Fitness Bottlenecks in Doxorubicin-Resistant Osteosarcoma

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Abstract

Osteosarcoma is a malignant, aggressive classification of primary bone tumor that is most typically found in children and adolescents under the age of 25. Thus, identifying novel methods of treatment is critical given the highly aggressive nature of the tumor. This is especially critical because in recent history there has been little progress in the improvement of prognosis, given extremely low survival rates of recurrent cancers. Current standard of care implements a combination of chemotherapy, radiation, and surgery. While undergoing treatment, these cancer cells experience extraordinary evolutionary pressures including but not limited to a multitude of vigorous chemotherapy exposures, which lead to a selective pressure emplaced onto a population of diverse cancer cells. This selective pressure ultimately leads to over competition, selecting for subclones with a specific fitness advantage and thus leading to the development of drug resistance. Therefore, this bottleneck effects permits the most fit subclone of cancer cell to out compete other populations, therefore taking over the cellular population.

Objectives

With osteosarcoma being one of the most common causes of death due to cancer in adolescents, it is critical to adopt novel practices, especially with regards to recurrent cases. One of the most attributable sources of poor survival rates is reliant upon cancer's resistance to standard multi-agent chemotherapy. Thus, one approach to identify positive prognosis of osteosarcoma is to elucidate the fitness bottleneck present through which chemoresistance is developed in order to overcome it. In order to do this, we have created a novel approach at identifying the pathway of single cells through a system of barcoding. We adapted a Genome Editing of Synthetic Target Arrays for Lineage Tracing (GESTALT) approach to investigate the evolutionary transformations of osteosarcoma cells while developing doxorubicin resistance. Altogether, we seek to leverage this evolutionary fitness bottleneck as a therapeutic vulnerability in doxorubicin-resistant osteosarcoma in order to enhance the standard of care that has remained at stagnant levels in recent years.

Methods

Initially, a system for identifying the behavior of specific clusters of cells by tracking cell proliferation through this evolutionary pathway was created. This is done via lentiviral transduction of a synthetic barcode array of ten Cas9/sgRNA targets. This barcode is integrated into the genome of the 143B human osteosarcoma cell line before transfection with a Cas9-expressing plasmid and GESTALT sgRNA. The result is a cell with a unique heritable barcoded region, allowing for single cell ancestry tracing.

One group of these barcoded 143B cells are subsequently adapted to doxorubicin by chronic exposure, while the control group is carried in DMSO. Once 143B cells are adapted to drug, retaining resistance against doxorubicin, they are released from the drug environment to create resistant cell lines.

DNA sequencing of barcodes was performed on resistant, sensitive, and released groups to further understand their compositions. RNA and whole exome sequencing were performed on these groups to identify gene regulatory networks important for the development of doxorubicin resistance. RNA and whole exome sequencing data were used to identify druggable nodes within network during differential timepoints of resistance development in order to identify genes critical in allowing cells to develop resistance to drug. These identified druggable nodes show possible vulnerabilities in doxorubicin resistance.

Results

Initially, an exposure-response relationship was identified by barcoded cells in which doxorubicin-resistant cells exhibited 0% cell death while doxorubicin-sensitive cells exhibited 87% apoptosis. Meanwhile, cell proliferation was significantly stunted in the doxorubicin-resistant group ($P<0.001$) suggesting some type of fitness cost related to developing resistance to drug. This fitness cost was consistent within the doxorubicin resistant group during the two and a half, four, and six week release time points. Yet, following these exposure release time points, the five week release group exemplified an increase in proliferation rate compared to the doxorubicin-sensitive and 1-week release groups ($P<0.001$). Through analysis of RNA-seq data of barcoded cell groups following differential time exposure after drug release, there was a consistent upregulation of ABC transporters and other known drug resistance genes. Therefore, suggesting a learned expression profile component of the drug resistance development pathway.

Following DNA sequencing of barcoded groups, an evolutionary fitness bottleneck as a result of drug adaptation resulted in the development of genetically unique subclones. Two specific subclones derived from the doxorubicin-sensitive population displayed immense growth within the doxorubicin-resistant population. These subclones initially constituted less than 0.2% of the doxorubicin-sensitive population together and accounted for around 90% of the doxorubicin-resistant population, indicating a fitness characteristic not possessed in alternative subclones. Further, one subclone specifically unique to the four week release line constituted over 40% of the population, whereas it was only identified as less than 0.1% of the sensitive, resistant, one and two week release populations thus demonstrating the rise of a proliferative subclone.

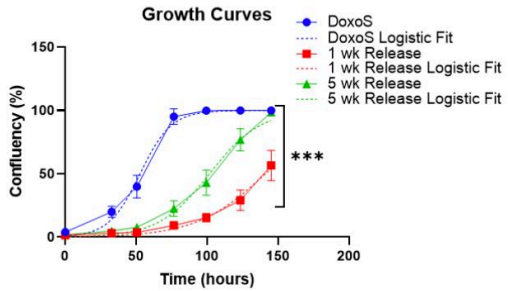

Fig 2. Growth curve displaying increased cell proliferation rates for doxorubicin-sensitive cells as compared to one week release doxorubicin resistant cells. Additionally, the increased proliferation of the five week release group resulting from the growth of a highly proliferative sub clone identified in the four week release population is plotted.

Utilizing bioinformatics tools, a network dependent pathway for development of drug resistance was elucidated. Integrating RNA-Seq and exome sequencing data, key gene regulatory networks and druggable network nodes displayed key genes heavily involved in this adaptation of resistance. Networks created with the gene mania cystoscope platform displayed the unique connections between high impact mutations within unique time points, differences in gene expression levels, and druggable nodes (Fig. 1). These networks were used to identify which genes showed significant upregulation or downregulation of gene expression against cells sensitive to doxorubicin to elucidate which genes are involved in the resistance pathway. Subsequently, high impact genes that showed high log fold changes from RNA seq data and were additionally identified as druggable nodes were recognized. These genes are target nodes for future experimentation. These actionable nodes are used to target the highly proliferative subclone identified in the four week doxorubicin-resistance population. It is the hope that inhibiting these nodes as well as further understanding the pathway-dependent maturation of resistance will help improve prognosis for osteosarcoma.

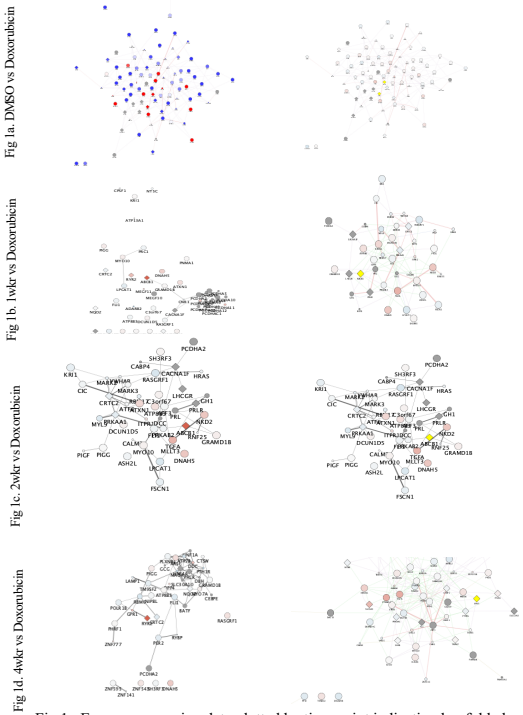

Fig 1. Exome sequencing data plotted by time point indicating log fold change differences of gene expression based on RNA seq data by continuous mapping filtered by highly expressed genes that display druggable nodes.

Conclusion

In order to elucidate the pathway to development of drug resistance, the GESTALT approach was implemented. Utilizing a barcoded, ancestry tracing protocol individual subclone populations were followed to study evolutionary fitness bottlenecks along the path to doxorubicin resistance in osteosarcoma cells. RNA-Seq, exome , and bioinformatics data, including the cystoscope platform, were utilized the to elucidate the networks created between high impact genes and identify druggable nodes involved in a high concentration of pathway connections. These data may be used to identify gene regulatory networks and druggable nodes to directly explore genes involved in resistance to standard od care treatment methods.

References

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