Exploiting Synthetic Lethality in RB1-Deficient Lung Cancer Cells Using Small Molecules and Nutritional Combinations

School of Molecular and Theoretical Biology 2026, Sabanci University, Istanbul, Turkey

INTRODUCTION

Cancer causes nearly 10 million deaths worldwide each year, accounting for about 1 out of every 6 deaths. While several therapeutic strategies currently exist for cancer treatment, their long-term efficacy is often limited by intrinsic resistance mechanisms and genetic alterations. Conventional chemotherapy effectively reduces tumor size but causes severe systemic toxicity, whereas targeted therapies and immunotherapies offer high precision and durable responses, yet remain constrained by cancer mutations and loss of target expression.

The concept of synthetic lethality

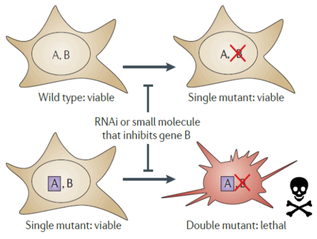


Figure 1. Scheme showing mechanism of synthetic lethality.

Synthetic lethality overcomes some of these limitations and aims to selectively eliminate cancer cells while leaving healthy cells unaffected. The strategy involves identifying genes whose inhibition is lethal only in cancer cells carrying an inactivated tumor suppressor gene. Specially designed drugs inhibit the proteins that synthetic lethal partner genes produce, effectively limiting cell proliferation.

RB1 is a tumor suppressor gene that encodes the retinoblastoma protein, which plays a key role in regulating cellular proliferation. In its active state, the RB protein binds to and inhibits the E2F transcription factor, thereby preventing the transcription of genes required for the S-phase in the cell cycle. RB1 is inactivated in approximately 15% of all human tumors, making it an important target for developing selective cancer therapies.

This study applies a synthetic lethality strategy to a pair of CRISPR/Cas9-generated, isogenic PC9 lung adenocarcinoma cell lines (RB1+/+ and RB1-/-). By combining targeted small-molecule inhibitors with specific amino acid restriction, we aim to induce selective cell death in RB1-inactivated tumors while bypassing the systemic toxicities of conventional therapies.

Рак ежегодно является причиной почти 10 миллионов смертей во всем мире. Также каждая шестая смерть приходится на человека, страдающего онкологическим заболеванием. Несмотря на то, что в настоящее время существует несколько терапевтических стратегий лечения рака, их долгосрочная эффективность часто ограничена механизмами резистентности опухоли. Традиционная химиотерапия обычно эффективно уменьшает размер опухоли, но вызывает тяжелую системную токсичность, тогда как таргетированная терапия и иммунотерапия обеспечивают высокую точность и длительность действия, однако их применение ограничено утратой экспрессии гена-мишени.

Синтетическая летальность преодолевает некоторые из этих ограничений и направлена на избирательное уничтожение раковых клеток без вреда для здоровых. Эта стратегия заключается в поиске генов, ингибирование которых летально только для раковых клеток с инактивированным геном-супрессором опухолей. Специально разработанные препараты подавляют белки, продуцируемые синтетически летальными парами, эффективно ограничивая клеточную пролиферацию.

RB1 - это ген-супрессор опухолей, кодирующий белок ретинобластомы, который играет ключевую роль в регуляции клеточной пролиферации. В активном состоянии белок RB связывается с фактором транскрипции E2F и ингибирует его, тем самым предотвращая транскрипцию генов, необходимых для S-фазы клеточного цикла. Ген RB1 инактивирован примерно в 15% всех опухолей человека, что делает его важной мишенью для разработки селективной противоопухолевой терапии.

В данном исследовании стратегия синтетической летальности применяется к паре изогенных клеточных линий аденокарциномы легкого PC9 (RB1+/+ и RB1-/-), полученных с помощью системы CRISPR/Cas9. Сочетая таргетированные низкомолекулярные ингибиторы с ограничением конкретных аминокислот, мы стремимся индуцировать селективную гибель клеток в опухолях с инактивированным RB1, избегая при этом системной токсичности, характерной для традиционной терапии.

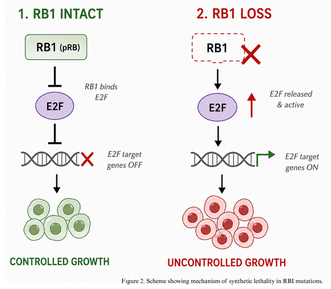


Figure 2. Scheme showing mechanism of synthetic lethality in RB1 mutations.

MATERIALS AND METHODS

1. Two human lung cancer PC9 cell lines were utilized in this study: an RB1-positive line and an RB1-knockout control line generated via CRISPR/Cas9 gene editing. Cell cultures were maintained in glucose-containing DMEM, and cell density and viability were determined using an EVE automatic cell counter with trypan blue staining.
2. Across all experimental conditions, cells were allowed to adhere for 24 hours post-seeding before media replacement and treatment administration. After that, cell numbers were evaluated 2 and 4 days post-treatment by measuring absorbance using Tecan plate reader following 0.05% crystal violet staining. Additionally, for experiment 3, cellular ATP levels were quantified via luminescent ATP assays (ATPlite).
3. Initial cell seeding densities were tailored specifically to the requirements of the downstream detection methods. For the crystal violet assay, cells were seeded at an initial density of 10,000 cells per well, whereas for luminescent ATP measurements (ATPlite assay), cells were seeded at 5,000 cells per well.
4. The efficacy of combinations of drugs and amino acid restrictions was independently investigated using Cloude Sonet 5. The graphs and diagrams were generated using the BioRender programme, while we used PRISM and Microsoft Excel to analyse the spectrophotometric data.

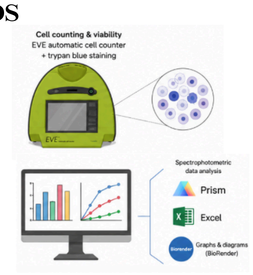


Figure 3. Scheme showing applied materials and methods.

Small molecules

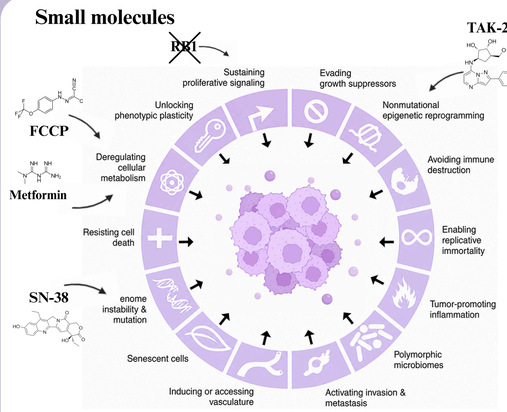


Figure 4. Scheme showing hallmarks of cancer and drugs which affect them

Nutrients

Selective cell death in cancer cells can be achieved by targeting specific dependencies of cancer cells.

- Methionine supports protein synthesis and SAM-dependent methylation, so its depletion can disrupt cellular metabolism and growth.
- Cysteine is required for glutathione synthesis, so its depletion reduces antioxidant capacity and increases oxidative stress.
- Glucose provides energy and biosynthetic intermediates through glycolysis, so its restriction can cause metabolic stress and impair cell proliferation.

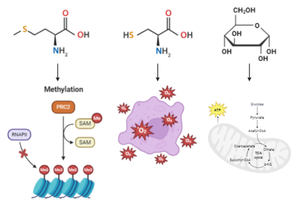


Figure 5. Scheme showing nutritents

EXPERIMENT 1

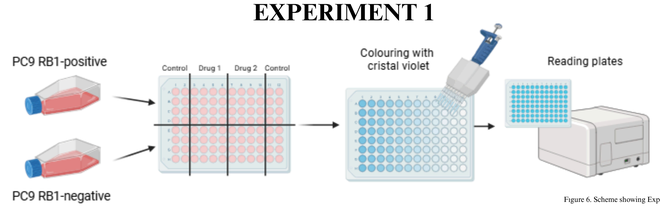
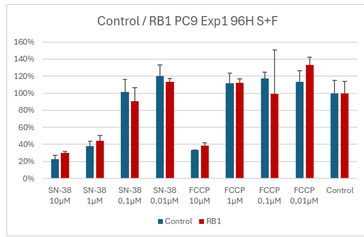


Figure 6. Scheme showing Experiment 1.

Our goal was to evaluate the effects of selected candidate drugs on cell proliferation and viability.
PC9 RB1 and PC9 control cells were cultured in a 96-well plate and treated with 4 drugs (SN38, TAK243, FCCP, Metformin) at different concentrations (from 1nM to 10μM) to examine their effect on the respective cell line. The number of cells was determined using a crystal violet assay.

RESULTS



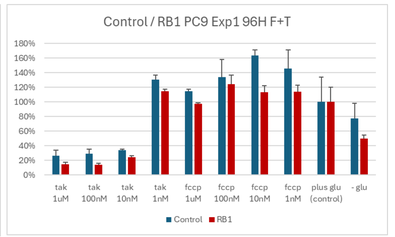


Figure 7 and 8. Experiment 1 results charts.

- Increasing SN-38 concentration reduced cell survival similarly in both cell types.
- Increasing TAK-243 concentration reduced cell survival, with slightly greater sensitivity in RB1-deficient cells.
- Increasing FCCP concentration reduced cell survival, with no consistent difference between cell types.
- Metformin did not produce a cell-death effect or selectivity.

Summary: SN-38, TAK-243, and FCCP were found to be effective at killing cells, but had little selectivity.

EXPERIMENT 2

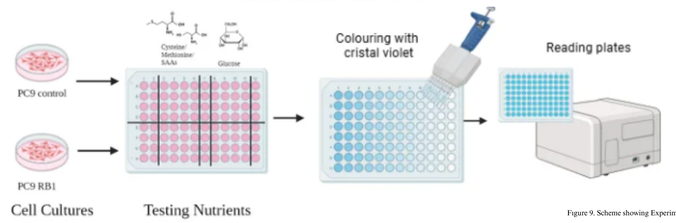
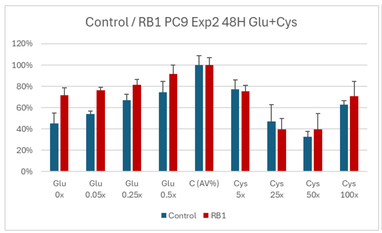


Figure 9. Scheme showing Experiment 2.

Our goal was to investigate the impact of increased concentrations of methionine, cysteine, and glucose on cell growth.

PC9 RB1 and PC9 control cells were cultured in a 96-well plate and treated with 3 nutrietients extra suplementations (Methionine and Cysteine from 5x to 100x, Glucose from 5x to 50x) and glucose restriction (from 0x to 1x) to examine their effect on the respective cell line. The number of cells was determined using a crystal violet assay.

RESULTS



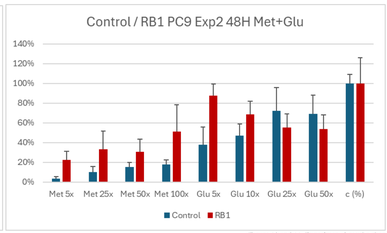


Figure 10 and 11. Experiment 2 results charts.

- Increased concentration of methionine selectively affects RB1-deficient cells.
- Glucose restriction results in cell death, with RB1 cells being more resistant.
- Increased cysteine concentration results in cell death (25x and 50x).
- RB1 cells are more immune to low glucose supplementation in comparison to control cells. In high supplementation, the effect is opposite.

Summary: Increased methionine and glucose restriction positively affected RB1 cells. Cysteine is not selective.

EXPERIMENT 3

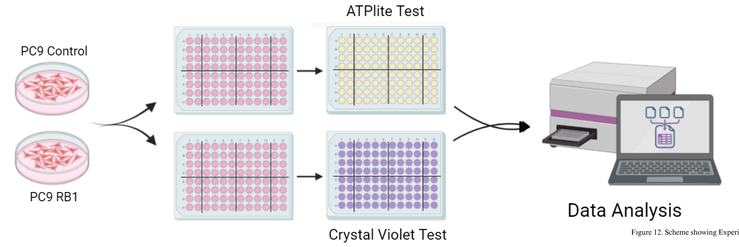
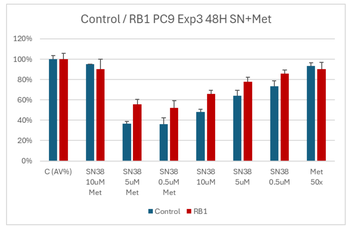
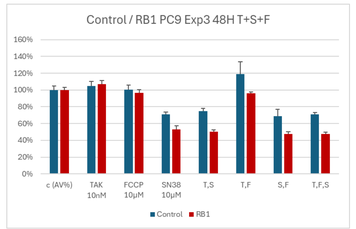


Figure 12. Scheme showing Experiment 3.

Our goal was to assess optimal combinations of small molecules and nutrient intervention mechanisms.
PC9 RB1 and PC9 control cells were cultured in a 96-well plate and treated with different combinations of drugs and nutrients based on the previous experiments. The number of cells was determined using the Crystal Violet assay and ATP-Lite test.

RESULTS





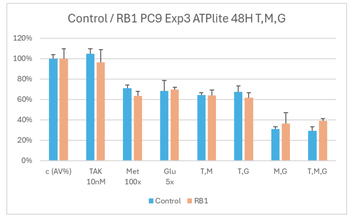


Figure 13, 14 and 15. Experiment 3 results charts.

- Combining three drugs (TAK, FCCP, and SN) causes a synthetic lethality response.
- SN-38 contributes to cell death in the majority of tests.
- The combination of glucose and methionine supplementation enhances the cytotoxic effect.
- The addition of methionine to SN-38 increases its selectivity, but in the opposite direction.

Summary: SN-38 selectively induces cell death only when iits combined wtih another drug

CONCLUSIONS

- SN-38 and TAK-243showed dose-dependent response with no selectivity.
- Metformin does not show any effect in single-drug trials, while FCCP shows a dose-dependent response with no selectivity.
- Glucose restriction causes cell death over time with no selectivity, while high concentrations of glucose did not affect cell proliferation.
- High concentrations of methionine accelerated cell growth in RB1-negative cells, while high concentrations of cysteine and both amino acids combined did not affect cells.

TAKE HOME MESSAGES

1) Methionine accelerates RB1-deficient cell proliferation.

2) SN-38 selectivity induces cell death when combined with other drugs.

3) Glucose restriction reduces cell survival.

4) RB1-deficient cells are protected against glucose deprivation - induced cell death.

ACKNOWLEDGEMENTS

Sabanci Universitesi

School of molecular and theoretical biology