PHARMACOLOGICAL INHIBITION OF LUNG CANCER METASTASES

By

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APPROVED

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ABSTRACT

Cancer has contributed to the annual mortality statistics worldwide, 90% of which are due to metastatic cancer, the spread of cancer cells to healthy tissues. Despite the significant advancements in cancer therapeutics, targeting metastasis remains challenging. Furthermore, 50% of lung cancer patients are diagnosed with metastatic lung cancer, ranking first in mortality among all tumors worldwide. This project aims to inhibit cell signaling that promote tumor cell migration of non-small cell lung cancer (NSCLC), the most common type.

By monitoring cell migration using the scratch wound assay in a 96-well plate and surrogate cellular assay, chemical compounds with known properties are tested. The scratch wound assay entails making a uniform scratch in each well, treating with agents, and observing the cells migrating to fill the gap or "wound". These agents, some of which are FDA-approved drugs, are kinase inhibitors obtained from a kinase screening library. Kinase inhibitors are of interest since a cascade of kinase reactions facilitates most cancer cell signaling. In the case that one or more of these kinase inhibitors slow down the spread of cells, those inhibitors are further studied in a cell viability assay, demonstrating occurrence of agent toxicity on A549 NucLight Red cells, and analyzed for synergy. These studies will indicate that the inhibition of one or more kinases could suppress aberrant pro-migratory signaling in A549 NucLight Red lung cancer cells and that these kinase inhibitors positively affect suppression of cancer cell migration pathway, thus contributing to lower mortality rates for cancer patients.

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INTRODUCTION

Lung cancer is a prevalent and devastating disease worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases (American Cancer Society). The ability of NSCLC to metastasize is a leading cause of mortality in patients, and despite advancements in treatment strategies, the prognosis for those with metastatic NSCLC remains poor (American Cancer Society; Riihimäki et al., 2014). Consequently, there is an urgent need to develop novel therapies that specifically target metastasis in NSCLC.

One promising approach to address the challenge of cancer involves the screening of small molecule kinase inhibitors. Kinases are enzymes that play a crucial role in cell signaling pathways and are often dysregulated in cancer, making them attractive targets for therapy (Schlessinger, 2000). These enzymes regulate various cellular processes such as cell growth, proliferation, and apoptosis. By identifying compounds that can inhibit the activity of specific kinases involved in cancer progression, researchers aim to discover effective treatments for cancer, including non-small cell lung cancer (NSCLC). Specifically, this study focuses on screening small molecule kinase inhibitors using the A549 NucLight red cell line (Hulkower & Herber, 2011; Grada et al., 2017).

Kinase inhibitors have shown significant therapeutic potential in the field of cancer research. Several kinase inhibitors have been developed and approved for the treatment of various cancers, such as imatinib for chronic myeloid leukemia and trastuzumab for HER2-positive breast cancer. These inhibitors can selectively bind to specific kinases, blocking their activity and disrupting the signaling pathways that promote cancer cell growth and survival (Zhang et al., 2009). By targeting key kinases involved in the dysregulated signaling networks of

cancer cells, kinase inhibitors can inhibit tumor growth and potentially improve patient outcomes.

Despite their therapeutic benefits, kinase inhibitors can also have side effects due to their broad effects on signaling pathways. Common side effects include gastrointestinal disturbances, fatigue, skin rashes, and hematological abnormalities (Sawyers, 2004). Some kinase inhibitors have been associated with cardiovascular toxicities, such as hypertension and impaired cardiac function (Liu et al., 2013). Additionally, kinase inhibitors can interact with other medications, leading to potential drug-drug interactions and affecting their efficacy or toxicity (Finocchiaro, Giovanna et al., 2015).

It is worth noting that the long-term effects of kinase inhibitors are still being studied, and there may be potential unknown side effects. As these inhibitors target specific kinases involved in normal cellular processes, their inhibition could disrupt physiological functions beyond cancer cells. Therefore, careful monitoring and further research are necessary to understand the full spectrum of side effects associated with kinase inhibitors.

Therefore, the screening of small molecule kinase inhibitors represents a promising approach for developing effective treatments for cancer, including NSCLC. By targeting dysregulated signaling pathways in cancer cells, these inhibitors can disrupt the growth and survival of tumor cells. However, it is important to consider the potential side effects of kinase inhibitors, both known and unknown, to ensure their safe and effective use in clinical practice.The A549 NucLight red cell line is a commonly used human lung adenocarcinoma cell line that serves as a valuable model for NSCLC (Wistuba et al., 2015). Through genetic modifications, these cells express a red fluorescent protein in the nucleus, allowing real-time imaging of cell behavior during migration and invasion (Wu et al., 2016; Grada et al., 2017).

Due to its highly metastatic nature, the A549 NucLight red cell line has been extensively studied in the context of NSCLC (Wu et al., 2016).

The global and U.S. statistics regarding metastasis and NSCLC remain alarming. In the United States, lung cancer continues to be the leading cause of cancer-related deaths in both men and women. According to the American Cancer Society, it is estimated that in 2022 there were approximately 230,200 new cases of lung cancer and 135,720 deaths resulting from the disease ("Key Statistics for Lung Cancer"). Worldwide, lung cancer still accounts for a staggering number of deaths each year, with an estimated 1.76 million deaths annually. Non-small cell lung cancer (NSCLC) remains the predominant type of lung cancer, representing the majority of cases (American Cancer Society).

Current treatments for NSCLC metastasis are limited and often ineffective. While chemotherapy is the most common treatment, it is associated with significant side effects and limited efficacy in metastatic disease (Cancer Research UK). Chemotherapy drugs target rapidly dividing cells, including cancer cells, but they can also affect healthy cells in the body, leading to various adverse effects. These side effects can range from mild to severe and may include nausea, vomiting, hair loss, fatigue, decreased blood cell counts, neuropathy, and increased risk of infection (National Cancer Institute).

Chemotherapy drugs work by disrupting the cell division process, but they can also impact normal cells that have a high turnover rate, such as those in the gastrointestinal tract, bone marrow, and hair follicles. As a result, patients undergoing chemotherapy often experience gastrointestinal disturbances, such as nausea and vomiting, as well as hair loss and decreased blood cell counts, which can lead to anemia, increased susceptibility to infections, and fatigue (American Cancer Society).

Furthermore, chemotherapy can have long-term effects on the body even after the treatment is completed. Some individuals may experience cognitive changes, commonly referred to as "chemo brain," which can include difficulties with memory, attention, and concentration (American Cancer Society). These side effects not only impact the physical well-being of patients but can also affect their overall quality of life.

Kinase inhibitors offer a promising alternative, as they can target specific dysregulated signaling pathways in cancer cells (Schlessinger, 2000). Unlike chemotherapy, which affects rapidly dividing cells, kinase inhibitors can selectively target cancer cells while sparing healthy cells (Schlessinger, 2000). Several pathways, including mTOR, c-Src, Abl, and PI3K, are frequently dysregulated in NSCLC and have known roles in cell migration and metastasis (Lara-Guerra et al., 2018).

The mTOR pathway, involved in cell growth and survival, has been found to promote migration and invasion in NSCLC cells (Lara-Guerra et al., 2018). The c-Src pathway, which regulates cell adhesion and migration, is upregulated in NSCLC cells (Lara-Guerra et al., 2018). The Abl pathway has been implicated in regulating cell migration and invasion in NSCLC cells (Lara-Guerra et al., 2018). The PI3K pathway, responsible for cell proliferation and survival, is dysregulated in NSCLC cells (Lara-Guerra et al., 2018).

One of the downstream targets of PI3K is the serine/threonine kinase Akt, which mediates cell growth through stabilization of cyclin D1 and downregulation of Cdk inhibitors p27 and p21 (Teng et al.). The PI3K/Akt/mTOR pathway is a canonical pathway involved in anti-apoptosis and prosurvival, which regulates a number of normal cellular activities, such as cell proliferation, survival, and migration (Ying et al.). Dysregulation of this pathway has been implicated in the development of various cancers, including NSCLC (Tan et al.). In NSCLC

cells, the PI3K/Akt/mTOR pathway is activated, leading to increased cell proliferation and survival (Zhang et al.). Therefore, targeting this pathway has been proposed as a potential therapeutic strategy for NSCLC (Tan et al.; Moore et al.). Several studies have investigated the role of the PI3K pathway in NSCLC, including its relationship to other signaling pathways (Wang et al.; Gong et al.; Xu et al. 1994). Mutations of genes in these pathways produced mutated proteins, overexpressed or deleted base pair, depending on the case, resulting in biological effects which increased proliferation, genomic instability, changed cytoskeleton and deminsed apoptosis and many cancerous process ("Pi3k Pathway Gene Polymorphism in Breast Cancer Tissues Proliferation").

Several compounds within the kinase library target these pathways, such as AZD8055 (a dual mTORC1/mTORC2 inhibitor), Dasatinib (a c-Src inhibitor), Bosutinib (an Abl inhibitor), and LY294002 (a PI3K inhibitor) (Lara-Guerra et al., 2018; National Cancer Institute). These compounds have demonstrated inhibitory effects on migration and invasion in other cancer cell lines, suggesting their potential efficacy in treating NSCLC metastasis (Lara-Guerra et al., 2018; National Cancer Institute). This study aims to assess the effectiveness of these compounds in inhibiting migration of A549 NucLight Red cells.

Therefore, the development of novel therapies targeting metastasis in NSCLC is crucial for improving patient outcomes. Screening small molecule kinase inhibitors represents a promising approach to identify compounds that can inhibit migration and invasion of NSCLC cells. The A549 NucLight red cell line serves as a valuable tool for studying the metastatic behavior of NSCLC cells. Kinase inhibitors have the potential to be more effective and less toxic than traditional chemotherapy, making them an attractive alternative for treating metastatic

NSCLC (Dong et al., 2021). Further research is necessary to determine the efficacy of these compounds in the treatment of NSCLC metastasis.

METHODS

Preparing A549NucLight Red Cells for Cell Wound Migration Assay

To prepare A549 NucLight Red cells for the cell wound migration assay, several steps were followed. Initially, the cells were cultured in a tissue culture flask supplemented with 1 µg/ml puromycin, a selective antibiotic used to retain the red fluorescence of the cells (Sartorius). The flask was maintained at a controlled environment of 37°C with 5% CO2 and 95% humidity until the cells reached a confluency of 80-90%, indicating that they had formed a monolayer (Sartorius). This ensured that the cells were in a suitable state for conducting the cell wound migration assay.

Once the desired confluency was achieved, the cells were trypsinized to detach them from the flask surface. Trypsinization involves using the enzyme trypsin to break the cell-cell and cell-substrate connections, allowing the cells to become suspended in a solution (Sartorius). The resulting cell suspension was then centrifuged at 1000 RPM for 5 minutes to pellet the cells (Sartorius). Centrifugation enables the separation of cells from the surrounding medium by applying centrifugal force, causing the cells to settle at the bottom of the tube.

Following centrifugation, the cell pellet was gently resuspended in fresh complete media. The complete media used for the resuspension consisted of Dulbecco's Modified Eagle Medium (DMEM)/Nutrient Mixture F-12 supplemented with 10% Fetal Bovine Serum (FBS) and 1 µg/ml puromycin (Sartorius). The resuspension in complete media provides the cells with essential nutrients, growth factors, and serum proteins required for their survival and proliferation.

The resuspended cells were allowed to grow in the complete media until the screening library was obtained for the subsequent cell migration assay. During this period, the cells proliferated and continued to maintain their red fluorescence due to the presence of puromycin in the culture medium..

Cell Migration Assay

Moving on to the cell migration assay, 35,000 A549 NucLight Red cells were plated per well in two 96-well plates. This ensured replicates for the top plate of the screening library, providing multiple data points for analysis (Sartorius). The cells were cultured in DMEM media supplemented with 10% FBS and 1 µg/ml puromycin to support their growth and maintain the red fluorescence.

The plated plates were then incubated in the IncuCyte S3 Live-Cell Analysis Instrument at 37°C with 5% CO2 and 95% humidity for 18 hours or until a confluency of 90% was reached. This allowed the cells to form a monolayer and reach the desired density for the subsequent steps of the assay (Sartorius).

Once the cells reached the desired confluency, a scratch or wound was created in each well using the Incucyte® WoundMaker. This specialized tool allowed for the generation of a uniform gap or wound in the cell monolayer, ensuring consistency across the wells (Sartorius). It is important to note that the WoundMaker was cleaned between the plates to prevent any potential cross-contamination.

After the wound was made, the wells were carefully aspirated to remove any debris and prepare them for treatment. Each well was then treated with 2.5 µM of its respective compound, which could be a Kinase Inhibitor or dimethyl sulfoxide (DMSO) as a control (Sartorius). The

treatment aimed to investigate the effects of the compounds on cell migration and wound healing.

Subsequently, the plates were placed into the IncuCyte® S3 Live-Cell Analysis Instrument for imaging every 3 hours for a total of 36 hours, equivalent to approximately 1 and a half days. The instrument captured images of the cells in each well, allowing for real-time monitoring of cell migration and wound closure over time (Sartorius).

To analyze the acquired images, the IncuCyte scratch wound assay software was utilized. This software utilized image analysis algorithms to quantify the closure of the wound area. Specifically, it evaluated the Red Object Wound Count (ROWC) in each well, indicating the presence of red fluorescence within the wound (Sartorius). By measuring the change in ROWC over time, the software provided quantitative data on cell migration and wound healing in response to the different treatments.

Hence, the preparation of A549 NucLight Red cells for the cell wound migration assay involved maintaining the cells, trypsinizing and resuspending them, and allowing them to grow. The cell migration assay itself included plating the cells, creating a wound, treating the wells, incubating and imaging the cells, and analyzing the acquired images using specialized software. These steps were essential for investigating the effects of compounds on cell migration and wound healing in a controlled and quantitative manner.

Figure 1: Summary of A549 NucLight Red Cell Sterile Culture and Wound Migration Assay (created in BioRender®)

RSULTS

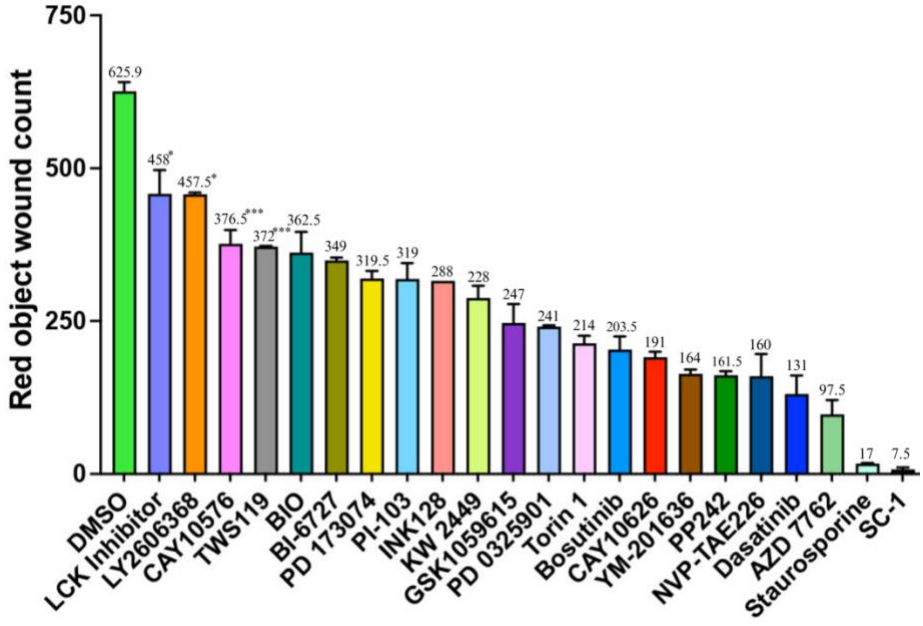


Figure 2: Wound migration assay of A549 NucLight Cells treated with 0.5% DMSO and 2.5µM compounds. Red Object Wound Count (ROWC) proved **high significance** via one-way ANOVA with Dunnett test for all the compounds listed in the bar graph, indicating a difference in wound closure compared to DMSO. The ROWC, which refers to the red cells found in the wound, averages are depicted above standard error bars.

A) B)

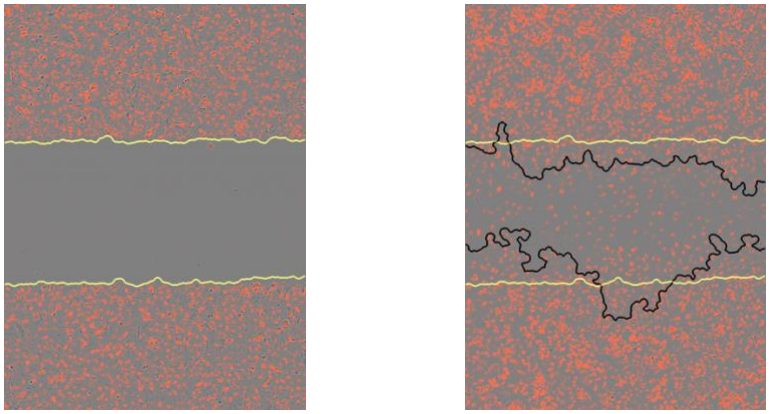
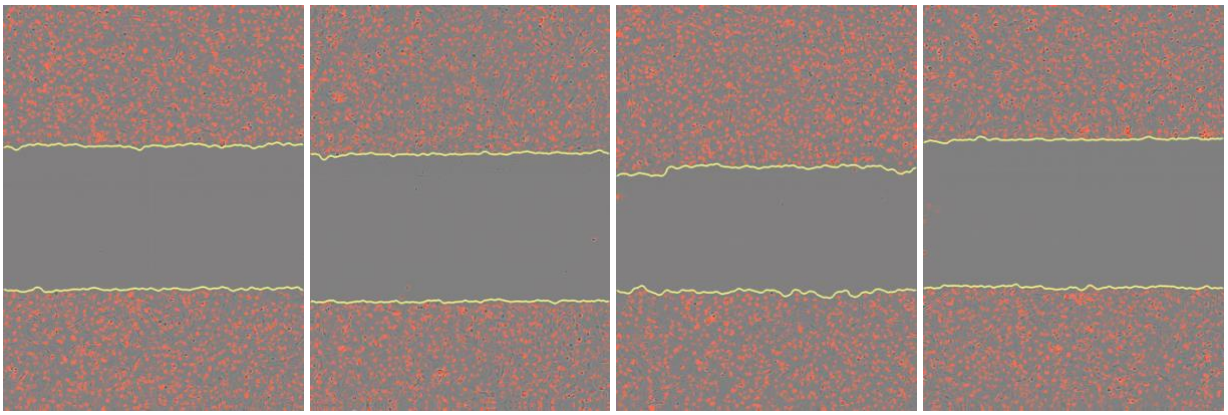


Figure 3: Cell migration assay of A549 NucLight Red Cells treated with 0.5% DMSO. The yellow lines indicated initial scratches at 0 h (A), while the black lines traced* migratory cell confluency at 36h (B).

A) PP242 CAY10626 Bosutinib BIO



B) PP242 CAY10626 Bosutinib BIO

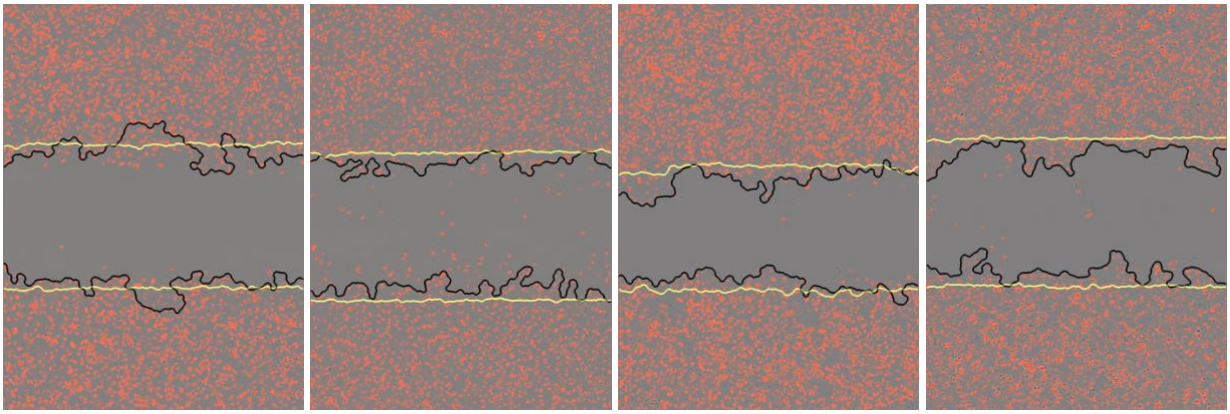
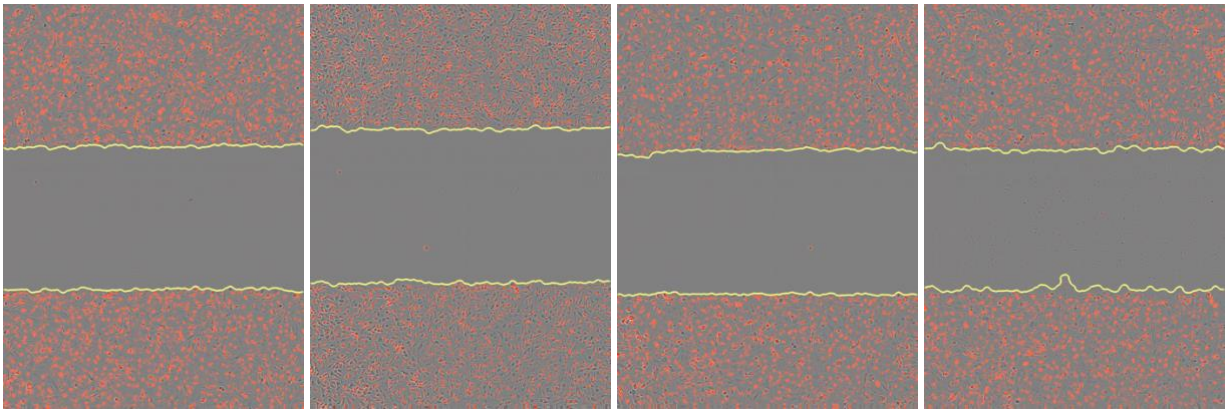


Figure 4: Cell migration assay of A549 NucLight Red Cells treated with 2.5 µM compounds. PP242, CAY10626, Bosutinib, and BIO all showed high potential at 2.5 µM concentration with PP242 showing highest potential. The yellow lines indicated initial scratches at 0 h (A), while the black lines traced* migratory cell confluency at 36h (B).

A) SC-1 Staurosporine NVP-TAE226 YM-201636



B) SC-1 Staurosporine NVP-TAE226 YM-201636

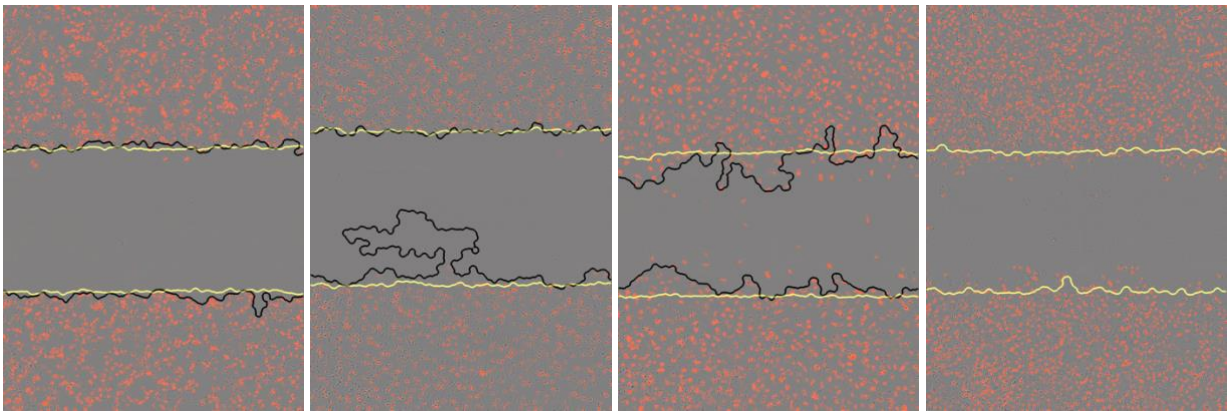


Figure 5: Cell migration assay of A549 NucLight Red Cells treated with 2.5 µM compounds. Shown compounds depicted A549 NucLight Red Cell death with different morphology thus due to various killing mechanisms. The yellow lines indicated initial scratches at 0 h (A), while the black lines traced* migratory cell confluency at 36h (B).

DISCUSSION

Upon conducting the Red Object Wound Count (ROWC) analysis and performing a one-way ANOVA with Dunnett test for the 2.5 µM LCK Inhibitor (LY2606368), CAY10576, TWS119, BIO, BI-6727, PD 173074, PI-103, KW 2449, GSK1059615, PD 0325901, INK128, Torin 1, Bosutinib, CAY10626, YM-201636, PP242, NVP-TAE226, Dasatinib, AZD 7762, Staurosporine, and SC-1 compounds against 0.5% DMSO, a p-value of less than 0.0001 was obtained. This p-value indicates a significant difference in wound closure between each compound and DMSO (see migration pattern of the control in Figure 3).

Further analysis of the IncuCyte S3 Live-Cell images at 0 h and 36 h revealed distinct categories for the compounds based on their potential. These categories are: high potential, possible potential, and no potential (with the highest cell death and various morphologies).

Among the compounds, those with the highest potential were ranked in descending order (Figure 4) as follows: PP242, CAY10626, Bosutinib, and BIO. These compounds exhibited minimal cell death, minimal migration, and maintained a healthy morphology.

The compounds with possible potential, listed in order of highest to lowest possible potential, are: AZD 7762, Dasatinib, Torin 1, GSK1059615, and PI-103. These compounds showed slightly more cell death and/or did not effectively inhibit migration.

Finally, the compounds with no potential (Figure 5), arranged from the highest to the lowest amount of cell death, are: SC-1, Staurosporine, NVP-TAE226, YM-201636, PD 0325901, KW 2449, INK128, PD 173074, BI-6727, TWS119, CAY10576, LY2606368, and LCK Inhibitor.

PP242 is a potent inhibitor of the mammalian target of rapamycin (mTOR) pathway, which is a key regulator of cell growth, proliferation, and survival. PP242 inhibits both mTORC1 and mTORC2, which are two distinct complexes of mTOR that have different downstream targets and functions (Vargova, Ingrid, et al.).

Non-small cell lung cancer (NSCLC) metastasis is a complex process that involves multiple signaling pathways, including the PI3K/AKT/mTOR pathway (Qu, Yunhui, et al.). The PI3K/AKT/mTOR pathway is frequently dysregulated in NSCLC, leading to increased cell proliferation, survival, and migration (Qu, Yunhui, et al.). PP242 inhibits the PI3K/AKT/mTOR pathway by blocking the activity of mTORC1 and mTORC2, which results in decreased cell growth and survival (Zeng, Zhihong, et al.).

In the context of NSCLC metastasis, PP242 may inhibit the epithelial-mesenchymal transition (EMT) process, which is a critical step in cancer cell invasion and metastasis (Qu, Yunhui, et al.). EMT is regulated by several signaling pathways, including the PI3K/AKT/mTOR pathway, and involves the loss of epithelial markers and the acquisition of mesenchymal markers (Qu, Yunhui, et al.). PP242 may block the PI3K/AKT/mTOR pathway

and prevent the activation of downstream transcription factors, such as Snail, which are involved in EMT (Qu, Yunhui, et al.).

In the Wound Healing Assay conducted with A549 NucLight Red Cells, PP242 at a concentration of 2.5 μM was found to be a potential inhibitor of NSCLC metastasis (Figure 4). This suggests that PP242 may be effective in blocking the migration and invasion of NSCLC cells, which are critical steps in the metastatic process.

Bosutinib is a dual Src/Abl kinase inhibitor that has been found to inhibit the growth of various cancer cells, including non-small cell lung cancer (NSCLC) cells (Tan, Daniel Shao-Weng, et al.). NSCLC metastasis is a complex process that involves multiple signaling pathways, including the Src/Abl pathway (Tan, Daniel Shao-Weng, et al.). Bosutinib inhibits the migration and invasion of NSCLC cells by targeting the Ack1 protein, which is a downstream effector of the Src/Abl pathway (Tan, Daniel Shao-Weng, et al.). Ack1 is a non-receptor tyrosine kinase that is overexpressed in NSCLC cells and promotes cell migration and invasion (Tan, Daniel Shao-Weng, et al.). Bosutinib inhibits Ack1 at an IC50 of 2.7 nM, which is much lower than its IC50 for Src and Abl kinases (Tan, Daniel Shao-Weng, et al.). Therefore, Bosutinib is a potent inhibitor of Ack1 and can effectively suppress NSCLC metastasis by blocking the Src/Abl/Ack1 signaling pathway.

BIO is a small molecule inhibitor of glycogen synthase kinase-3 (GSK-3) Lai, Xianghong, et al.). GSK-3 is a serine/threonine kinase that plays a crucial role in various cellular processes, including cell proliferation, differentiation, and apoptosis. In NSCLC, BIO has been shown to inhibit cell proliferation, induce apoptosis, and suppress cell migration (Lai, Xianghong, et al.). The typical IC50 of BIO varies depending on the cell line and experimental conditions, but it ranges from 0.5 to 10 μM (Lai, Xianghong, et al.).

One of the key pathways involved in NSCLC metastasis is the VEGF/VEGFR2 signaling pathway (Lai, Xianghong, et al.). VEGF is a potent angiogenic factor that promotes tumor growth and metastasis by stimulating the formation of new blood vessels. VEGFR2 is a receptor for VEGF that is expressed on the surface of endothelial cells and plays a critical role in angiogenesis. Inhibition of the VEGF/VEGFR2 pathway has been shown to suppress NSCLC metastasis (Lai, Xianghong, et al.).

BIO inhibits NSCLC metastasis by downregulating Ki-67 and VEGF, inducing apoptosis by activation of cleaved-Caspase-3 and cleaved-Caspase-9, and suppressing cell migration by downregulating MMP-2 and VEGF (Lai, Xianghong, et al.). These effects are mediated by the inhibition of GSK-3, which regulates the expression and activity of various downstream effectors, including β-catenin, NF-κB, and c-Myc (Lai, Xianghong, et al.). Inhibition of GSK-3 by BIO leads to the suppression of these effectors, which in turn inhibits NSCLC metastasis.

SC-1 and Staurosporine are two compounds that have been observed to induce similar morphological changes associated with cell death (Figure 5), but further investigation is needed to determine the specific type of cell death they induce in the context of non-small cell lung cancer (NSCLC) and its known kinase pathways (Lara-Guerra et al. 2018). The precise cell death mechanism and morphology caused by SC-1 and Staurosporine in NSCLC are yet to be fully elucidated (Lara-Guerra et al. 2018). However, previous studies have reported that Staurosporine, a broad-spectrum kinase inhibitor, can induce apoptotic cell death characterized by cell shrinkage, chromatin condensation, and formation of apoptotic bodies, which was observed in Frigure 5 (Fares et al. 2020). These observations suggest that Staurosporine may activate apoptotic pathways in NSCLC cells (Fares et al. 2020). Similarly, SC-1 has been observed to induce cell death morphology characterized by cell shrinkage and membrane

blebbing (Figure 5), which are indicative of apoptotic or necrotic cell death (Hulkower and Herber 2011). Understanding the specific cell death mechanisms triggered by SC-1 and Staurosporine and their relationship to NSCLC kinase pathways could provide valuable insights into potential targeted therapies for NSCLC metastasis (Lara-Guerra et al. 2018). Further research is needed to determine the exact mechanisms and pathways involved in the cell death induced by these compounds in NSCLC (Lara-Guerra et al. 2018).

NVP-TAE226 and YM-201636 are two small molecule kinase inhibitors that have been investigated for their effects on cell death mechanisms in non-small cell lung cancer (NSCLC). When studying the impact of NVP-TAE226, it was observed that this compound induced significant cell death in NSCLC cells. The specific cell death morphology induced by NVP-TAE226 remains to be determined. However, it is worth noting that despite its ability to induce cell death, NVP-TAE226 still exhibited some residual migration and cell survival. This suggests that NVP-TAE226 may not completely inhibit the migration of NSCLC cells and may have varying effects on different signaling pathways involved in cell survival and migration.

On the other hand, YM-201636 also displayed the ability to induce cell death in NSCLC cells. The exact cell death morphology caused by YM-201636 has not been characterized in the context of NSCLC. To better understand the mechanisms underlying the cell death induced by YM-201636, further investigation is required. It is important to consider that the observed effects on cell migration and survival may be influenced by the dysregulated kinase pathways present in NSCLC cells.

Therefore, the Red Object Wound Count (ROWC) analysis and subsequent analysis of the IncuCyte S3 Live-Cell images have provided valuable insights into the potential of various compounds as inhibitors of non-small cell lung cancer (NSCLC) metastasis. The one-way

ANOVA with Dunnett test revealed a significant difference in wound closure between each compound and the control (0.5% DMSO). The compounds with the highest potential to inhibit NSCLC metastasis were PP242, CAY10626, Bosutinib, and BIO. These compounds exhibited minimal cell death, minimal migration, and maintained a healthy morphology. The compounds with possible potential were AZD 7762, Dasatinib, Torin 1, GSK1059615, and PI-103, showing slightly more cell death and/or less effective inhibition of migration. The compounds with no potential included SC-1, Staurosporine, NVP-TAE226, YM-201636, PD 0325901, KW 2449, INK128, PD 173074, BI-6727, TWS119, CAY10576, LY2606368, and LCK Inhibitor, which exhibited varying degrees of cell death and impaired morphology.

It is important to note that the specific mechanisms of action and cell death induced by some of these compounds in the context of NSCLC are not fully understood and further research is needed to elucidate their effects on NSCLC metastasis.

CONCLUSION

The results of the study conducted aimed to investigate the efficacy of various small molecule kinase inhibitors in inhibiting the migration of A549 NucLight Red cells, which serve as a valuable model for studying metastatic behavior in non-small cell lung cancer (NSCLC). The significance of this research lies in the urgent need for novel therapies that target metastasis in NSCLC, as it is associated with high mortality rates. Currently available treatments for NSCLC metastasis are limited, and there is a demand for more effective and less toxic alternatives to chemotherapy.

By screening a range of small molecule kinase inhibitors, the study aimed to identify compounds that could effectively inhibit the migration and invasion of NSCLC cells. Kinases are

enzymes that play crucial roles in cell signaling pathways and are frequently dysregulated in cancer, making them attractive targets for therapeutic interventions.

The findings of the study revealed several compounds that exhibited high potential in inhibiting the migration of A549 NucLight Red cells. Notably, PP242, CAY10626, Bosutinib, and BIO demonstrated promising results by significantly inhibiting cell migration while maintaining cell viability. The compounds, at a concentration of 2.5 µM, exhibited a significant inhibition of wound closure compared to the control group treated with 0.5% DMSO, with a p-value of less than 0.0001.

Of particular interest was PP242, a dual mTORC1/mTORC2 inhibitor, which demonstrated the highest potential among the tested compounds. It showed remarkable efficacy in blocking the migration and invasion of NSCLC cells while preserving cellular morphology. This suggests that PP242 may hold promise as a therapeutic agent for inhibiting metastasis in NSCLC.

On the other hand, some compounds, such as Dasatinib, NVP-TAE226, YM-201636, and GSK1059615, exhibited substantial cell death and reduced cell viability compared to the control group. The impact of these compounds on cell viability warrants consideration, particularly their respective IC50 values, before further analysis can be conducted. Additionally, compounds like Staurosporine and SC-1 showed similar morphological changes associated with cell death, highlighting the need for further investigation to determine the specific type of cell death induced by these compounds.

In conclusion, the study demonstrated the potential of small molecule kinase inhibitors in inhibiting the migration of A549 NucLight Red cells, serving as a model for NSCLC metastasis.

The identification of compounds such as PP242, CAY10626, Bosutinib, and BIO as effective inhibitors of cell migration provides a promising avenue for developing targeted therapies for NSCLC metastasis. The dysregulation of specific signaling pathways in NSCLC makes them attractive targets for intervention, and small molecule kinase inhibitors offer a more targeted and potentially less toxic approach compared to traditional chemotherapy.

Further research is warranted to evaluate the efficacy of these compounds in the treatment of NSCLC metastasis. Continued investigation and optimization of small molecule kinase inhibitors may lead to the development of novel therapies that can improve patient outcomes and reduce the burden of metastatic NSCLC. The findings of this study contribute to the growing body of knowledge in the field of NSCLC metastasis and provide a basis for further exploration of targeted therapies.

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