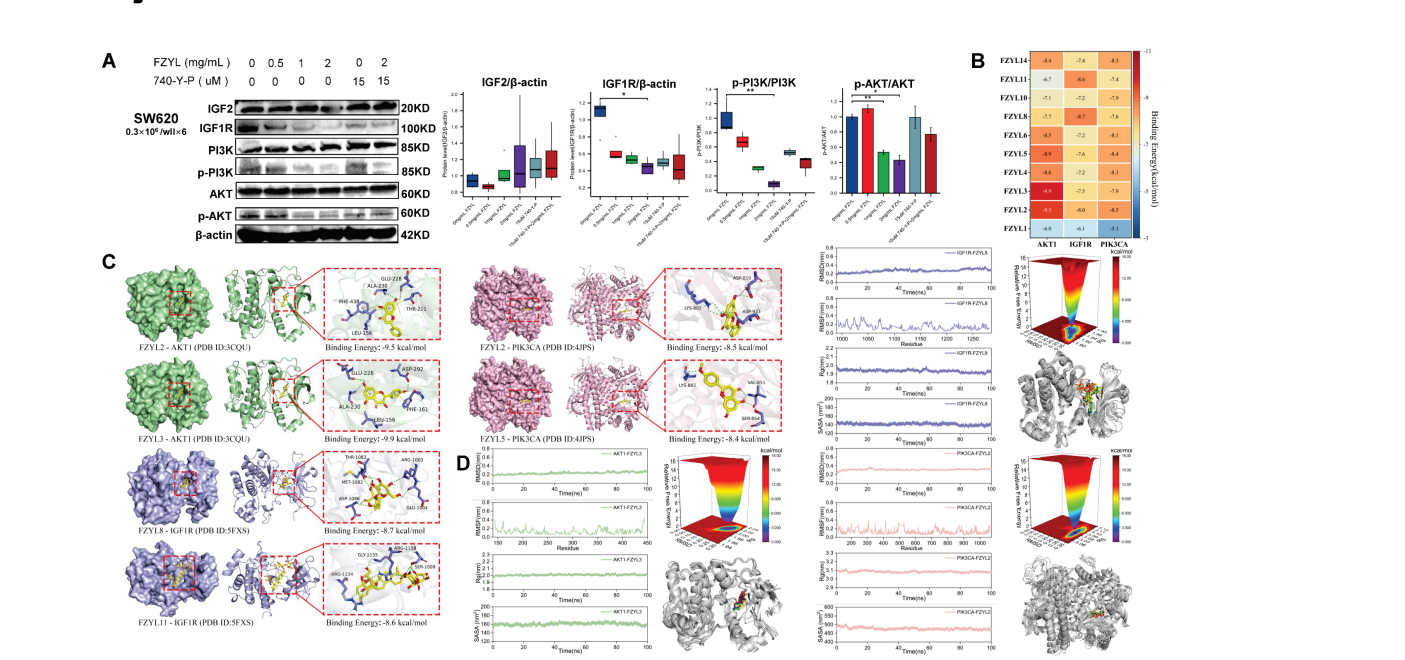
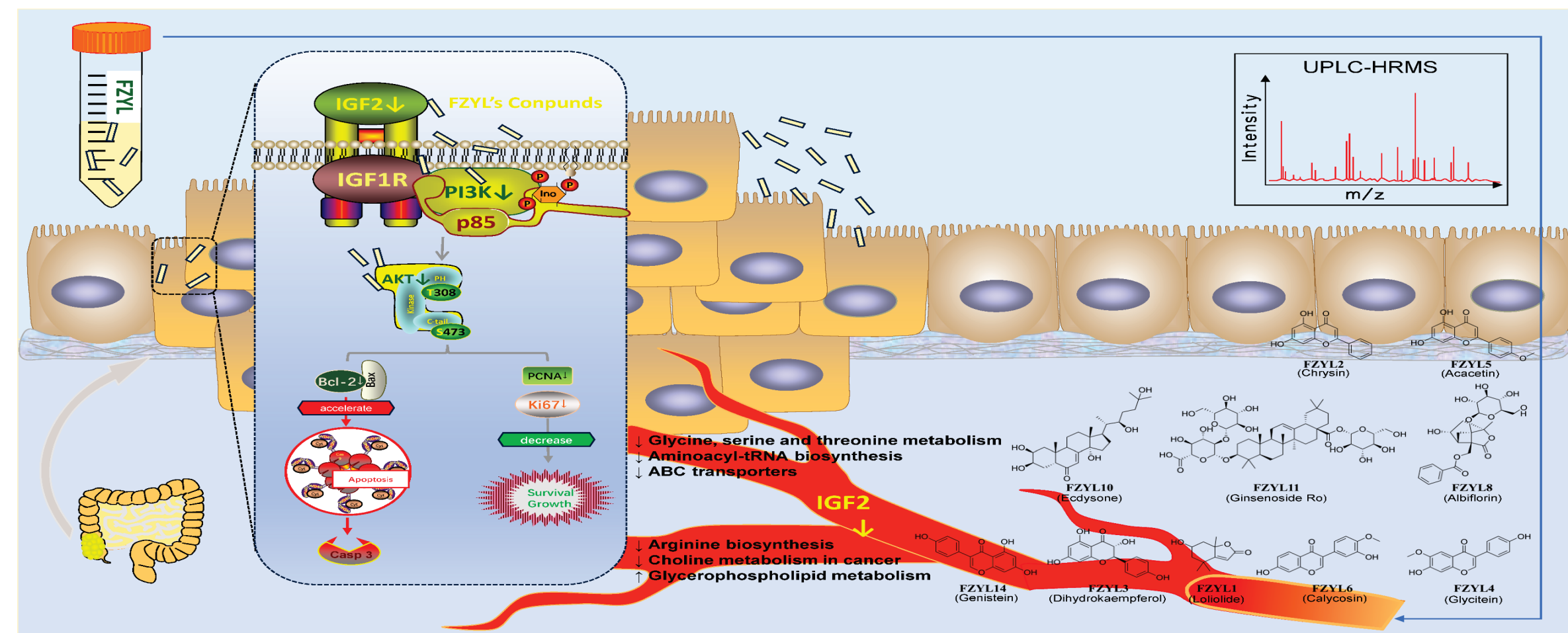


Figure 9. (A) Western blot analysis showing the effect of FZYL on the protein levels of the IGF2/IGF1R/PI3K/AKT pathway. (B) Heatmap of molecular docking results of the 10 core components of FZYL with AKT1, IGF1R, and PI3K. (C) Docking models of the top 2 compounds with AKT1, IGF1R, and PI3K. (D)Molecular dynamics analysis results of the best combination. RMSD curve, which reflects the extent of structural fluctuations in the complex. RMSF curve, indicating the degree of fluctuation of individual amino acid residues. Rg curve, which illustrates the degree of expansion of the complex system. SASA curve, reflecting the degree of folding and bending of the protein structure. Free energy landscape, where the concentration of energy clusters reflects the stability of the complex state. Comparative analysis of complex structures at five time points (0, 25, 50, 75, and 100 ns) during the molecular dynamics simulation process. NS; *p < 0.05; **p < 0.01; ***p < 0.001.

Discussion and Conclusion



The findings demonstrate that FuZheng YiLiu (FZYL) decoctions effectively inhibit colorectal cancer proliferation and enhance apoptosis, potentially by downregulating the IGF2/IGF1R/PI3K/AKT signaling pathway. Comprehensive analyses, including tumor measurement, biochemical assays, molecular docking, and protein expression evaluations, substantiate the therapeutic mechanisms of FZYL. This study provides valuable insights into FZYL's role as a promising candidate for colorectal cancer treatment, highlighting its potential to mitigate disease progression while elucidating the underlying biological mechanisms. Overall, FZYL represents a significant advancement in therapeutic approaches for colorectal cancer management.

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Acknowledgments

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