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Screen to identify heterochromatin promoting drugs

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by

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The thesis of Andre Christopher Loyola is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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I dedicate this thesis to Charity and Sunday for their love and support.

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ABSTRACT OF THE THESIS

Screen to identify heterochromatin promoting drugs

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Heterochromatin is a tightly packed form of DNA associated with gene silencing and plays a significant role in the regulation of gene expression, segregation of chromosomes during mitosis and protection of genome stability and integrity. Heterochromatin is also becoming more recognized to be implicated in cancer and may serve as a potential target for cancer therapy. To our knowledge, there are no drugs that

are well-established to promote heterochromatin. Here, we describe and perform two simple screening methods to identify compounds that may promote heterochromatin. We screened Oncology Set III, a set of FDA approved oncology drugs, using DX1 mutant flies whose eye color phenotype is sensitive to heterochromatin levels. This screen produced no hits and was inconclusive. We then screened Natural Set II, a set of natural compounds, using a cell based method. One drug, streptonigrin, increased the brightness and induced the formation of visible foci of DNA stain Hoechst 33258 in HeLa cell nuclei, indicative of increase in heterochromatin levels. We then showed that Heterochromatin Protein 1 alpha (HP1α) foci co-localizes with DNA foci in streptonigrin treated cells, further indicating the drug affects heterochromatin formation. The induction of heterochromatin reorganization by streptonigrin was also seen to be concentration dependent in 3T3 cells by observation of nuclear morphology. These results show that streptonigrin may promote heterochromatin formation. Interested in looking at the capabilities of streptonigrin to prevent cancer growth without significantly affecting cell proliferation or viability, we also established non-lethal concentrations of streptonigrin *in vivo*.

I.

Introduction

Heterochromatin

In eukaryotic cells, DNA is folded with histone and non-histone proteins in order to form chromatin. Nucleosomes, the basic units of chromatin consist of 146 bp of DNA wrapped around a histone octomer core and linker histone protein H1, the core being made up of two copies of H2A, H2B, H3 and H4 histone proteins. This chromosomal template is the foundation for many important cellular processes, such as gene transcription, DNA repair and recombination (Luger 1997). Generally, chromatin is found in two different assortments, euchromatin and heterochromatin. In 1928, Heitz first distinguished between these different chromosomal states cytologically by observing the differential staining of chromatin at interphase. Euchromatin stains lightly at interphase, indicating a less condensed, accessible and transcriptionally active form of chromatin, while heterochromatin stains more strongly and indicates a more condensed, inaccessible and transcriptionally silent chromatin (Passagre 1979).

Heterochromatin, the more condensed and transcriptionally silent form of chromatin, can be further divided into two categories: constitutive and facultative heterochromatin. Constitutive heterochromatin domains occur in regions that are highly dense in repetitive DNA elements and possess structural importance. These regions remain condensed throughout the cell cycle. They are typically found at centromeres and telomeres and help to facilitate separation of chromosomes during mitosis and protect the ends of chromosomes from deterioration, respectively. Facultative heterochromatin domains are important for development and the chromatin state of these regions can change in response to cellular signals and gene activity (review by Grewal and Jia 2007).

An example of facultative heterochromatin is X-chromosome inactivation in female mammals, a phenomenon in which one of the X chromosomes is condensed as facultative heterochromatin and formed into what is known as a Barr body (Rego 2008).

Various phenomena such as methylation of CpG islands or post translational modification of histone proteins are associated with the dynamic transitions of chromatin states (review in Goll and Bestor 2005). A "histone code" is described in which particular posttranslational modifications can dictate the chromosomal state (Allis 2001). Tri-methylation of histone 3 lysine 9 and recruitment of HP1 chromo domain proteins is well established to be associated with heterochromatin and epigenetic silencing (review in Grewal and Jia 2007). These proteins are strong indicators of and are strong biomarkers for heterochromatin formation.

Heterochromatin and tumor suppression

It has become increasingly evident that heterochromatin is implicated in cancer. It has been shown that in certain biological contexts, HP1 reduction can lead to progression of various types of tumors, conceivably due to changes in centromere stability, telomere capping and regulation of heterochromatic gene expression. Decreased HP1 levels are also evident in many cancer cell lines (Dialynas 2008). In particular, it has been shown that BRCA1, a tumor suppressor gene well established to be associated with breast and ovarian cancer, prevents tumor formation through heterochromatin mediated silencing (Zhu 2011). Retinoblastoma (Rb) tumor surpressor, a protein that suppresses transcription

of E2F genes upon DNA damage in order to prevent the division of damaged cells, also mediates its effects through stabilizing heterochromatin (Talluri 2014).

In our lab, we have identified a non-canonical JAK-STAT pathway in which un-phosphorylated STAT promotes and stabilizes heterochromatin by binding to the HP1 chromodomain protein (review in Li 2008). We have shown that increasing global heterochromatin levels through up regulation of HP1 or un-phosphorylatable STAT5A can suppress colon cancer growth in mouse xenograph models. Interestingly, while many genes down-regulated by STAT5A and HP1 are shown to be overexpressed in or are drivers of human cancers, genes that are involved in cell cycle progression and apoptosis are not down-regulated by STAT5A or HP1 (Hu 2013). Our results support the idea that heterochromatin may offer a unique target for human cancers and could offer a treatment that is associated with less side effects compared with cytotoxic oncology drugs.

With an interest in identifying compounds for further research and targeting heterochromatin for cancer therapy, we designed and performed two simple screens in order to identify small compounds that can promote heterochromatin formation. Upon discovery of compounds that are suspected to promote heterochromatin formation, we will identify the mechanisms by which these drugs accomplish this. We will also investigate the ability of these compounds to suppress cancer.

II.

Results

DX1 mutant fly screening of Oncology Set III

We first began our search for heterochromatin promoting drugs by looking into Oncology Set III, a set of 97 FDA approved oncology drugs provided to us by the National Cancer Institute (NCI) Developmental Therapeutics Program (DTP). We screened this library using DX1 mutant *Drosophila melanogaster*. DX1 consists of seven tandem copies of a P[white+] reporter transgene inserted into a euchromatic region which induce heterochromatin formation at the insertion locus. The degree of variegation in the eye color of these mutants is sensitive to heterochromatin levels and increases when heterochromatin is promoted (Dorer 1994). We performed this screen with the hypothesis that compounds that promote heterochromatin should cause significantly more variegation in the eye color phenotype of DX1 mutants. We crossed 3 DX1/CyO flies with 3 virgin W1118 wild-type flies lacking the white gene that codes the transporter that carries precursors of the red and brown pigments into developing eyes during pupation at room temperature (Mackenzie 1999). Two days after the cross, we threw away the parent flies and pipetted 60 uL of 33% DMSO in water containing compound at 10 uM concentration or just 33% DMSO in water as a control. We did this once more four days after crosses were set up. Once the flies eclosed, we scored each DX1/W1118 male on a scale from 1 to 5, 1 being most variegated and 5 being least variegated (Figure 1 a, b). We then averaged the scores of all the DX1/W1118 males in each vial. Three trials were done per compound and were represented in bar graph (Figure 1c). We identified hit drugs as compounds that received a score that was two standard deviations below the mean of the control. The control received a score of 3.80 and had a standard deviation of 0.79, therefore a drug that receives a score less than 2.22 would be considered a hit. The

drug that caused the most variegation in this set was E7 and was represented in a bar graph with the control (Figure 1d). Representative pictures of E7 and the control were taken through microscope (Figure 1e). E7 was identified to be methotrexate and received a score of 2.43. Although E7 was the best drug, based off of our definition it is not considered a hit.

Small drug screen identifies streptonigrin to induce DNA reorganization in hela cells

While our *in vivo* screen using *Drosophila* melanogaster offers a sensitive way to screen for drugs, it is a tedious method and the barriers introduced by *in vivo* screening may cause us to miss potential heterochromatin promoting drugs. With an interest in creating an easier method that can ensure we identify drugs if they promote heterochromatin, we designed a simple screen using HeLa cells. We screened Natural Products set II, another set of compounds provided by the National Cancer Institute (NCI) Developmental Therapeutics Program (DTP). Natural Products Set II consists of 120 natural compounds that were chosen from the DTP's repository of 140,000 compounds based on origin as a natural product, purity, structural diversity and availability of the compound.

For this method, we seeded HeLa cells at a density of 7,000 cells/well in 96 well plates and then treated with 10 uM concentration of compound for 6 hours. After treatment, cells were fixed and stained with DNA stain Hoechst 33258. We then observed the nuclear morphology of the cells through fluorescence microscopy to see if we could identify compounds that induced a dramatic reorganization of DNA in HeLa cell nuclei (Figure 2A). We used a high concentration of drug with the intentions of testing lower

concentrations of compounds if suspected to increase heterochromatin levels. Out of the 117 compounds, a single drug was identified as inducing a dramatic change in nuclear morphology. A general increase in brightness of the stain and formation of large foci was observable compared to DMSO-only treated controls, indicative of an increase in heterochromatin in these cells (Figure 2b). Our single hit, streptonigrin, is an aminoquinone antitumor antibiotic isolated from *Streptomyces flucculus* (Figure 2c). These results show that streptonigrin induces reorganization of DNA in the nuclei and may alter heterochromatin organization.

HP1α co-localizes with DNA in streptonigrin treated cells

Although we have demonstrated that streptonigrin can induce the reorganization of nuclear morphology in HeLa cell nuclei through DNA staining with Hoechst 33258, we wanted to further verify that the drug's effects correlated with change in heterochromatin organization. To look into this, we performed immunoblot experiments against HP1α. In streptonigrin-treated HeLa cells, we observe the co-localization of HP1α and DNA (figure2a). These results support the idea that streptonigrin is inducing heterochromatin reorganization in HeLa cells.

Nuclear reorganization of streptonigrin-treated 3T3 cells is concentration dependent

Next, we wanted to look at the concentration and time dependent effects of streptonigrin on heterochromatin organization. Because HeLa cells do not possess easily observable heterochromatin foci we decided to use 3T3 cells, an immortalized mouse fibroblast cell line. This cell line possesses easily discernible pericentric heterochromatin

foci and is more sensible to use when observing slight changes in nuclear morphology (Muller 2009). Also, for this experiment we used DNA stain Hoechst 33342 instead of Hoechst 33258. Hoechst 33342 is more membrane permeant and is better suited than Hoechst 33258 for live staining (Chazotte 2011). We decided to perform live stains for this experiment at this point because the procedure saved time and reduced effort.

3T3 cells were seeded into 24 well plates at a density of 50,000 cells/well, treated with different concentrations of streptonigrin and live stained with DNA stain Hoechst 33342 in order to observe nuclear morphology. 3T3 cells were treated with 1 nM, 10 nM, 100 nM, 1 uM, 10 uM concentrations of streptonigrin or DMSO for 4 hours. We live stained the cells and observed a concentration dependent effect of streptonigrin on nuclear morphology in 3T3 cells (Figure 4a). Higher concentrations of streptonigrin (10 uM, 1 uM) induced the formation of large heterochromatin foci at shorter incubation time while lower concentrations showed less degree of change in nuclear morphology.

Interested in looking at the effects of low concentration of streptonigrin, we treated 3T3 cells with 1 nM of streptonigrin for 16 hours then and live stained with Hoechst 33342. We saw that while 1 nM concentrations had little effect at four hours, with longer incubation times increase in brightness and formation of foci is observable (Figure 4b).

Effects of streptonigrin on cell proliferation and viability of HeLa cells

One goal of this project is to identify whether heterochromatin promoting drugs that can suppress cancer growth without significantly impacting cell proliferation and

viability. Upon verifying the ability of streptonigrin to affect heterochromatin formation even at lower concentrations, we worked to identify concentrations of streptonigrin that are non-lethal *in vivo*.

We assessed cell proliferation by plating HeLa cells in 24 well plates at a density of 20,000 cells/well and allowed cells to adhere onto plates for 12 hours. Cells were then incubated with 1 nM, 10 nM and 250 nM of streptonigrin for 24 hours. Cells were counted with hemocytometer at 24, 48 and 72 hours after initial exposure to drugs and cell proliferation was accessed by counting cells with hemocytometer in trypan blue (Figure 5A, Table 1).

Cells treated with streptonigrin 10 nM showed a decrease in cell counts by 72 hours and cells treated with streptonigrin 250 nM showed decrease in cell counts as early as 24 hours. These results suggest that streptonigrin at these concentrations are causing HeLa cells to enter apoptosis and unattach from the plate. On the other hand, cells treated with streptonigrin 1 nM seem to proliferate normally. Based on student t-test, cell counts are significantly lower at the 24 hour time point but return to non-significant levels at the 48 and 72 hour time points. The administration of drugs may have caused this slight decrease and once removed the cells were able to proliferate normally.

We assessed cell viability by seeding HeLa cells at a density of 25,000 cells/well into 24 well plates and allowed them to adhere and grow for 36 hours. They were then treated with 1 nM, 10 nM or DMSO for 48 hours. After treatment we counted and calculated the percentage of cells that were in late apoptosis with trypan blue, a negative stain that is positive for late apoptotic cells (figure 4b). Cell viability for DMSO, 1 nM

and 10 nM streptonigrin treated cells were 96.72%, 95.78% and 91.95%, respectively. We found that 10 nM streptonigrin treated cells showed a significant decrease in cell viability ($p < 0.05$). P-values for 1 nM and 10 nM streptonigrin treated cells compared to DMSO control were 0.52 and 0.023, respectively.

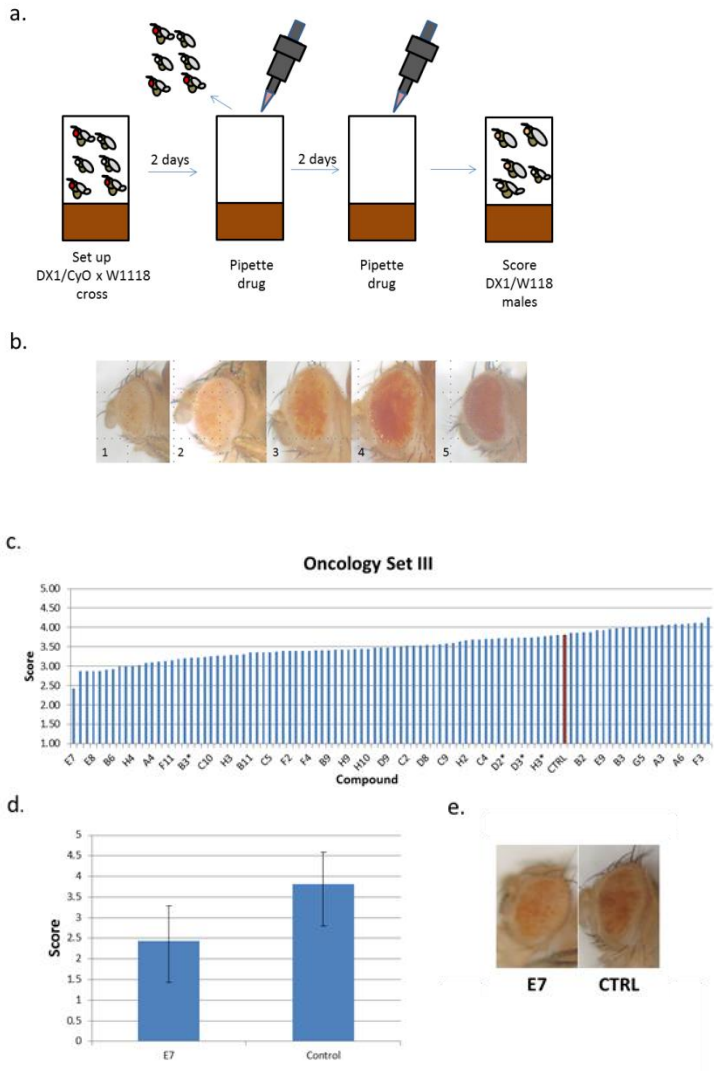

Figure 1. *Drosophila* screen of Oncology Set III

(a) *Drosophila* screen methodology. Three DX1/CyO males and three virgin W1118 were crossed at room temperature and allowed to lay eggs for two days. Parent flies were then removed and 60 uL of 33% DMSO in water with 10 uM compound was pipetted onto food two days and four days after crosses were first set up. The eye phenotype of DX1/W1118 male progeny were then scored

(b) Eye phenotype scoring scale. Representative pictures representing the score assigned to flies based off of eye color variegation.

(c) Screen of Oncology Set III. Triplicates of each sample were performed represented on bar graph. Control is represented as a red bar.

(d) Bar graph of E7 and Control. Average score of E7 was 2.43 and average score of control was 3.80

(e) Representative pictures of the eyes of E7 and control DX1 mutants.

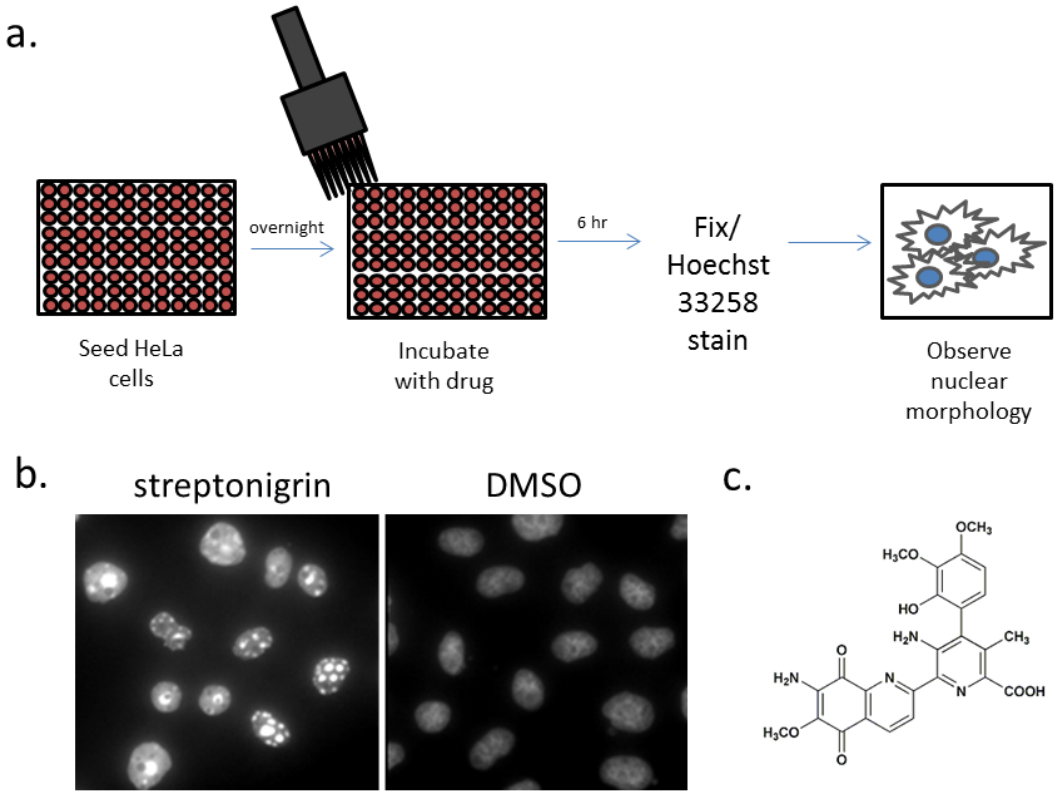

Figure 2. Drug screen method and identification of hit drug streptonigrin in Natural Set III

(a) Drug screening method. HeLa cells were seeded into 96 well plates at a density of 7,000 cells/well and left to adhere overnight. They were then treated with 10 uM of compound for 6 hours, fixed, stained with DNA stain Hoechst 33258 and nuclear morphology was observed.
(b) Fluorescence microscopy pictures of streptonigrin and DMSO treated HeLa cells
(c) Chemical structure of hit drug streptonigrin.

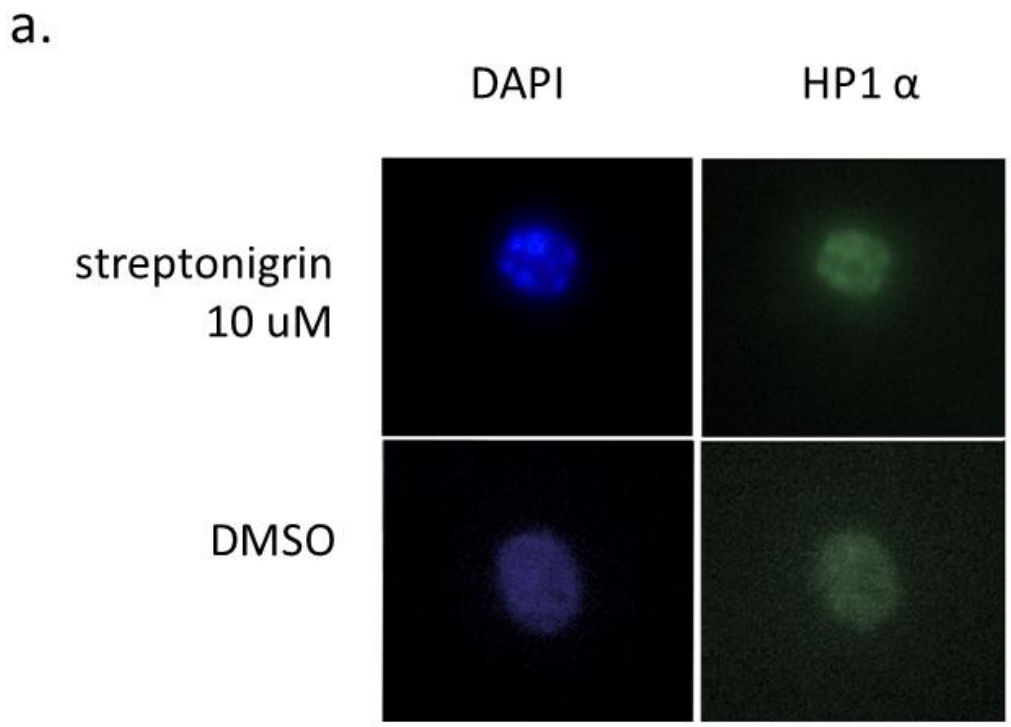

Figure 3. HP1α and DNA co-localize in streptonigrin treated HeLa cells

(a) Immunofluorescence of DMSO and streptonigrin treated HeLa cells. HeLa cells were treated with DMSO or 10 uM streptonigrin for 6 hours and were stained with DAPI (blue) and primary antibodies against HP1α (green).

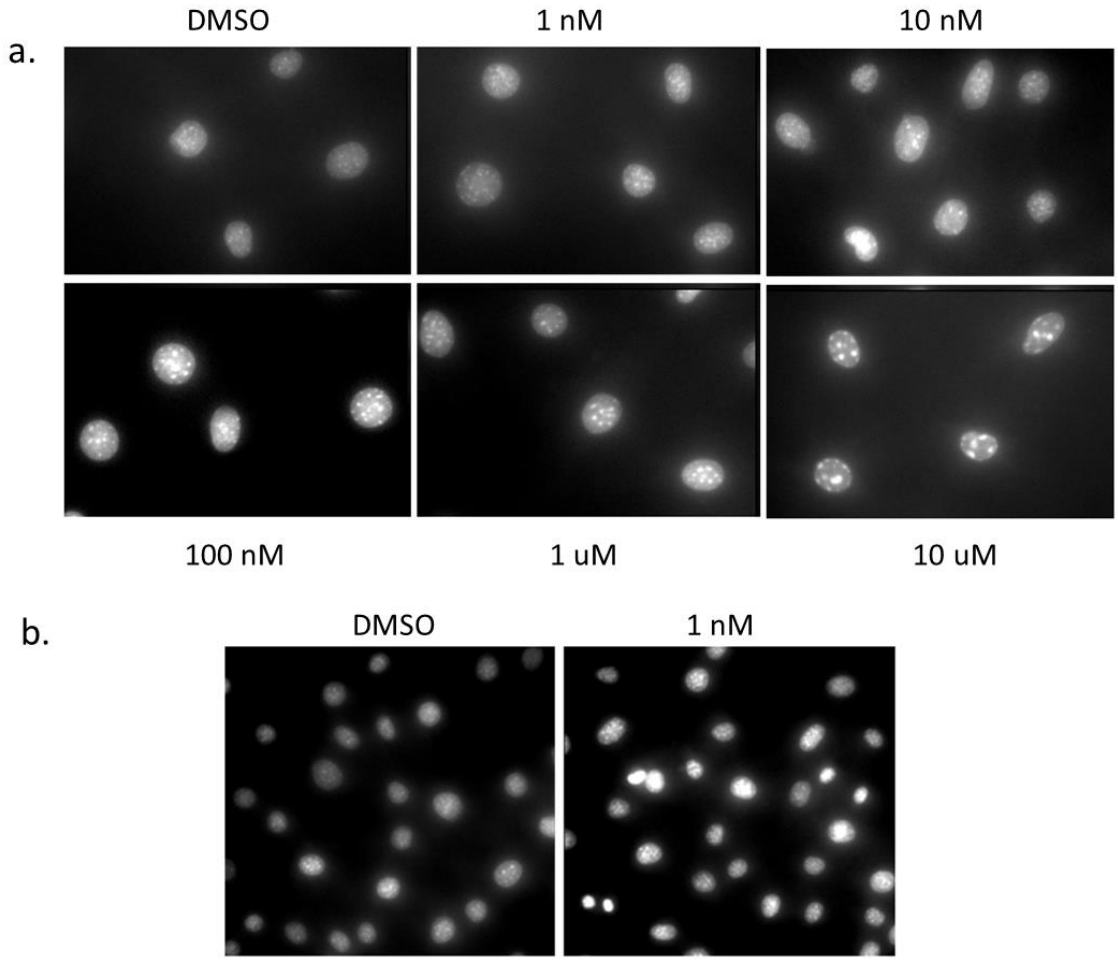

Figure 4. Nuclear reorganization of streptonigrin-treated 3T3 cells is concentration and time dependent

(a) 3T3 cells live stained with DNA stain Hoechst 33342. Cells were treated with log concentrations of streptonigrin or DMSO for 4 hours.
(b) Fluorescence microscopy of 3T3 cells live stained with DNA stain Hoechst 33342. Cells were treated with 1 nM of streptonigrin or DMSO for 16 hours.

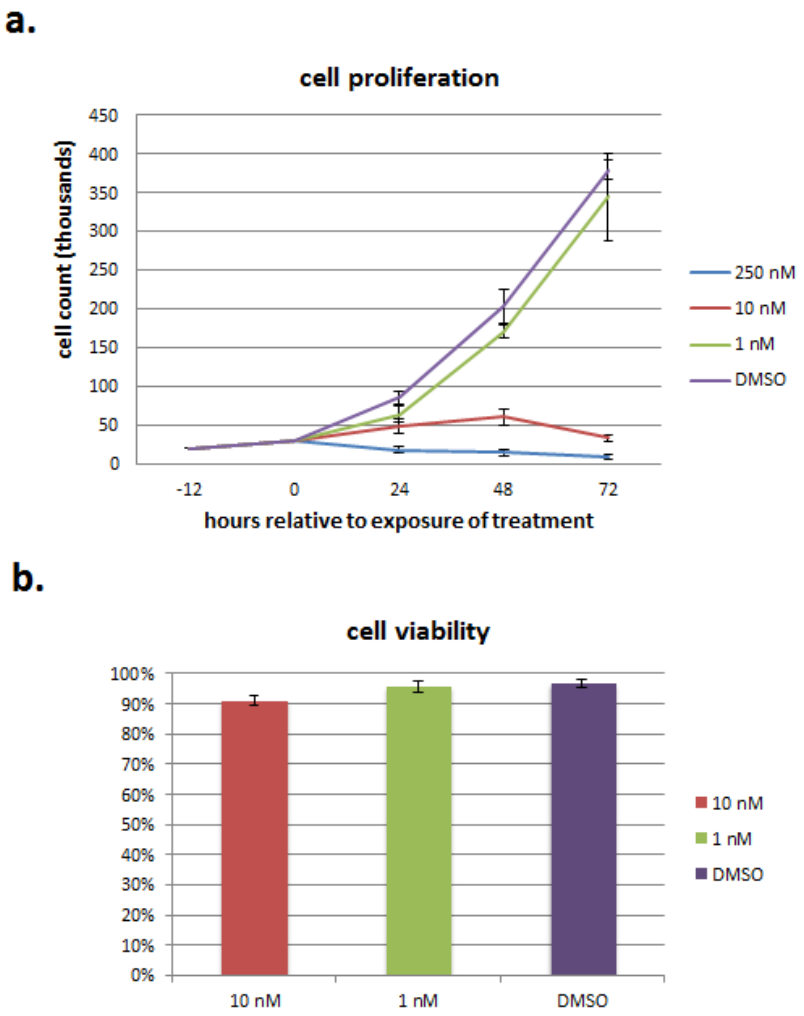

Figure 5. Effects of streptonigrin on cell proliferation and viability in HeLa cells

(a) Cell proliferation assay of DMSO and streptonigrin treated HeLa cells. Cells were seeded at a density of 20,000 cells/well in 24 well plates and allowed to adhere for 12 hours. Cells were then treated with DMSO, 1 nM, 10 nM or 250 nM streptonigrin for 24 hours. Cells were counted at 24, 48 and 72 hrs after initial exposure. Triplicate samples of each time point were counted by hemocytometer, averaged and plotted. Error bars represent s. e. m.

(b) Cell viability assay of DMSO and streptonigrin treated HeLa cells. Cells were seeded at a density of 25,000 cells/well in 24 well plates and allowed to adhere and grow for 36 hours. Cells were then treated with DMSO, 1 nM or 10 nM streptonigrin for 48 hours. After treatment, media with dead, unadhered cells was collected and remaining cells were trypisinized. Viable cells were counted with trypan blue. Triplicates of each sample were counted and averaged. Error bars represent s. e. m

Table 1. Cell counts 24, 48 and 72 hours after initial exposure to DMSO, streptonigrin 1 nM, 10 nM or 250 nM for one day. P-values compared to DMSO are represented in parenthesis. Data reported as mean ± standard deviation (n=3).

Treatment	**24 hours**	**48 hours**	**72 hours**
DMSO	85500 ± 7937.25	203158 ± 22133.1	379315 ± 11816.62
streptonigrin 1 nM	64000 ± 9987.49 (0.043)	169908 ± 8365.53 (0.107)	344470 ± 56076.29 (0.39)
streptonigrin 10 nM	4800 ± 9367.49 (0.0066)	60182.5 ± 10942.23 (0.0023)	32917.5 ± 4348 (8.29E-05)
streptonigrin 250 nM	17482.5 ± 3814.99 (0.0011)	14297.5 ± 3776.47 (0.0037)	8645 ± 3047.41 (0.00015)

III.

Discussion

Here, we describe an *in vivo* screening method using DX1 mutant *Drosophila melanogaster* in order to identify compounds that may promote heterochromatin based off of the degree of variegation they cause in the eye color phenotype (figure 1a,b). We performed this screen on Oncology Set III (figure1c). E7, later to be identified as methotrexate caused the highest increase in variegation and scored a 2.43 but did not score two standard deviations below the average and was not identified as a hit (figure 1d, e). The results of this screen were inconclusive. We also designed an *in vitro* drug screen method in order to identify compounds that affect the organization of DNA in the nuclei of HeLa cells and may promote heterochromatin (figure 2a). We screened Natural Set III, a small set of natural compounds provided by the NCI DTP using this method. By observation of nuclear morphology in HeLa nuclei with DNA stain Hoechst 33342, we identified streptonigrin as a compound that induces the formation of visible DNA foci and increases the general brightness of DNA staining compared to DMSO controls, indicating that this drug may increase heterochromatin levels (figure 2 b, c). We also performed immunofluorescence experiments against HP1α in HeLa cells and observed the co-localization of HP1α and DNA in streptonigrin treated cells, further verifying that streptonigrin is indeed inducing changes in heterochromatin organization (figure 2). Furthermore, we verified the concentration and time dependent effects of streptonigrin on heterochromatin formation by observing the live staining of 3T3 mouse immortalized fibroblast cell line with Hoechst 33342 (figure 3). Finally, we established that 1 nM concentrations of streptonigrin do not significantly affect cell proliferation and viability *in vivo* (figure 4) in hopes to further investigate the ability of this drug to prevent cancer growth without affecting cell proliferation and viability *in vivo*.

Improving our drug screening method

While our *drosophila* drug screening method is a slow approach to identifying compounds, it is very sensitive and allows us to test compound *in vivo*. We plan to use our cell screening method, which is cheap, easy to perform and is high throughput and then upon identifying hits use the *drosophila* screening method as a supplementary screen. We plan on using 3T3 cells for future screens, as their easily discernible foci make detection of less drastic changes on nuclear morphology possible. Also, because of the discernible pericentric heterochromatin foci 3T3 cells possess, we may be able to quantitively measure changes in heterochromatin organization using analysis software. More work must be done in order to determine how we can quantify our results and how to define which drugs significantly affect heterochromatin organization. We are currently improving our technique and are in the process of screening the Mechanistic Diversity Set II, a set of 817 compounds provided by the DTP for more drugs that may promote heterochromatin.

Further investigating streptonigrin's effects on heterochromatin organization

We have demonstrated that streptonigrin affects heterochromatin organization, but the mechanism in which this is occurring is not clear. Speculating from recent literature, a group developed and performed a fluopol-ABPP HTS assay that identified streptonigrin as a potent, selective and irreversible PAD4 inhibitor (Knuckley 2010). PAD4, peptidyl arginine deiminase 4 is an enzyme that converts Arg or monomethyl-Arg to citrulline in histones and has been shown to be associated with chromatin decondensation in neutrophils (Leshner 2012). The 7-amino-quinoline-5,8-dione core of

streptonigrin has been identified to be a highly potent pharmacophore that acts as a pan-PAD inhibitor by examining a library of 32 analogues of streptonigrin (Dreyton 2014). While it is unclear whether streptonigrin's ability to inhibit PAD4 is responsible for the reorganization of heterochromatin that we have observed, this library of analogues may prove useful into providing insights to the mechanism in which heterochromatin is being affected by streptonigrin. Also, a group developed and performed a phospho-flow single-cell drug screen using NCI Natural Products Set I and identified streptonigrin as an inhibitor of STAT signaling in U937 monocytic cell line (Krutzik 2008). Streptonigrin could be inducing heterochromatin formation by preventing phosphorylation of STAT proteins. Regardless, further research must be done in order to determine that heterochromatin levels are indeed increasing and in what manner. In the future, we plan to perform western blots against H3K9me3 and HP1 in order to verify an increase in heterochromatin levels. Also, DNAse assays using methylation sensitive restriction enzymes could be performed to look into whether DNA methylation is playing a role in the formation of heterochromatin.

Further investigating streptonigrn's effects on cancer growth *in vivo*

As we have demonstrated the ability of HP1 and un-phosphorylated STAT5A to suppress cancer growth without affecting genes that control cell cycle and apoptosis, we also hope to verify that our hit drugs have the ability to suppress cancer growth *in vivo* without affecting cell proliferation and viability. We have established low concentrations of streptonigrin that do not affect cell proliferation and viability and plan on performing mouse xenograft experiments in order to determine whether these drugs can suppress

cancer growth *in vivo*. Discovering a concentration that is capable of doing this could offer unique treatments that result in fewer side effects compared to modern chemotherapy treatments.

IV.

Materials and Methods

Cell Culture

HeLa human cervical cancer cell line and 3T3 immortalized mouse fibroblast cell line were cultured in Dulbecco's Modified Eagle Medium (DMEM,) containing 100ug/uL penicillin, 100 ug/uL streptomycin and supplemented with 10% (v/v) FBS.

Cell proliferation/viability assays

For cell proliferation assays, HeLa cells were plated onto 24 well plates at a density of 20,000 cells/well and allowed to adhere for 12 hours. Cells were then treated cell with 1nM, 10nM, 250 nM streptonigrin or DMSO for 24 hours. Cells were detached by trypsinization using 100uL 0.05% trypsin-EDTA at 37C for 8 minutes and then trypsin was deactivated with 200uL of DMEM. Cells were counted physically at 24, 48 and 72 hrs after initial exposure using hemocytometer. Triplicates were done for each treatment and counts were averaged. Data is represented by line graph made in Microsoft Excel 2010. Errors bars represent s. e. m.

For cell viability assays, HeLa cells were seeded into 24 well plates at a density of 25,000 cells/well and allowed to adhere for 36 hours. Cells were then incubated with 1 nM, 5 nM , 10 nM streptonigrin or DMSO-only. Media pooled with detached cells was saved and remaining adhered cells were then detached by incubating cells with 100 uL of 0.05% trypsin-EDTA at 37C for 8 minutes, deactivated with 400 uL of DMEM and media with detached-cells was recollected. Viability was then assessed by diluting cells x1.1 into 0.4% trypan blue in ddH20 and at least 300 cells in each sample were counted. Triplicates were done for each treatment and averaged. Data is represented by bar graph was made in Microsoft Excel 2010. Error bars represent s. e. m.

Cell Screening Method

HeLa cells were plated onto 96 well plates at a concentration of 7x10^3 cells per well in 180ul media and allowed to adhere overnight. The next day, 20 uL media with drugs were added to the wells to make a final concentration 10uM in 200ul media and allowed to incubate for 6 hours. After treatment, cells were washed with PBS then fixed in 100uL 3.7% (v/v) formaldehyde for 15 minutes, permeabilized with 100uL 0.3% PBT in PBS for 20 minutes and stained with 100uL 1ug/ml Hoechst 33258 in PBS + .3% PBT for 15 minutes. 30uL of 70% glycerol in dd$H_2$0 was then added to the wells for preservation and nuclear morphology was observed under fluorescence microscope. Out of the 120 drugs in Natural Set III, one drug, streptonigrin was observed to induce nuclear reorganization based on observation of nuclear morphology.

Drug Library

Compound Library was obtained through the National Cancer Institute Developmental Therapeutics Program. We screened Natural Products Set II, a set consisting of 120 compounds of natural origin which were selected from the DTP Open Repository collection of 140,000 compounds. The compounds were delivered in 96-well polypropylene (PP) microtiter plates with 60 compounds per plate. The plates were stored dry at -20C and contained 0.20 uMol of compound + 1 uL of glycerol. 19uL of DMSO was added to each well to obtain 20uL of 10mM stock solutions.

Drosophila screening method

Three male DX1/CyO and three female virgins W1118 flies were crossed in room temperature. Flies were allowed to lay eggs for two days. Parent flies were then removed and 60 uL of 33% DMSO in water with 10 uM compound was pipetted onto food day two and day four after crosses were set up. When flies came out DX1/W1118 males were scored on a scale of 1 to 5 and the scores were averaged for each vial were averaged. Triplicates of each sample were performed, averaged and represented in bar graph. Representative pictures were taken using a digital camera through microscope lense.

Immunofluorescence

HeLa cells were plated onto 6 well plates at a concentration of 0.3x10^6 cells/well and allowed to adhere overnight. The next day cells were treated with 10 uM streptonigrin or DMSO for 6 hours. Afterwards cells were fixed with 500uL 3.7% (v/v) formaldehyde for 15 minutes, permeabilized with 500uL of 0.3% PBT and 1% BSA in PBS for 20 minutes. 500 ul of primary antibody against HP1α was then spread and allowed to incubate overnight at 4C. The next day, cells were then incubated with secondary antibody at room temperature for 120 minutes, and mounted on coverslip with DAPI and observed under fluorescence microscope.

Live staining experiments

3T3 cells were seeded at a density of 100,000 cells/well in 24 well plates and allowed to adhere overnight. Cells were then incubated with 1 nM, 5 nM, 10 nM, 100 nM, 1 uM, 10 uM streptonigrin or DMSO only. Live staining was then performed by

washing the cells with PBS and then incubating them with 10 uM of Hoechst 33342 in DMEM for 30 minutes after drug treatment. Nuclear morphology was then observed by fluorescence microscopy.

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