DRUG INHIBITION OF PROSTATE CANCER METASTASES

By,

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ABSTRACT

Although there are drugs specified for the suppression of cancerous properties such as proliferation and cell death resistance, there are no current treatments targeting cellular metastasis. For cancer to metastasize, tumor cells detach from their original location, survive the circulatory system, accommodate to new surroundings, and avoid immune responses (Fares et. al., 2020). Signal transduction cascades are processes that lead to cellular responses, such as regulating cell migration, which is controlled by protein kinases. By using kinase inhibitors, I can target protein kinases and theoretically lower cancer cell migration, which will hopefully contribute to future anti-metastatic therapies and lower the mortality rate of cancer patients.

Prostate cancer is the second leading cause of cancer-related deaths and is the most common cancer in men (Tai et. al., 2011). Findings show that overexpression of protein kinases may drive metastasis in prostate cancer (Feng, 2016). The PC3 cell line I am using is a stage 4 prostate cancer cell line that is an androgen independent small cell carcinoma. Due to androgen independence, hormone therapies cannot be used, and androgens are not a possible drug target. By running scratch wound assays and testing kinase/FDA drug libraries, I will identify a drug (or a combination of drugs) that inhibits metastasis. The scratch wound assay creates physical barriers in each cell plate and using the INCUCYTE machine, I will image and analyze the effects of each drug and how it lowers migration between cancer cells.

OVERALL HYPOTHESIS: One or more of the kinase inhibitors may suppress pro-migratory signaling in prostate cancer (PC) cells. The data could provide insights of the mechanism of cell migration in PC and could guide new possible uses of currently FDA approved kinase inhibitors.

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BACKGROUND

In cancer, metastasis occurs when tumor cells that originated in one site, or organ, in the body move to other parts of the body and proceed to colonize those organs. There are targeted drugs specified for the suppression of other cancerous properties such as proliferation (cell reproduction) and resistance to cell death, however there are none for metastasis. Since cancer migration to other parts of the body causes over 90% of mortality in cancer patients, it is necessary to find treatments suppressing this activity (Fares et. al., 2020).

For cells to metastasize, the tumor cells must first detach from its original location, then survive the circulatory system, accommodate to its new surroundings in the new site, and avoid immune responses that would kill the cell (Fares et. al., 2020). Many of these processes include signal transduction cascades, which essentially refer to a domino effect beginning with the binding of a cellular receptor and continuing within the cell until a certain response is achieved within the cell. Thus, inhibiting any part of a signal transduction cascade may stop the sequence of events at a certain point and stop the cellular goal from being achieved. These cascades are extremely informative because many of them include molecules called inhibitors, which prohibit certain reactions from taking place, and kinases, which are commonly used enzymes frequently found in these cascades. Metastatic signal transduction is not well understood and understanding these processes play an influential role in how cancer therapies are developed, especially because drugs used in chemotherapies are used to slow down cancer growth.

The main objective for researching prostate cancer cells is to use experimental methods that will hopefully help us identify the pathways that allow prostate cancer cells to metastasize. Using laboratory methods such as scratch wound assays and testing kinase/FDA drug libraries, we will try to identify a drug that inhibits metastasis (or a combination of drugs) that can be

applied to cancer treatments. From this, we can hopefully contribute to future anti-metastatic therapies and lower the mortality rate of cancer patients.

As mentioned previously, kinases play a key role in several signal transduction events and are now a large target for cancer therapies. Kinases can turn several activities on or off and regulate various interactions within the cell, including cell migration. Our DNA can be altered by kinase activity which changes how proteins are manufactured and carry out certain functions. Out of 538 protein kinases that our genes encode, many protein (enzymes are proteins) kinases are involved in cancer growth and the targeting of these kinases is gaining more involvement in clinical trials (Bhullar et. al., 2018). Using kinase inhibitors, we can target these protein kinases and stop them in the signal cascade, thus ending the domino effect. This in turn should lower cell migration because the pathway is stopped. Therefore, kinases are an extremely favorable target for cancer drug treatments due to their abundance in the cell and inhibition of kinases have shown anti-proliferatory mechanisms in cancer (Bhullar et. al., 2018).

The prostate cancer cell line we will be using is the PC3 cancer cell line. Prostate cancer is the second leading cause of cancer-related deaths and is the most common cancer in men (Tai et. al., 2011). The PC3 cancer cell line in specific is a stage 4 prostate cancer cell that is a small cell carcinoma (meaning it begins in skin/tissue) and is androgen independent. Androgen independence means this cancer cell does not respond to hormone therapies because this specific cell line does not need an androgen to complete its signal transduction cascade. Thus, androgens cannot be used as a target. Findings have shown that the overexpression- and thus overabundance- of protein kinases may drive metastasis in prostate cancer (Feng, 2016). Because kinase inhibitors are beginning to enter the clinical setting (meaning that it can now be utilized for human treatment), it is imperative that research is done on prostate cancer cells in the

laboratory to study the effects they have on cancer cells before making a conclusion on how they can be utilized for cancer treatments.

MATERIALS AND METHODS

In preparation of the advanced laboratory techniques required by this research proposal, I was taught various techniques to practice skills necessary in the laboratory. I was able to master pipetting skills, BCA assays for protein quantification, and SDS-PAGE for protein separation by mass. I also received training in sterile human cell culture techniques and laboratory safety guidelines.

In this study we will be analyzing how cells migrate using scratch wound assays and a library of kinase inhibitors. First, this requires culturing and growing the prostate cancer cells for the cellular assay alongside the guidance of my principal investigator.

Sterile Tissue Culture: In order to test the PC3 cell line against various treatments, it is necessary to grow and maintain the cell line outside of their natural environment and inside of a laboratory environment. Using a sterile environment allows researchers to create a more controlled environment where one can control the growth of the cells and reduce contamination of the cell line. Contamination can alter how cells react to treatments and will likely lead to the flask of cells being discarded. For this experiment, I utilized an immortalized cell line, meaning that the cells are altered such that they can divide indefinitely by bypassing cellular death mechanisms. This is different from normal cells in the human body- which are programmed to die. In contrast, cancer cells obtain genetic mutations that allow them to proliferate and migrate at advanced capabilities (Immortalized Cell Line). Immortalized cells are not necessarily cancer cells because they are not transformed for enhanced proliferation, however the cells we used were cancerous (Immortalized Cell Line). Although immortalized cell lines are helpful for scientific research, genetic mutations may accumulate, and these cell lines may behave differently than *in vivo*.

The project began by receiving the human PC3 cell line from our distributor and thawing the cells from the frozen vial.

Thawing: Thawing cells includes placing the vial of cells in a warm bath for about 3 minutes and then resuspending the cells and adding media. The cellular media I used is called RPMI + Fetal Bovine Serum. RPMI contains a pH indicator and creates favorable environments for redox reactions to occur due to reducing agent glutathione. Although there are vitamins present in RPMI, it is considered an incomplete media because there are no lipids, proteins, or growth factors. These nutrients are substituted by adding fetal bovine serum. The cells are then resuspended, centrifuged, and the supernatant is removed. The cells are then fed and placed into a new flask.

While thawing cells, it is critical to work fast due to the addition of DMSO, Dimethyl Sulfoxide. This agent reduces ice and prevents cell death during the thawing process but may induce apoptosis due to increased cell permeability if left for too long.

Feeding Cells: In order to feed cells, I removed the old media and replaced it with 10 mL of new RPMI media with Fetal Bovine Serum.

Splitting Cells: Cells were split when they were too confluent, which was around 80-90% confluency for the PC3 cell line. After checking the confluency, I washed the flask with phosphate buffered saline (PBS) twice. PBS removed cellular debris and kept cells from shriveling up. Then, I added 3 mL trypsin, which is an agent used to detach the cellular monolayer from the flask. When using trypsin, it is important to be cautious of the time it is incubated because if left for too long, trypsin strips the cells of proteins which can lead to cell

death. After incubating, 3mL RPMI + Fetal Bovine Serum is added to neutralize the trypsin and cells are fed as usual. When adding media, it is important to add the same amount or more of media than the amount of trypsin was added to ensure trypsin is neutralized.

Scratch Wound Assay: We plated 37,000 PC3 cells in each well of a 96-well plate and incubated overnight. Each treatment had two replicates. After incubation, the Incucyte Woundmaker Tool was used to create a "scratch," or physical gash between every well in the plate, as equally as possible. After the scratch is created, 0.5% DMSO was added to every well and 2.5 µl of each treatment was added to the experimental wells. For the control groups, 0.5% DMSO was added since DMSO does not have any inhibitory qualities and no compound was added as treatment. The negative control group will show what PC3 cell growth should look like without any inhibition i.e., the gap should be mostly filled with cells. Using the IncuCyte S3 Live- Cell Analysis, a picture was taken of the plates every 3 hours for 48 hours.

Live Cell Analyses: The IncuCyte S3 is a Live-Cell Analysis system that images cell plates over a period of time at certain intervals and then can analyze the data by calculating the cell proliferation, migration, and can show images of the cell morphology over time, all without disturbing the cells. The IncuCyte is a highly reliable machine that produces reproducible results, especially since the cells are not moved or manipulated while being observed.

Relative Wound Density Measurements: In order to analyze the migration of each treatment, I used relative wound density. Relative wound density measures the ratio of the occupied area of the gap to the total area of the initial gap. In other words, if the gap is closing, the relative wound density would increase because there are more cells in the scratch area. If the scratch is not being

filled by cells, the relative wound density would be low since the ratio of cells occupying the area of the gap is not much higher than the initial area occupied (none).

RESULTS

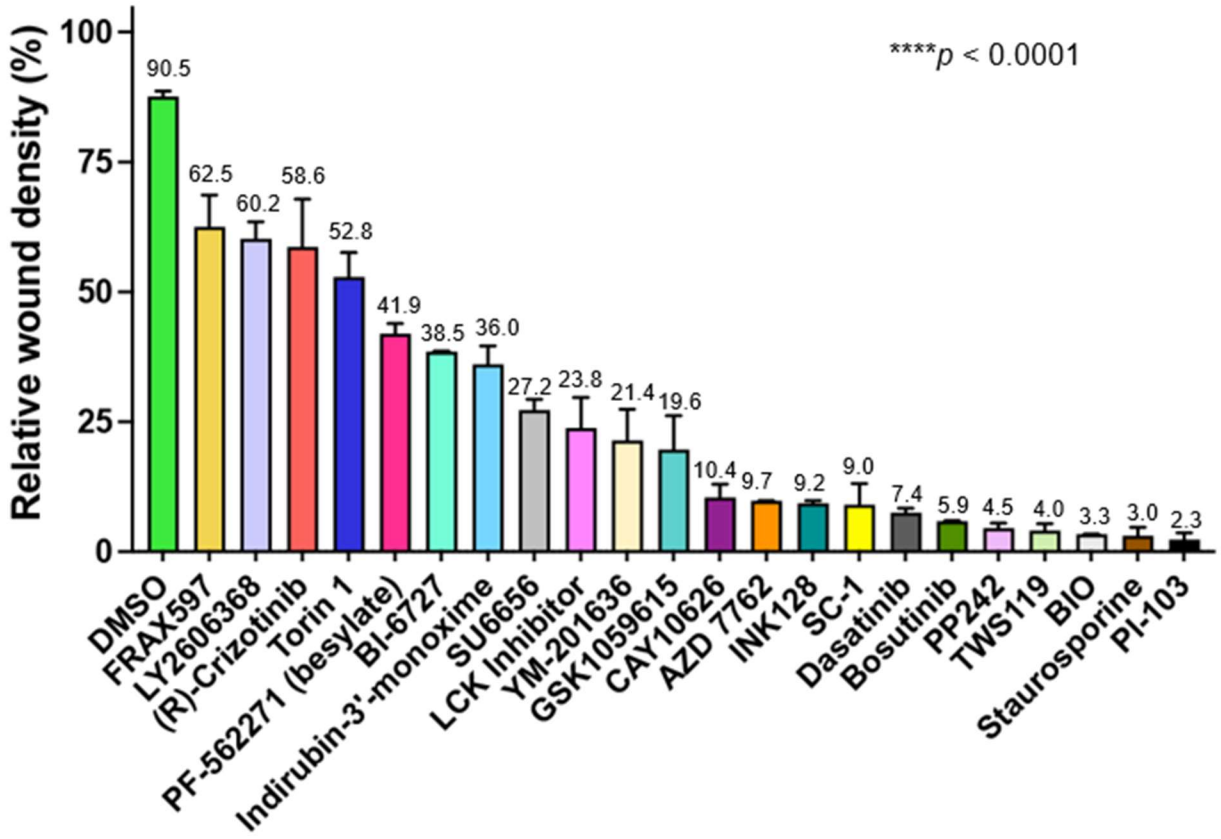

Figure 1. Relative Wound Density of 2.5 µM per compound at 36 h. Images were taken every 3 hours using the IncuCyte S3 live-cell analysis system. All compounds have a four-star p-value of < 0.0001, which shows that all compounds are statistically significant in comparison to DMSO. The numbers above the bars are the average relative wound density based on two replicates for each compound. Error bars are based on the two replicates of each compound.

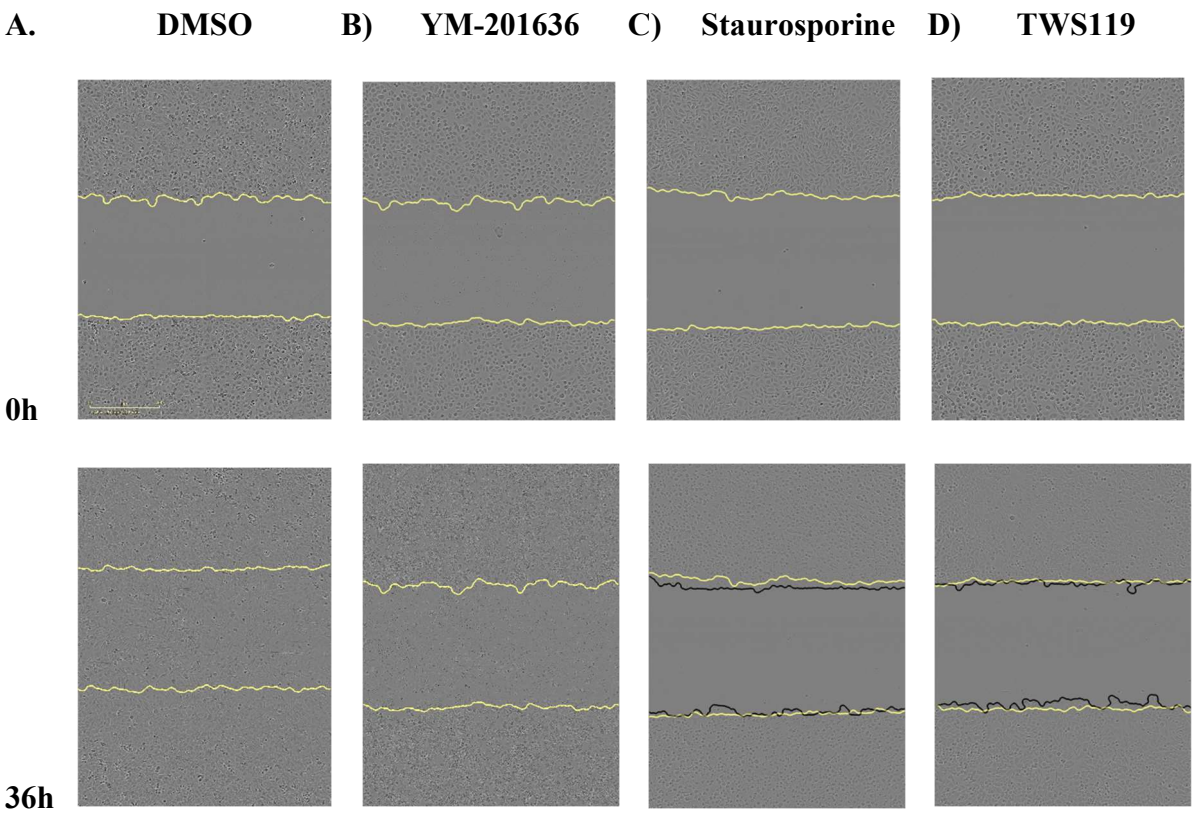

Figure 2. IncuCyte images are taken every 3h for 36h. Yellow lines indicate the initial wound at 0h and black lines indicate cell migration into the wound area at 36h. Scale bar is present at 0h DMSO, marking every 400 µM**. (A)** DMSO (0.5%) control does not inhibit metastasis and shows cell growth without inhibition. Most of the wound is filled with PC3 cells. DMSO has a high wound density, and all other compounds have a significantly lower wound density, indicating stronger inhibition. **(B)** 2.5 µM YM-201636 killed cancer cells based on the bubbles and rounded-up cell morphology. **(C)** 2.5 µM Staurosporine shows cell inhibition and cell death due rounded up cell morphology without any bubbles. **(D)** 2.5 µM TSW119 displays PC3 inhibition with live cancer cells.

Graph of Relative Wound Density (%) for each compound

The images were monitored until 36 hours because that is when Relative Wound Density began greatly decreasing, hence inhibition was highest at this time. In order to determine which compounds, leave viable cells, we referred to the Incucyte images and analyzed cellular morphology. As the compounds decrease on the graph (moving away from DMSO), the inhibition increases because there are less cells occupying the area of the gap.

Checkpoint Related Treatments

When cells are multiplying, there are various stops in the cell cycle that regulate division. Mechanisms within these checkpoints are controlled by a series of phosphorylation's, one of which is kinase activation (cyclin binding to the appropriate cyclin-dependent kinase) and the other is checkpoint control (Collins, K). When the various stages of the cell cycle detect DNA damage and other flaws in replication, division may be halted or delayed, whereas cancer cells evade CDK and checkpoint controls to divide uncontrollably (Collins, K). The following compounds are in some way related to checkpoint related pathways.

FRAX597

FRAX 597 is a p21-activated kinase (PAK) inhibitor selective for group I PAKs also inhibiting YES1, RET, SCR1R, and TEK (Frax597). By reducing PAK1 phosphorylation, FRAX597 halts the G1 phase of the cell cycle and prevents Schwann cells from proliferating, reducing both proliferation and survival of ovarian and pancreatic cancer cells (Licciulli). FRAX597 is not currently used in clinical trials; however, it shows high potential for therapeutic effects (Licciulli). As seen in Figure 1, this treatment had 62% relative wound density compared to the 90.5% density of non-inhibitory DMSO, showing that the compound is inhibiting migration.

LY2606368

LY2606368 is a checkpoint kinase 1 (Chk1) inhibitor studied in various colorectal cancer lines and causes double stranded DNA to break, leading to cell damage then death (Ly2606368). In p53- deficient HeLa cells, this compound inhibits doxorubicin-activated G2/M checkpoints and, at 25nM, induces apoptosis in gastric cancer cells. This treatment is being used in phase II clinical trials with ovarian cancer, small cell lung cancer, and solid advanced tumors.

AZD 7762

By competitive inhibition and reversible binding of ATP-binding sites, AZD 7762 is a selective inhibitor of checkpoint kinases 1 and 2 and eliminates the S and G2 checkpoints (Azd 7762). By inhibiting DNA repair at these sites, proliferation may be stopped, thus favoring cancer therapeutics. In phase I trials, tolerance of AZD 7762 was tested and stopped from further development due to potential cardiac toxicity (Sausville, E).

mTOR Related Treatments

The mammalian target of rapamycin (mTOR) regulates cell division, apoptosis, and gene transcription. This protein is critical in tumor metabolism and present in other pathways such as PI3K and AKT (Zou, Z). In cancerous cells, mTOR is overexpressed and upregulated. There are two complexes called mTOR1 (which regulates cell growth when energy is available) and mTOR2 (controlling cell survival and multiplication)(Zou, Z).

Torin 1

Torin 1 is a selective mTOR inhibitor, showing high specificity for mTOR over PI3Kα and other kinases (Torin 1). Since mTOR is activated in many cancers, it is an attractive target

for anticancer therapies and has been used in pre-clinical trials (Sun, S.). Studies have demonstrated that Torin 1 exerts superior antiproliferative effects and induces pronounced impairment of cellular growth when compared to rapamycin. (Sun, S.).

CAY10626

CAY10626 is a potent PI3Kα/mTOR inhibitor that prevents the production of PtdIns-(3,4)-P2 and PtdIns-(3,4,5)-P3, which regulate downstream targets in many cancers (Cay10626). It has shown synergistic effects in increasing cytotoxicity for pancreatic cancers through dual inhibition and should be considered for clinical trials due to strong cancer regression (Tian,Y).

INK128

By blocking TORC1 and TORC2 phosphorylation, this compound is an ATP-dependent inhibitor of mTOR kinases, thus interfering with rapamycin and pan-PI3K inhibitor resistant cell line growth (Ink128: Norisuke Shibuya). This treatment has shown inhibition of angiogenesis and studies have demonstrated future use of INK128 in preventing colorectal cancer recurrences and enhancement of Dasatinib efficacy in synergy (Shi-Yong Sun).

PP242

This treatment inhibits mTORC1 and mTORC2 alongside showing greater potency than rapamycin in leukemia cells (PP242). Although no clinical trials have been completed, studies have shown tumor inhibition in vivo for cancers such as colon cancer (Rashid, M).

PI3K Related Treatments

The PI3K/ AKT pathway is among the most overregulated pathways in tumor progression, contributing to cancer cell invasion and metastasis, among other cancerous properties (Rascios, F). The two components of the pathway are phosphatidylinositol-3-kinase (PI3K), a lipid enzyme triggered by phosphorylation and extracellular signals, and a serine/threonine protein kinase B (PKB) (Rascio, F). PI 3-kinase α (PI3Kα) is a critical regulator of cell growth and transformation and its signaling pathway is the most commonly mutated pathway in human cancers (YM-201636). Once PI3K is activated, the downstream effector is serine/threonine kinase AKT. AKT is involved in cancer progression, GSK inhibition, and together with PI3K, can target mTOR (Rascios, F). Hyperactivation of this pathway leads to angiogenesis, drug resistance in tumors, and many invasive cancer properties. This pathway is highly regulated by microRNAs and usually related to issues with the p53 tumor suppressor gene.

BI-6727

This treatment is a dihydropteridine that inhibits PIK1, 2, and 3, which are serine/threonine kinases that are prominent in cell cycles (Bi-6727). BI- 6727 induces mitotic arrest and apoptosis, inhibiting cell proliferation in many cancer cell lines. It has also been found to reverse "ABC-efflux transporter-mediated multidrug resistance activity through inhibition of the transport activity of ABCB1 and ABCG2, thus promoting mitotic arrest in resistant cancer cells," (Bi-6727). In clinical trials, the drug was used for ovarian cancer patients in a randomized phase II trial (Bi 6727 (Volasertib) randomized trial in ovarian cancer).

YM-201636

PIKfyve, a FYVE-type zinc finger domain with phosphoinositide kinase (PIK) binding phosphatidylinositol 3-phosphate (PI3P), is involved in membrane trafficking and reorganizing the cytoskeleton (YM-201636). YM-201636 is a cell-permeable and selective inhibitor of PIKfyve, mimicking the effects when PIKfyve is depleted with siRNA and reversibly stops endosomal trafficking in NIH3T3 cells (YM-201636). In addition, retroviral exit is blocked in budding cells through endosomal sorting complex interference and 2-deoxylglucose uptake is also inhibited. Studies have shown inhibited tumorigenicity and high potential for clinical trials (DoĞan E).

GSK1059615

As a potent, reversible, ATP-competitive, thiazolidinedione inhibitor of PI3Kα, and an activating mutant of p110α, GSK1059615 prevents proliferation and reduces MAPK signaling (GSK1059615). Phase 1 clinical trials have been conducted, and studies have shown death of head and neck squamous cells with this drug using mitochondrial necrosis pathways (Xie, J).

SC-1

SC-1 inhibits both RasGAP and ERK1 in a collaborative manner to block differentiation through ERK1 differentiation and increase Ras signaling through PI3-kinase pathway to promote self-renewal (SC-1). No clinical trials were found.

PI-103

PI-103 is a "potent cell-permeable ATP-competitive inhibitor of phosphatidylinositol 3-kinase (PIK3)," showing antiproliferative activity of glioma cells (PI-103). Although there are no

clinical trials at the moment, preclinical trials have tested the efficacy of PI-103 and studies show anti-proliferative properties (Luo, L). There seems to be little bioavailability for PI-103 *in vivo* in comparison to other derivatives.

GSK3 Related Treatments

Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase interconnected with other pathways such as mTOR, PI3K, and cyclin in checkpoint kinases (Duda, P). GSK3 degreades proteins when phosphorylated, and thus can either promote or suppress cancers depending on the mutation. AKT can target GSK3, thus inactivating it and enhancing mTOR production and proliferation since mTOR will no longer be degraded by GSK3 (Duda, P). On the other hand, overexpression of GSK-3 may result in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis resistance (Duda, P). GSK3 may be inhibited by insulin, cell specification factors, growth factors, and cell adhesion (TWS119).

Indirubin-3'-monoxime

A potent inhibitor of glycogen synthase kinase 3β, Indirubin-3'-monoxime prevents tau phosphorylation as in Alzheimer's disease and inhibits cyclin-dependent kinases (CDKs) at high concentrations (Indirubin-3'-monoxime). This compound reversibly arrests cycling in the G2/M phase and thus inhibits cell proliferation. As a proteasome inhibitor, this derivative of Indirubin has shown an increase in cytotoxicity and clinical studies have shown promising results of reversing drug resistance in cancer cell lines.

TWS119

This treatment inhibits GSK3β as a 4,6 disubstituted pyrrolopyrimidine (TWS119). Although there are no clinical trials, TWS119 has shown association with the canonical Wnt-β-catenin signaling pathway in T-cell differentiation (Yu, Z). There is a unique effect of this compound on cytokine production and proliferation and shown inhibition of the A549 lung cancer cell line (Yu, Z). There is high potential for TWS119 in clinical applications for immunotherapy treatments.

BIO, GSK3 Inhibitors

A cell-permeable compound, BIO is a reversible and selective ATP- competitive inhibitor of GSK3α/β, resulting in Wnt signaling activation (Bio; Augello, G.). BIO has been tested for its specificity against cyclin-dependent kinases and may be involved in self-renewal of embryonic stem cells involved in differentiation (Augello, G). BIO may be used in anti-cancer response but needs further research.

SRC Tyrosine Kinase Related Treatments

Src is a non-receptor tyrosine kinase that is deregulated in many cancers and inhibits this protein. Src plays a significant role in cancer metastasis due to its regulation of the cytoskeleton, invasion, adhesion, migration, and regulating the tumor's microenvironment (Zhang, S.). Src degrades the cellular matrix and manipulates cytoskeletal elements that promote cancer metastasis (Zhang, S). There have been clinical trials that have demonstrated low benefits in phase II trials with single Src inhibitors, however, upon selecting the correct biomarkers there is immense potential for cancer therapies with Src inhibition (Zhang, S).

SU6656

SU6656 is a Src tyrosine kinase inhibitor-proto-oncogene that ends cell division and accumulates DNA via endomitosis while increasing CD41 and CD62 expression on the membrane (SU6656: Blake, R. A). Some kinases SU6656 inhibits include Src, Yes, Lyn, and fyn and has not been used in clinical trials, however, studies have shown that SU6656 is a strong Src inhibitor and should be considered for preclinical and clinical trials (Blake, R. A).

LCK Inhibitor

This drug is a pyrrolopyrimidine blocking two LCK kinase forms and a member of the Src non-receptor protein tyrosine kinases, necessary for initial T-cell signaling in cytokine production (Lck Inhibitor; Crean-Tate, K). LCK inhibitor weakly inhibits kinases similar to Src, Kdr, Tie-2. EGFR, PKC, CDC2/B, and ZAP-70. In preclinical trials, LCK Inhibitors have demonstrated slowed tumor growth (Crean-Tate, K).

Dasatinib

Dasatinib is a potent inhibitor of Abl and Src tyrosine kinases that can block other kinases with and without receptors (Dasatinib). Phase II and III clinical trials are currently being executed to test this treatment with patients of chronic myeloid leukemia and the effect of Dasatinib on stopping cancer cell growth (Testing Pembrolizumab with Existing Cancer Therapy in Patients with Evidence of Residual Chronic Myelogenous Leukemia).

Bosutinib

This treatment inhibits c-Src, Abl, EPHB2, TrkA, TrkB, TXK, and other kinases which stop cell proliferation and reverse Src transformation (Bosutinib: Bosutinib in Pediatric Patients

With Newly Diagnosed Chronic Phase or Resistant/Intolerant Ph + Chronic Myeloid Leukemia). Phase II and III clinical trials are being performed to test efficacy and tolerability in pediatric patients with newly diagnosed chronic myeloid leukemia (Bosutinib in Pediatric Patients With Newly Diagnosed Chronic Phase or Resistant/Intolerant Ph + Chronic Myeloid Leukemia).

Other Treatments

(R)-Crizotinib

(R) Crizotinib is a aminopyridine derivative that is a ATP-competitive dual inhibitor of c-MET and ALK receptor tyrosine kinases showing both cytoreductive antitumor activity and antitumor efficacy in mouse tumor models ((r)-crizotinib). This treatment is being used in clinical trials for non-small cell lung cancer and anaplastic large cell lymphomas/ neuroblastomas in phase I and II ((r)-crizotinib). The compound showed 58.6% relative wound density in Figure 1.

PF-562271 (besylate)

This compound is an ATP-competitive reversible inhibitor of Focal adhesion kinase (FAK) and inhibits FAK phosphorylation (PF-562271 (besylate)). FAK is a non-receptor tyrosine kinase that is prevalent in many oncogenic pathways (PF-562271 (besylate)). PF-562271 has been used in phase I clinical trials for pancreatic cancer, head and neck cancer, and other neoplasms (Study of PF-00562271).

Staurosporine

A potent inhibitor of protein kinase C (PKC) in rat brains, Staurosporine is non-selective, meaning it inhibits various other protein kinases (Staurosporine). This compound is relatively

cytotoxic and regulates smooth muscle. This treatment may not be used in the future due to cytotoxicity and mitochondrial damage and induced apoptosis, however the complex pathways Staurosporine uses in apoptosis may help bypass chemoresistance (Stepczynska A). Derivatives of this compound may show better use in clinical trials for anti-tumor therapies.

CONCLUSION

This experiment has demonstrated significant potential for future PC3 cancer therapies utilizing compounds that exhibit high inhibition in the Scratch wound assay while remaining viable. Many of these compounds are particularly intriguing due to their ability to inhibit multiple overlapping pathways. The mTOR/PIK3/GSK3/AKT pathways, in particular, are of great interest due to the potency of the compounds and their ability to target multiple pathways simultaneously in prostate cancer cells.

In Figure 2.A, only DMSO treated cells (hence used as a control lacking any inhibitor) occupy 90.5% of the wound gap area at 36 hours, indicating no inhibition of cancer invasion since DMSO alone is non-inhibitory. YM-201636 in Figure 1 shows a relative wound density of 21.4%, significantly lower than the negative control DMSO. However, the presence of bubbles and rounded cell morphology in Figure 2.B suggests that this treatment inhibits cellular migration by inducing cell death, which is an undesired outcome. Figure 2.C demonstrates Staurosporine with rounded cell morphology and no bubbles, indicating dead cells. Although the relative wound density is only 3%, this compound is too potent and potentially cytotoxic at this dosage. The absence of bubbles may indicate a different cellular process compared to YM-201636 Both compounds induce cell death in PC3 cells, but YM-201636 is a PI3K inhibitor, while Staurosporine is a PKC inhibitor, leading to differences in cell morphology due to distinct mechanisms of cell killing. In comparison, Figure 2.D shows TWS119 at 36 hours with living cells exhibiting a healthy morphology and a high inhibition of only 4% relative wound density. This indicates a highly potent compound that inhibits the GSK3β pathway.

Based on the graph, we were able to identify compounds that resulted in viable cells after 36 hours of treatment. Each compound's effect on cell morphology was evaluated based on the

targeted pathway and whether the cells exhibited the typical appearance of a living or dead cell. These compounds are kinase inhibitors that modulate the phosphorylation of kinases involved in cell proliferation and renewal.

The compounds that inhibited PC3 cancer cell migration without inducing cell death were SU6656, LCK Inhibitor, GSK1059615, CAY10626, AZD 7762, INK128, SC-1, Bosutinib, and TWS119. These inhibitors primarily target Src kinases, mTOR, PIK3, Checkpoint Kinases, GSK3β, Protein Kinase C, and PI3P pathways.

The treatments that inhibited cellular growth but resulted in dead cell morphology were YM-201636, Dasatinib, PP242, BIO, PI-103, and Staurosporine. These compounds are likely cytotoxic to the cells at a dosage of 2.5 µl, explaining their inhibition of cellular migration (as seen in Figure 2.C). When used in combination with other treatments or at lower dosages, their potency may vary.

Future Directions

Compounds of high interest for future research include INK128, SC-1, Bosutinib, and TWS119, as they exhibit high inhibition of PC3 migration while maintaining viable cells with normal morphology. These compounds are inhibitors in the mTOR, PIK3, Src, and GSK3β pathways which are interconnected. Further investigation into the inhibition of these pathways and their downstream effects is warranted due to their crucial roles in cancer metastasis and high potency. Notably, Bosutinib (5.9% relative wound density in Figure 1) is intriguing, considering the high inhibition demonstrated by other Src inhibitors. Conducting a cytotoxicity assay on this treatment may provide additional information for anti-metastatic therapies.

n future research, it is recommended to conduct cytotoxicity assays to determine the lethal dosage of various compounds. Each compound should be tested, with particular emphasis

on those exhibiting the lowest wound density in Figure 1, to identify their most potent concentration or ascertain if they are simply cytotoxic. Once the appropriate dosages are determined, synergy assays can be performed using the IncuCyte system to evaluate whether the combination of two drugs exhibits greater efficacy than each drug individually.

Through further testing, we can refine our understanding of specific pathways, in addition to those investigated in this study, which contribute to PC3 metastasis inhibition. This will enable the identification of drugs or drug combinations that exhibit the most potent inhibition of PC3 cancer cell migration. It is conceivable that certain drugs may target multiple pathways, thus offering potential for application across various cancer types and mutations. By leveraging these compounds, we can potentially make significant contributions to future anti-metastatic therapies, ultimately reducing the mortality rate among cancer patients, particularly those afflicted with prostate cancer.

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