Synergistic and Antagonistic Effects of Drug Combinations on Tumor Cell Migration in a Fibroblast Scratch Assay Model

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Abstract: This study examined the effects of 12 biochemical agents, including chemotherapies, a dilution series of Etoposide, copper (II) ions, and other bioactive compounds, on tumor cell migration using a fibroblast scratch assay. Agents were applied to adult mouse fibroblasts, and migration was quantified via microscopy and cell counts. Chemotherapies such as 5-Fluorouracil, Cytarabine, Etoposide, and Cycloheximide showed varied effects based on mechanisms like DNA synthesis inhibition and apoptosis induction. Copper (II) ions and Vanadate promoted mitochondrial damage and tumor suppressor activation, while Retinoic Acid inhibited proliferation and metastasis-related signaling. Resveratrol demonstrated the strongest inhibition of cell migration, with logistic growth modeling suggesting lasting effects on tumor proliferation. These results highlight how Resveratrol can act as a dual-action therapeutic and support further investigation into its use in cancer treatment.

Keywords: Fibroblasts, tumor cell migration, therapeutic, cancer treatment

1. Introduction

Cancer involves uncontrolled cell growth and the ability of malignant cells to migrate and invade other tissues. The fibroblast scratch assay is a common in vitro model used to study this behavior. While agents like Resveratrol, Curcumin, and EGCG have shown promise in inhibiting tumor cell migration through pathways such as inflammation modulation and cell cycle arrest, most studies focus on single agents and suffer from inconsistent scratch methods, cell death, and low-resolution data. This project addresses these limitations by testing dual-agent combinations, standardizing scratch techniques, replenishing media to enhance cell viability, and collecting high-frequency data for improved analysis of wound closure dynamics.

2. Method and Experimental Details

Mouse fibroblasts were evaluated using a scratch assay to test single and dual agent effects on migration and proliferation, using 14 wells cultured to confluence. To simulate wound healing, a vertical scratch was made across the monolayer of mouse fibroblast cells using a pipette tip guided with a stencil.

After the initial scratch, drugs were delivered at (0.1%–1%?) concentrations in wells containing 500 µL of medium. Treatments measurements were the following: 2 µL of Resveratrol; 2 µL of AICAR; 2 µL of 5-FU; 2 µL of Curcumin; 2 µL of Etoposide; 2 µL of Temozolomide; 2 µL of Retinoic Acid; 2 µL of Talazoparib; 2 µL of Cobimetinib; 2 µL of Gefitinib; 2 µL of Doxorubicin; 2 µL of Ara-C; 2 µL of Cycloheximide; 2 µL of 2-Deoxyglucose; a

total of 2 µL of 5-FU plus Ara-C; and a total of 2 µL of Etoposide plus Doxorubicin. With the drugs added to the scratched mediums, initial images were taken with an (electron?) microscope to visualize the scratches, acting as the 0 hour set of images.

After hour 0, the wells were incubated for 24 hours before another set of images were taken of the (14?) wells. The imaging was repeated again at the 48 hour mark. The 0, 24, 48 hours were then processed using Fiji/ImageJ.

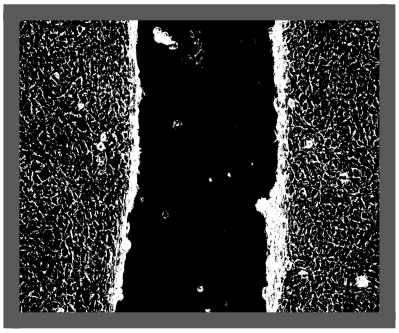

Fig 1. Scratch Width of 3T3-L1 Cell (Treated with 5-FU) at Baseline

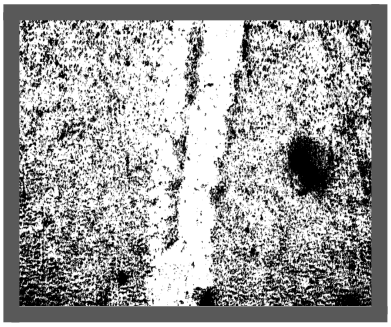

Fig 2. Scratch Width of 3T3-L1 Cell (Treated with 5-FU) at 24 hours

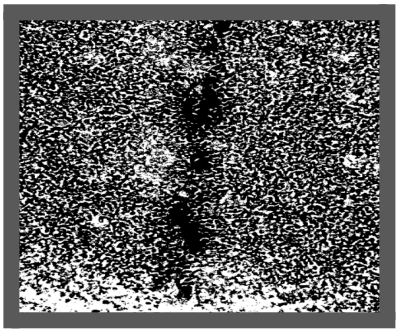

Fig 3. Scratch Width of 3T3-L1 Cell (Treated with 5-FU) at 48 hours

3. Results and Discussion

The scratch assay experiments revealed that different biochemical agents influence fibroblast migration in highly distinct ways. Chemotherapy drugs such as 5-Fluorouracil and Cytarabine produced a clear reduction in wound closure, which is consistent with their known role in disrupting DNA synthesis and slowing down rapidly dividing cells. These outcomes suggest that the assay is sensitive enough to capture the cytostatic properties of clinical chemotherapeutics.

Retinoic Acid showed reliable inhibition of migration as well. Its effects can be understood through its ability to promote cell differentiation and suppress genes associated with invasion and metastasis. The consistency of this result across replicates points to its value in further testing, especially as an agent that may help restrain the spread of malignant cells. Natural compounds like Resveratrol produced some of the strongest suppression of migration, and the computational modeling supported its long-term inhibitory effects. Since Resveratrol is known to act through multiple mechanisms, including mitochondrial regulation and anti-inflammatory signaling, its strong performance in this setting aligns well with prior studies.

The results with copper and vanadate ions suggest that metabolic stress can also reduce migration. Copper likely disrupted mitochondrial function, while vanadate may have interfered with phosphatase activity that is required for cell proliferation. These pathways do not directly overlap with DNA synthesis inhibition, showing that fibroblast motility can be disrupted through several independent routes.

Not every compound demonstrated a clear inhibitory effect. Talazoparib and Doxorubicin produced more irregular patterns of wound closure, and in some cases the migration appeared to continue despite treatment. These outcomes may reflect differences in how fibroblasts respond

compared to cancer cell lines, or they may simply point to the limits of a short-term, 48-hour assay. Longer observations or tumor-derived cells could reveal effects that were not visible here. Variability in scratch width at baseline also introduced some inconsistency, reminding us that method standardization will be important in future work.

Overall, the findings suggest that fibroblast scratch assays are useful for identifying both promising and unreliable compounds when it comes to inhibiting cell migration. By pairing the imaging results with computational models of population growth, the study added a layer of validation that supports the biological interpretations. At the same time, the differences among agents highlight the complexity of designing combination therapies, since some compounds may reinforce each other while others interfere.

4. Conclusion

This study showed that biochemical agents can produce very different effects on fibroblast migration in a scratch assay model. Chemotherapeutics such as 5-Fluorouracil consistently reduced migration, while compounds like Retinoic Acid and Resveratrol also demonstrated strong and reliable inhibitory effects. Metals such as copper and vanadate impaired motility through stress on mitochondrial and signaling pathways. In contrast, agents like Talazoparib and Doxorubicin gave less consistent results, which may have been influenced by assay limits or model choice.

Taken together, these outcomes suggest that the assay can serve as an effective screening tool to measure how drugs influence wound closure and cell proliferation. The results also reinforce the idea that the biological impact of any compound depends on both its molecular mechanism and the model system in which it is tested. Moving forward, extending the analysis to tumor-derived cells, incorporating longer time frames, and testing direct drug combinations will help clarify which agents show true promise for inhibiting tumor spread.

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