Multiscale Analysis of Histone Acetylation Variability in the Cancer Genome to Predict Changes in Chromatin Function and Architecture

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Background

Epigenetic regulation of gene expression by histone modifications plays a critical role in determining cellular state and fate. Specifically, histone acetylation affects chromatin structure by relaxing chromatin and altering transcription factor (TF) binding accessibility. This epigenetic variability is reflected by alteration of nuclear morphology, gene expression, and nucleosome conformation [1] [2]. Growth arrest and apoptotic events are important functions of histone acetylation, and as such, the cancer epigenome shows a wide array of variability in histone acetylation levels [5]. The delicate balance of histone acetylation and deacetylation in cancer cells is disturbed, causing aberrant TF binding and RNA polymerase II recruitment [4]. This effect can be seen in the morphology of cancerous cells' irregular size, shape, and enlarged nucleus, indicating dysregulation of the epigenome. Upregulation of histone deacetylases (HDACs) has been shown to be characteristic of tumorigenesis and metastasis, further cementing the regulatory role of histone acetylation and chromatin architecture in cancer [4] [5].

Currently, it is unclear how the extent and occurrence of aberrant histone acetylation and deacetylation affects the epigenetic landscape that pushes a cell to a cancerous state. Furthermore, there is a distinct lack of computational modeling utilizing imaging data to determine the relationship between chromatin architecture, nuclear morphology, and cancerous states. This relationship can be used to inform computational models to develop a therapeutic dosage for epigenetic drugs to improve clinical outcomes. Since toxicity increases with dosage, therapeutic dose is of great interest to inform adequate treatment protocols. In a disease such as cancer, a therapeutic dose would be administered at levels that would cause cancer cell death while preserving non-cancerous cells.

Clinical trials are costly and laborious, therefore, modeling approaches that can predict the therapeutic dosage of a drug are recommended as an initial approach. However, the experimental data required to fit these models is not always easily available or generated. As cell culture and subsequent imaging of nuclei are relatively inexpensive, we propose to develop a model that can predict therapeutic dosages based on the data generated by our pipeline.

Aim 1 Establish how nuclear morphology changes as a function of acetylation levels in HCT116 and fibroblast cell lines treated with a histone deacetylase inhibitor sodium butyrate.

We treated human colorectal cancer cell lines (HCT116) with a global epigenetic inhibitor of histone deacetylases (HDACi) - sodium butyrate (NaBu). HDACi have been shown to kill cancer cells by changing chromatin architecture, exposing physically inaccessible regions to allow gene transcription to occur. These regions are typically those of tumor suppressor genes, however our intent is to correlate morphology with histone 3 acetylation (AcH3) levels and measure changes to nuclear shape to changing AcH3 levels. HCT116 cells were treated with two concentrations of NaBu for 12 and 24 hours, and immunohistochemistry was performed with a nuclear stain (DAPI) and AcH3. This experiment is currently being performed once more, but with the addition of 36 and 48 hour time points, and an apoptotic marker to identify when and how changes in AcH3 levels, nuclear morphology, and apoptotic events coincide.

In order to quantify changes in nuclear morphology, we developed and optimized an image analysis pipeline that makes use of classic computer vision techniques to segment heavily clustered nuclei stained for DAPI (Figure 1a) [6]. It then fits an ellipse to each segmented nuclei in order to determine geometric features such as area, eccentricity, and perimeter (Figure 1b). Previously, nuclei were classified as being normal or dysmorphic based on the curvature [3]. However, we needed to expand the definition of dysmophic to include other aspects of nuclear morphology such as the size. Therefore, along with the total number of nuclei, the following aspects of nuclear morphology are reported for each nucleus: area, eccentricity, mean negative curvature, max negative curvature, and relative concavity. These metrics should encapsulate all of the facets of nuclear morphology that should allow us to define what is a normal or dysmorphic nucleus.

Preliminary Results

Due to the high variability between experiments, there was not a significant difference between the number of nuclei belonging to cells that were treated with 0, 1, or 5 mM of NaBu for 12 or 24 hours (Figure 1c). Similarly, there appeared to be no aspects of nuclear morphology that distinguishes between any of the experimental conditions (Figure 1d). Because there was not a clear, quantifiable difference in the nuclear morphology of untreated and treated cells, it was not possible to classify nuclei as normal or dysmorphic without using manually labeled data. However, additional experiments with fibroblasts will provide better control data that may allow us to use semi-supervised learning to automatically classify nuclei as normal or dysmorphic.

Future Work

In addition to performing additional experiments, we will investigate if there are other quantifiable aspects of nuclear morphology that could be impacted by NaBu. Additionally, we will further optimize our image analysis pipeline.

Aim 2 Mathematical Model

We specified two subpopulations within cancer and non-cancer cells: those with normal nuclei and those with abnormal (dysmorphic) ones. Cells with abnormal nuclei were assumed to spontaneously die while cells with normal nuclei were allowed to divide. Moreover, as a drug proxy, we took NaBu to be responsible for accelerating the rate at which nuclei become abnormal. Too great a dose, and both cell populations would die off quickly. Not enough, and cancer cells might proliferate once again after removal of the drug. Our model is summarized as the following reaction network where N and A correspond to cells with normal and abnormal nuclei respectively.

$$N \xrightarrow{\alpha} 2N$$
$$N \underset{k_{-1}}{\overset{\text{NaBu} \cdot k_1 + k_0}{\rightleftarrows}} A$$
$$A \xrightarrow{\omega} \emptyset$$

The networks corresponding to cancer and non-cancer cells (control) are naturally expected to have different parameters (α, k_1, k_{-1}, k_0, and ω)

To determine whether there are parameter regimes in which cancer cells become extinct before control cells, we ran stochastic simulations of our system and did mathematical analysis. The time course of a cell population (N + A) shows that, for a high enough dosage of NaBu, the population goes extinct (see Figure 2a). Control and cancer populations as plotted in Figure 2b show a case within the therapeutic dose whereby cancer cells go extinct before control cells. The distribution of extinction times for a fixed amount of NaBu is shown in Figure 2c. For this set of parameters, control cells survive on average longer than cancer cells. By iterating over concentrations of NaBu, we note that the control cells consistently outlived cancer cells (see Figure 2d). However, these results do not hold for other parameter sets. Mathematical analysis of our model provides necessary conditions on the parameters and NaBu that guarantee the extinction of cancer cells, the extinction of cancer cells before control cells, and the extinction of cells with abnormal nuclei before those with normal nuclei (see below for details on the conditions). These conditions, in turn, allow us to limit the parameter search space which increases the efficiency of our fitting routines.

Being able to predict therapeutic drug dosages with modeling and not just through clinical trials accelerates drug discovery and development. Moreover, having easily available data to fit a model can improve the efficiency and decrease the cost of developing drug candidates. Our model shows that by comparatively simple image analysis of nuclear morphology, we can theoretically estimate therapeutic dosages for drugs. There has not been sufficient attention in drug development to linear models based on nuclear morphology despite their simplicity and cheap implementation. Such models could also complement deep-learning models when training data is limited.

As we are still working on collecting experimental data, it is unclear whether this model can correctly predict the therapeutic dosage of a drug in practice. However, the validation of this model is expected to be quite straightforward as it consists of the original experiment with different concentrations of NaBu. Once the necessary data is available, we will be able to quickly validate the predictions of this model and modify it accordingly. A validated model could then be extrapolated to other drug candidates and cell types since it only requires few imaging samples of the cell nuclei. In the case of unforgiving drugs with low therapeutic indices (a measure of the relative safety of a drug), using therapeutic dosages is risky. In this case, a personalized dosage for the patient is needed and models of this nature could provide an initial step in determining personalized dosages from imaging the cells of individual patients.

References

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Appendix

The condition required for the extinction of cancer cells is given by

$$\mathrm{NaBu} > \frac{\alpha k_{-1} + \omega(k_0 + \alpha)}{k_1 \omega}.$$

The extinction of cancer cells before the control cells occurs when the leading eigenvalue λ of a solution to a network of control cells satisfies

$$\bar{\lambda} < \lambda,$$

where $\bar{\lambda}$ corresponds to the leading eigenvalue of of a solution to a network of cancer cells. The solution to the leading eigenvalue is given by

$$\lambda = \frac{(\alpha - k_0 - k_{-1} - k_1\beta - \omega)}{2} + \sqrt{k_{-1}\alpha - (k_0 - \alpha + k_1\beta)\omega + \frac{(k_0 + k_{-1} - \alpha + k_1\beta + \omega)^2}{4}}.$$

Lastly, the extinction of cells with abnormal nuclei before those with normal nuclei (in the limit under deterministic conditions) will occur if

$$\mathrm{NaBu} < \frac{2k_{-1} + \alpha + \omega - 2k_0}{2k_1}.$$

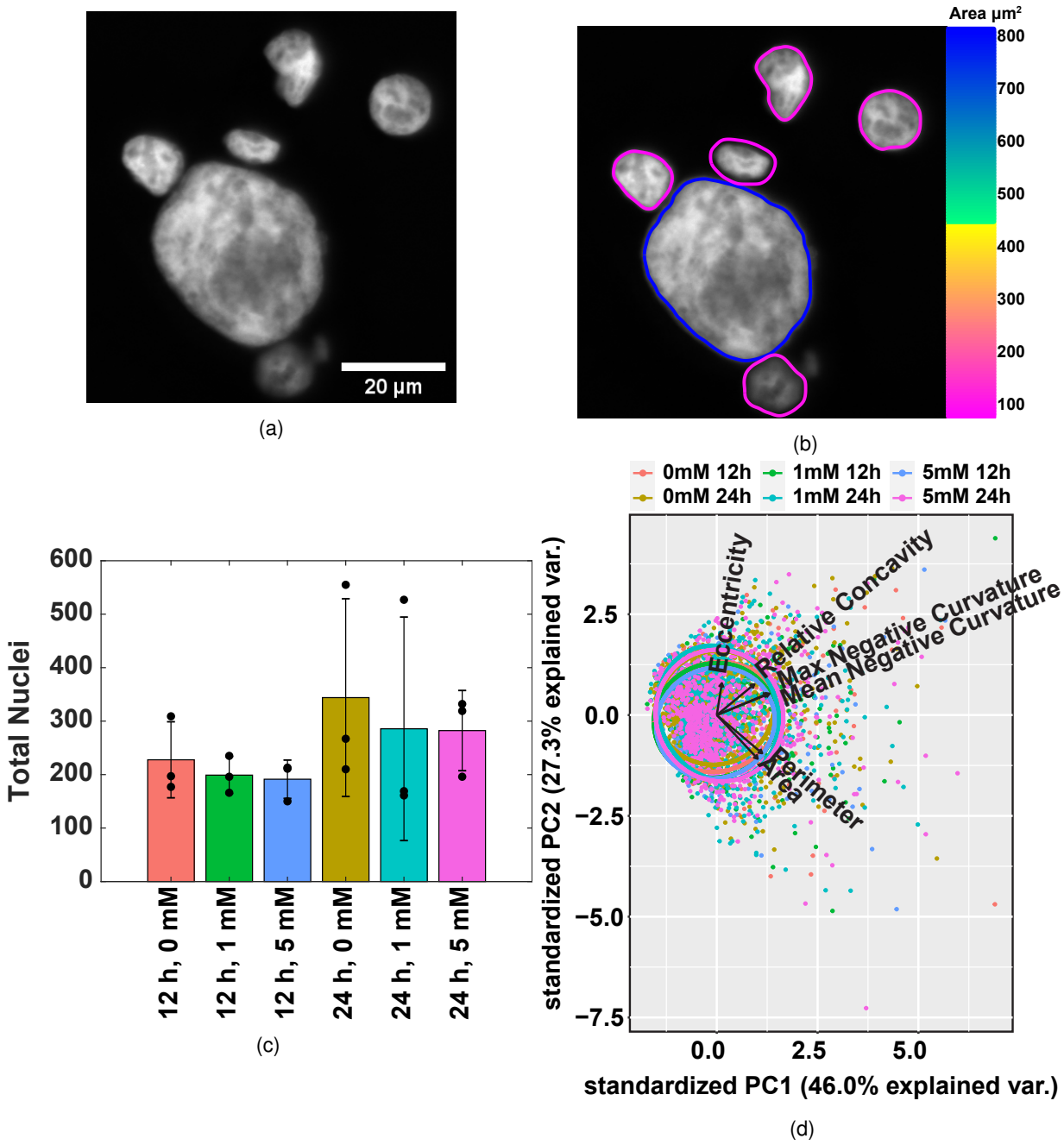

Figure 1: Preliminary Results for HCT116 cells treated for 12 or 24 hours with 0, 1, or 5 mM of NaBu. 1a Representative image of HCT116 cells treated with 5mM of NaBu for 24 hours and 1b the detection results colored by area. 1c Total number of nuclei detected for each condition, where each point represents a coverslip (ANOVA p-value 0.6382). 1d Principle component analysis of nuclear morphology metrics for each nucleus.

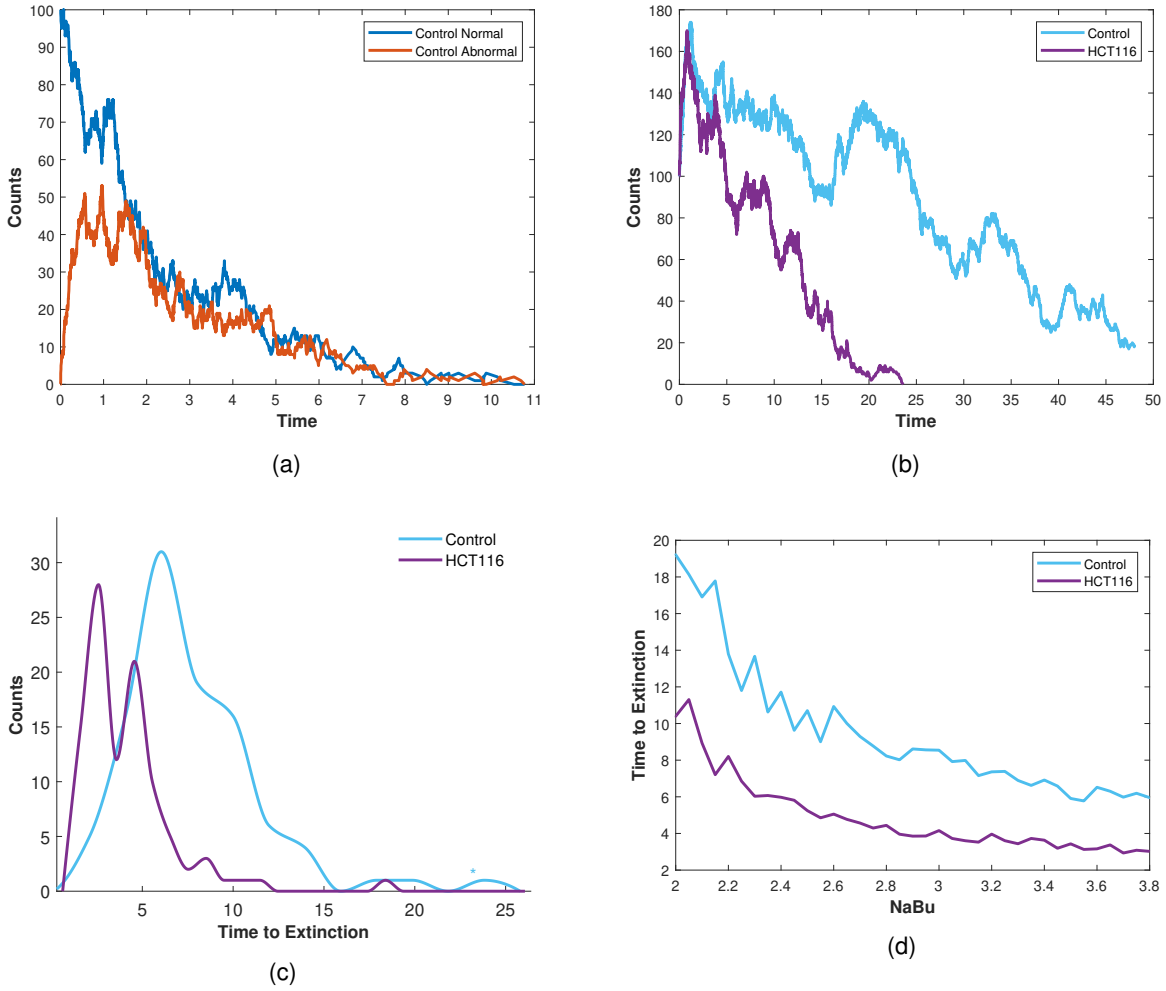

Figure 2: A simple mathematical model could predict optimal dosages of an epigenetic drug from nuclear morphology markers. (a) Populations of cells with normal and abnormal nuclei over time. In the presence of NaBu, both populations eventually go extinct. (b) Entire cell population (N + A) for control and cancer cells over time. Cancer cells become extinct before control cells for this given dosage of NaBu. (c) Distributions of times to extinction for control and cancer cells. On average, cancer cells become extinct before control cells. (d) Time to extinction for different dosages of NaBu. As the dosage increases, cells reach extinction faster. For this particular choice of parameters, control cells outlive cancer cells for every dosage of NaBu.