

Evolutionary Mechanisms and Reversal Strategies of Acquired Resistance to RC48 in Bladder Cancer



Chengming Yang, Lushan Yang, Bufan Ying, Shu Bai, Dan Xie, Yuchen Feng, Xingyi Song, Xinyue Yu, Huiyun Deng, Xinrui Shang, Siyi Gao, Jiaoxue Guo, Cheng Zhong, Zihan Zhao, Lianghua Wang, Sheng Zhang, Jianhua Luo, Meng Guo, Yu Kong, Mingjuan Sun*^{*}

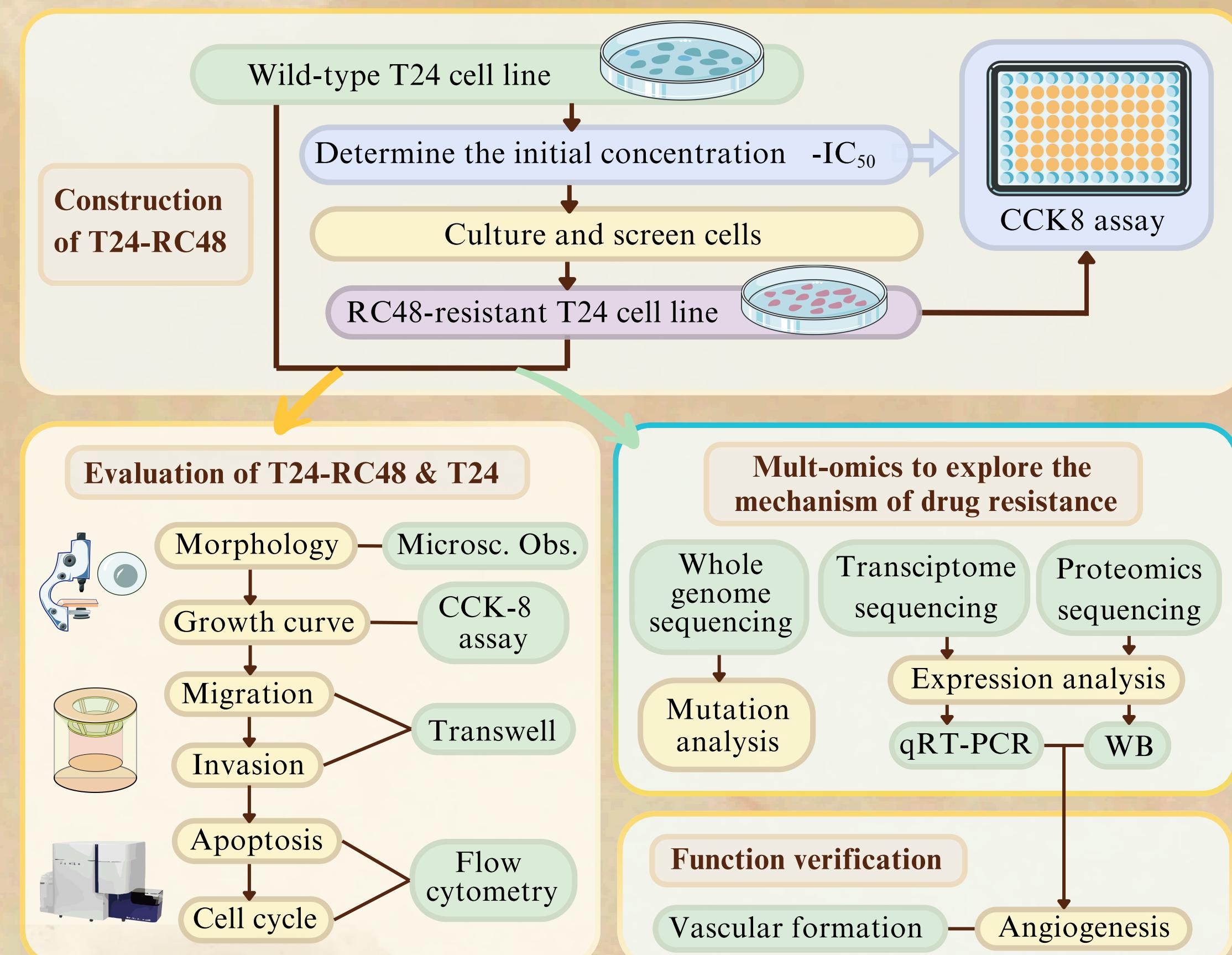
Department of Biochemistry and Molecular Biology, College of Basic Medical Sciences, Naval Medical University, 200433, China

Introduction

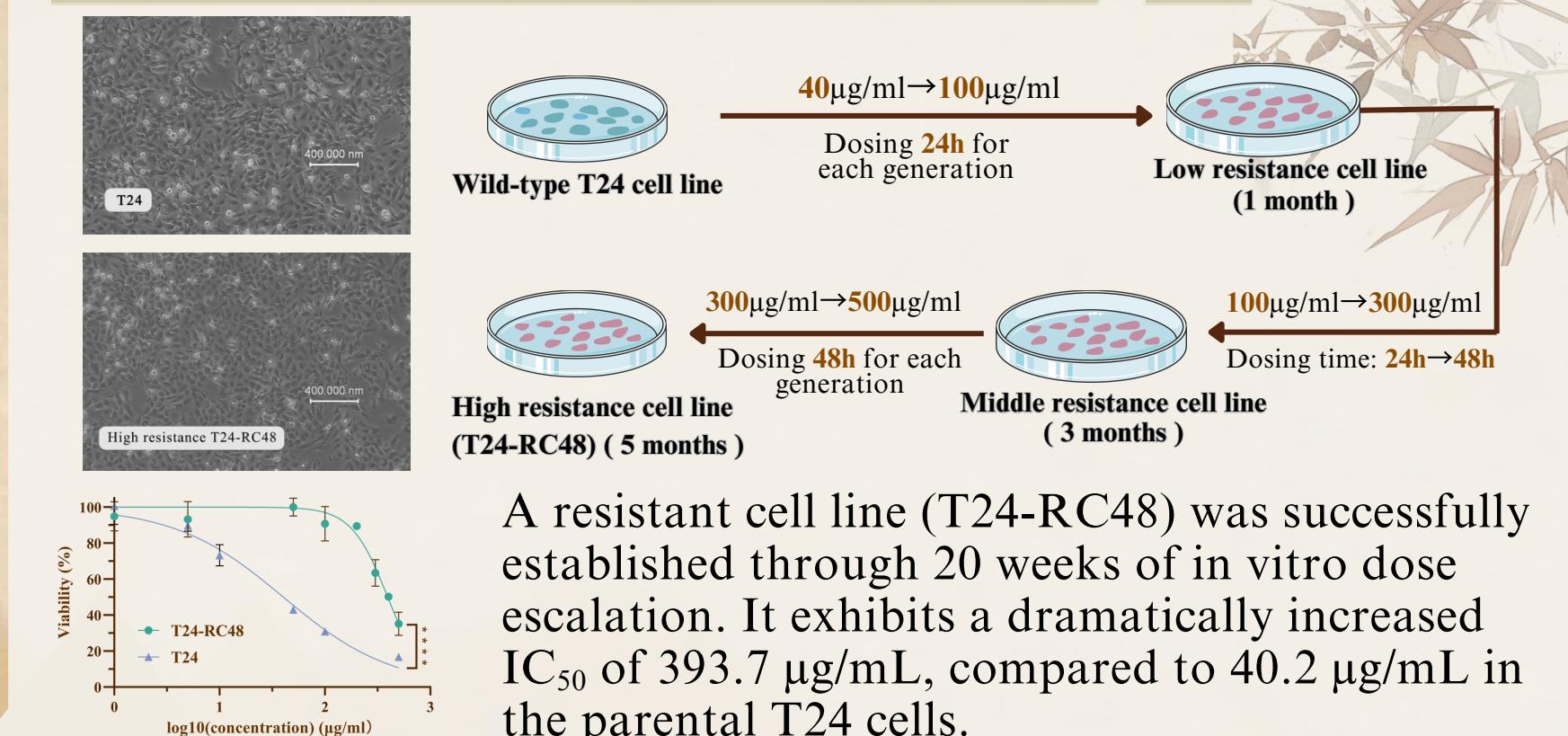
Bladder cancer, particularly bladder urothelial carcinoma (BLCA), remains a lethal malignancy with limited therapeutic options. Antibody-drug conjugates (ADCs) such as Disitamab Vedotin (RC48), which targets HER2-Positive tumors, have shown promising efficacy. However, Acquired resistance poses a major clinical challenge, and the underlying mechanisms are poorly understood.

To preempt this challenge, we employed a strategy of "Evolve to Counter Evolve". Not just observing resistance, we induced it. We generated T24-RC48 (an RC48-resistant cell line) through directed evolution and used multi-omics analysis to uncover key resistance mechanisms. By exploiting resistance pathways as vulnerabilities, rational combinations like RC48 plus Ivonescimab can overcome drug resistance.

Material & Methods

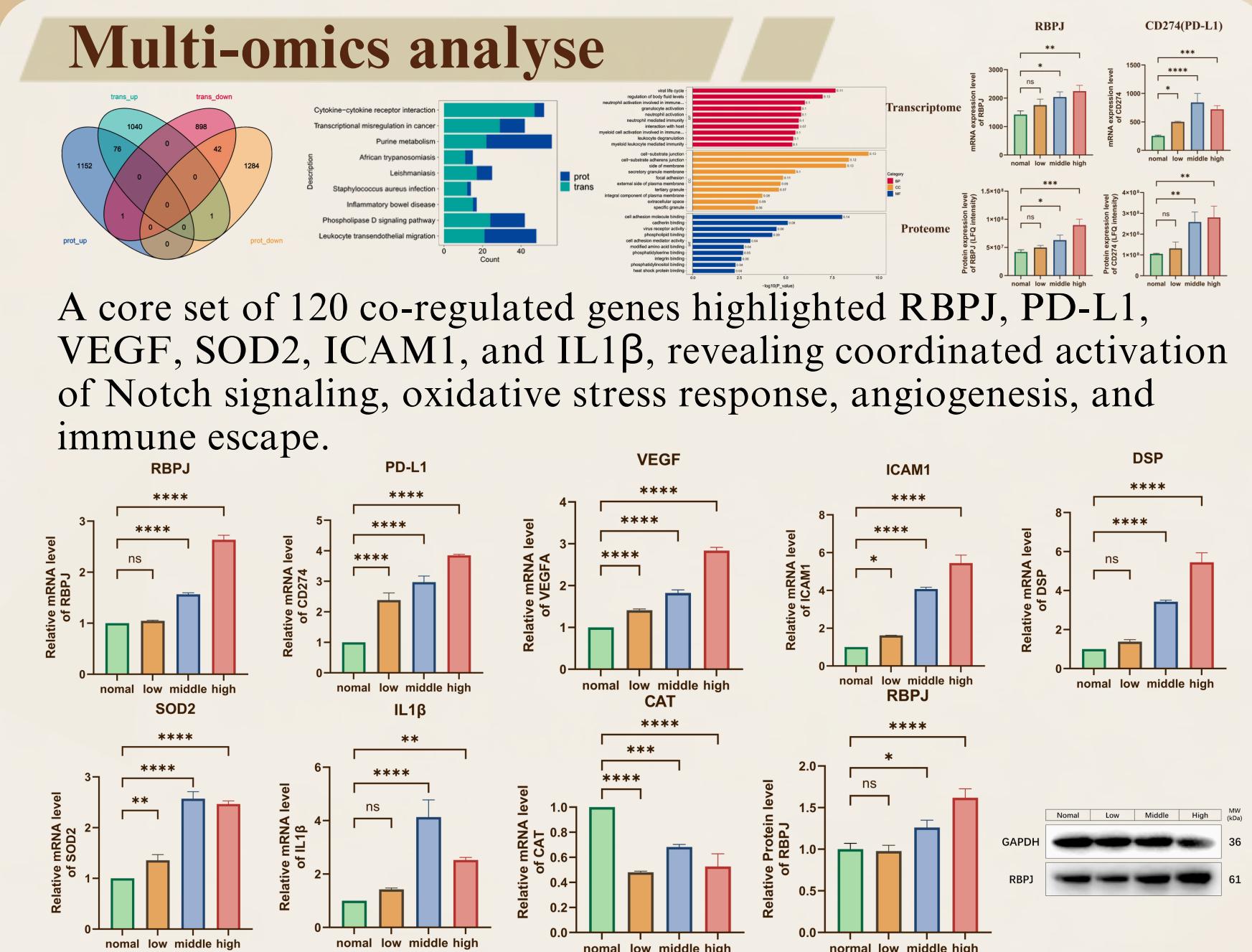


Directed evolution of T24-cell line



A resistant cell line (T24-RC48) was successfully established through 20 weeks of in vitro dose escalation. It exhibits a dramatically increased IC₅₀ of 393.7 μg/mL, compared to 40.2 μg/mL in the parental T24 cells.

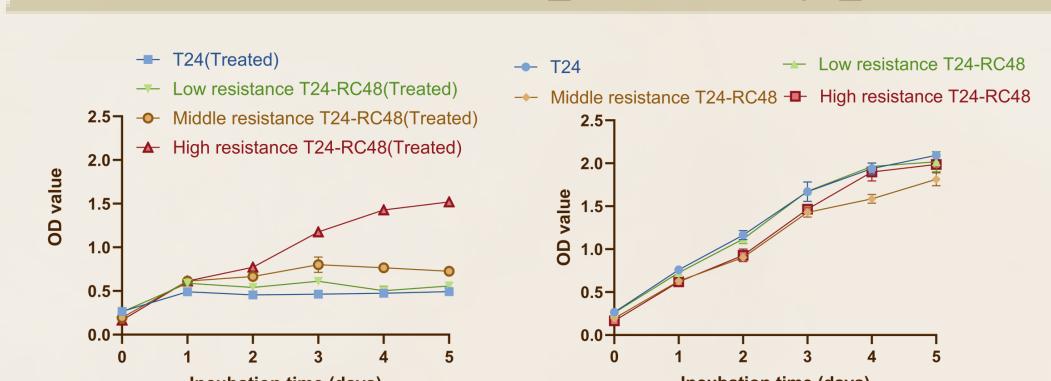
Multi-omics analyse



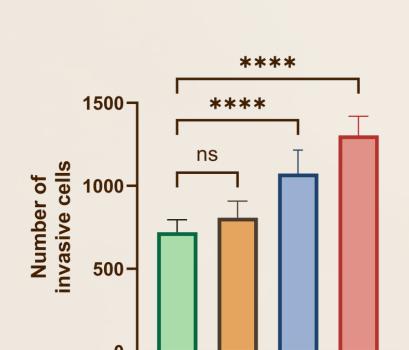
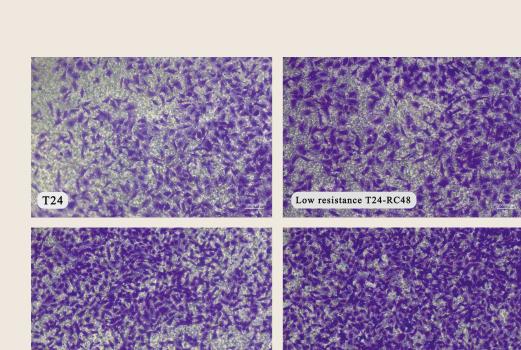
A core set of 120 co-regulated genes highlighted RBPJ, PD-L1, VEGF, SOD2, ICAM1, and IL1β, revealing coordinated activation of Notch signaling, oxidative stress response, angiogenesis, and immune escape.

The qRT-PCR confirmed upregulation of RBPJ, PD-L1, VEGF, ICAM1, SOD2, and IL1β in RC48-resistant cells, with CAT downregulated. While Western Blot validated stepwise increase in RBPJ protein, confirming Notch pathway activation.

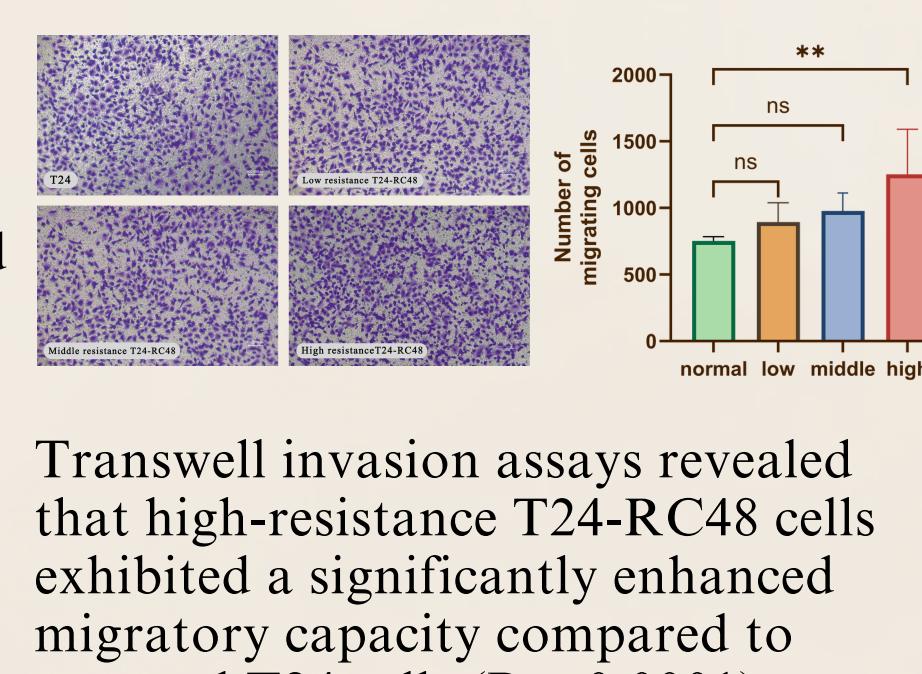
Detection of phenotypes



T24-RC48 cells displayed a resistance-dependent increase in migratory ability, with high-resistance T24-RC48 showing significantly elevated migration compared to parental T24 cells ($P < 0.001$).

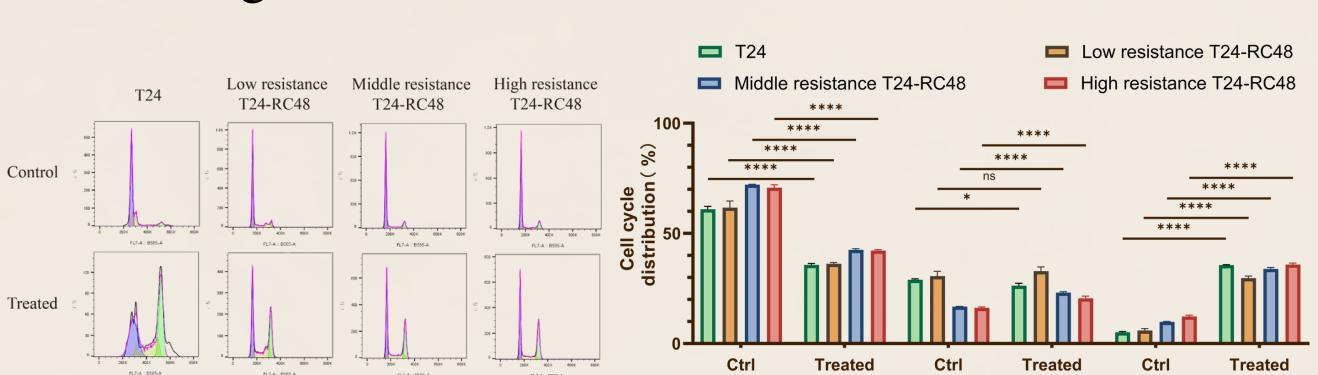


Under RC48 treatment, T24-RC48 maintains robust proliferation, while T24 wild-type shows significant growth inhibition, highlighting the acquired resistance phenotype.



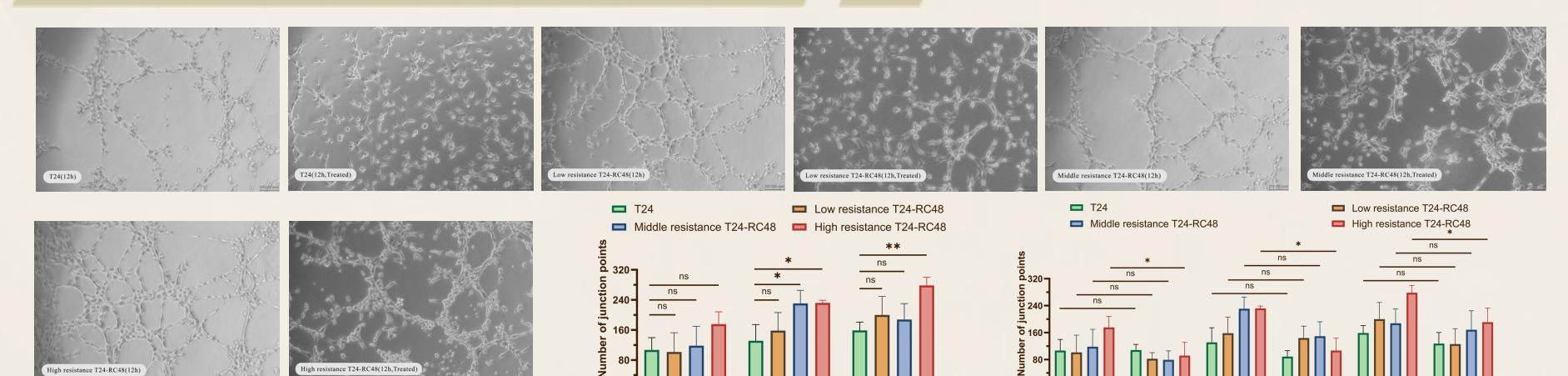
Transwell invasion assays revealed that high-resistance T24-RC48 cells exhibited a significantly enhanced migratory capacity compared to parental T24 cells ($P < 0.0001$).

T24-RC48 cells demonstrated significantly enhanced apoptosis resistance both with and without RC48 treatment and intensified with increasing resistance levels.



Flow cytometry revealed that T24-RC48 cells exhibited attenuated G2/M arrest and altered S-phase dynamics compared to parental T24 upon RC48 treatment.

Function verification

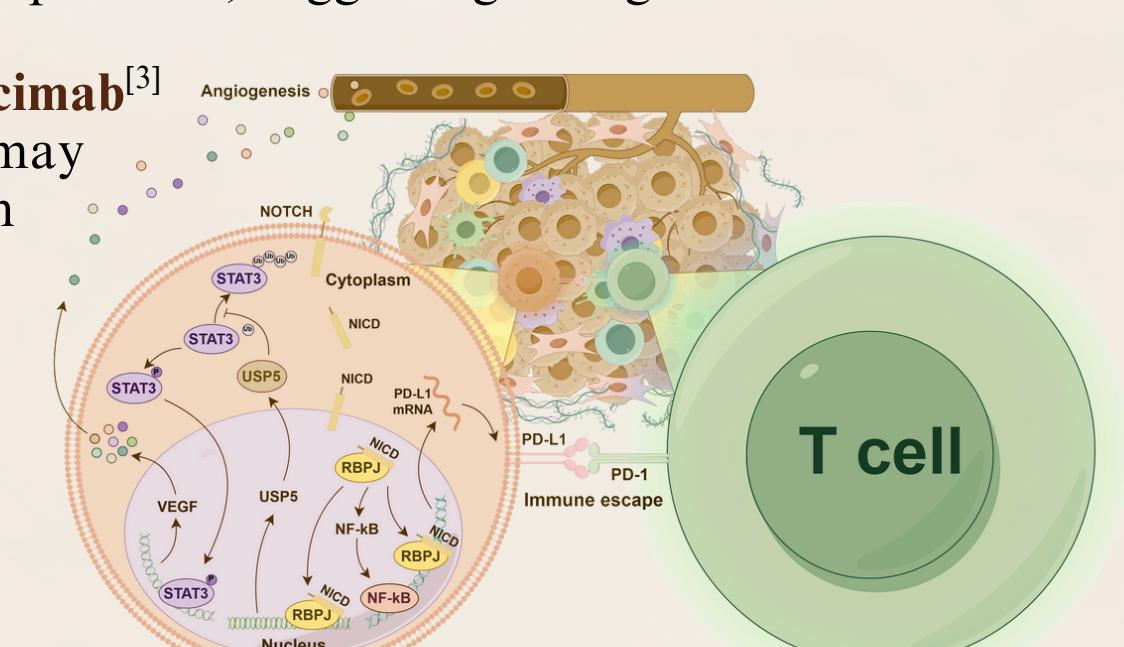


Conditioned media from T24-RC48 cells significantly enhanced HUVEC tube formation, which can be inhibited by Ivonescimab, most notably in the high-resistance group. demonstrating a potential anti-angiogenic strategy to overcome resistance.

Conclusion & Outlook

T24-RC48 showed a 10-fold increase in IC₅₀, with increased resistance to apoptosis, enhanced migration, invasion, and reduced G2 arrest. Our study reveals that RC48 resistance in bladder cancer is driven by Notch pathway activation^[1] via RBPJ, immune evasion and angiogenesis^[2]. We proposed a novel signaling model in which Notch activation transcriptionally enhances VEGF and PD-L1 expression, suggesting a targetable axis for sequential therapy. Combining RC48 with ivonescimab^[3] (anti-PD-1/VEGF antibody) may improve outcomes and sustain precision therapy in BLCA.

Future work will focus on exploring clinical combinations of RC48 with Ivonescimab to overcome resistance and improve outcomes in BLCA patients.



Reference

- [1] Shi Q, Xue C, Zeng Y, Yuan X, Chu Q, Jiang S, Wang J, Zhang Y, Zhu D, Li L. Notch signaling pathway in cancer: from mechanistic insights to targeted therapies. *Signal Transduct Target Ther.* 2024 May 27;9(1):128. doi: 10.1038/s41392-024-01828-xIF: 52.7 Q1 . PMID: 38797752IF: 52.7 Q1 ; PMCID: PMC1128457IF: 52.7 Q1 .
- [2] Targeting Angiogenesis in Bladder Cancer. *Cancer Discov.* 2017 Nov;7(11):OF2. doi: 10.1158/2159-8290.CD-NB2017-140IF: 33.3 Q1 . Epub 2017 Oct 4. PMID: 28978558.
- [3] Herbst RS, Chen L. The evolving immuno-angiogenic paradigm in NSCLC: lessons from ivonescimab. *Nat Rev Clin Oncol.* 2025 Jul;22(7):461-462. doi: 10.1038/s41571-025-01024-yIF: 82.2 Q1 . PMID: 40329050.