Computational Drug Repositioning Identifies Potentially Active Therapies for Chordoma

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BACKGROUND: Chordomas are aggressive bone tumors that often recur despite maximal resection and adjuvant radiation. To date there are no Food and Drug Administration (FDA)-approved chemotherapies. Computational drug repositioning is an expanding approach to identify pharmacotherapies for clinical trials.

OBJECTIVE: To identify FDA-approved compounds for repurposing in chordoma.

METHODS: Previously identified highly differentially expressed genes from chordoma tissue samples at our institution were compared with pharmacogenomic interactions in the Comparative Toxicogenomics Database (CTD) using ksRepo, a drug-repositioning platform. Compounds selected by ksRepo were then validated in CH22 and UM-Chor1 human chordoma cells in Vitro.

RESULTS: A total of 13 chemical compounds were identified in silico from the CTD, and 6 were selected for preclinical validation in human chordoma cell lines based on their clinical relevance. Of these, 3 identified drugs are FDA-approved chemotherapies for other malignancies (cisplatin, cytarabine, and linciclib). Cytarabine, a deoxyribonucleic acid polymerase inhibitor approved for the treatment of various leukemias, exhibited a significant concentration-dependent effect against CH22 and UM-Chor1 cells when compared to positive (THZ1) and negative (venetoclax) controls. Tretinoin exhibited a significant concentration-dependent cytotoxic effect in CH22, sacral chordoma-derived cell lines but to a much lesser extent in UM-Chor1, a cell line derived from skull base chordoma.

CONCLUSION: Cytarabine administration reduces the viability of human chordoma cells. The equally effective reduction in viability seen with tretinoin seems to be cell line dependent. Based on our findings, we recommend the evaluation of cytarabine and tretinoin in an expanded set of human chordoma cell lines and animal models.

KEY WORDS: Chordoma, Computational drug repositioning, Chemotherapy, ksRepo, Cytarabine, Tretinoin

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Chordomas are malignant tumors arising from remnants of the embryological notochord and most commonly arise in the clivus and sacrococcygeal region.¹ They are rare tumors with an incidence rate of 0.18 to 0.84 million persons per year, with a male predominance.² Gross total resection

of the tumor followed by high-dose adjuvant radiotherapy is considered standard of care in good surgical candidates. Loco-regional recurrence, however, continues to occur in >50% of patients following complete resection with or without adjuvant radiation.³ There is currently no evidence to support the use of cytotoxic chemotherapy in the management of chordoma outside of two reported pediatric cases.^{4,5} As a result, there are few treatment options to control disease in these patients following surgery and radiation.

Techniques for discovering new indications for drugs already approved by the Food and Drug Administration (FDA), known as “drug repositioning,” have emerged to reduce the time between drug discovery, development, and

ABBREVIATIONS: CDK, cyclin-dependent kinases; CTD, Comparative Toxicogenomics Database; DEG, differentially expressed gene; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; IRB, Institutional Review Board; RNA-Seq, ribonucleic acid sequencing

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availability while also reducing costs. Specifically, strategies for computational high-throughput analysis to integrate genetic and chemical compound data are increasingly being explored to accelerate drug discovery.⁶ Among these is ksRepo,⁷ a novel, open-source repositioning platform that applies a modified Kolmogorov-Smirnov test for gene enrichment analysis. The ksRepo package identifies pharmacogenomic interactions from the Comparative Toxicogenomics Database (CTD)^{8,9} based on characteristic differentially expressed genes (DEGs). Since its original validation,⁷ ksRepo has been applied to repurpose pharmacotherapies for vestibular schwannoma.¹⁰ However, this approach has yet to be applied to human chordoma. In this preclinical study, we implemented ksRepo to compare DEGs in chordoma previously identified by our institution^{11,12} with identified gene interactions in the CTD. To our knowledge, this is the first attempt to identify potential therapies for human chordoma using computational drug repositioning.

METHODS

Chordoma Ribonucleic Acid Sequencing Dataset

An overview of our methodology is described in Figure 1. All data from human subjects were extracted from previously published data

repositories with prior Institutional Review Board (IRB) approval.^{11,12} No IRB approval was needed for the described study as there was no involvement with human subjects. See “RNA-Seq analysis” in **Supplemental Digital Content 1**, which details our procedure for ribonucleic acid sequencing (RNA-Seq) analysis of chordoma tissue samples and identification of biomarkers. Chordoma gene regulation is summarized by a heatmap in Figure 2.

ksRepo Processing

Drug candidates were selected from identified chordoma biomarkers with the ksRepo package in the R statistical software environment. ksRepo (<https://github.com/adam-sam-brown/ksRepo>)⁷ is an open-source computational drug repositioning platform that identifies drug candidates based on pharmacogenomic interactions using a modified Kolmogorov-Smirnov enrichment test. The ksRepo platform was originally validated on 5 prostate cancer datasets and the CTD.⁷ Select genes were ranked according to the degree of differential expression for each of the 3 datasets for skull base chordomas, spine chordomas, and those identified in both subtypes. The CTD dataset was selected for drug-gene interaction comparisons. The CTD is a public dataset established to advance the understanding of the interactions between chemical compounds and the human genome.⁸ The default number of 1000 bootstrap samples was used for calculation of *P*-values. To correct for multiple comparisons, the Bonferroni correction method was used to adjust *P*-values from the ksRepo output.¹³ An adjusted *P*-value < .05

was considered to be significant. We implemented ksRepo comparisons of drug-gene interactions in 3 separate groups from the identified DEGs: (1) skull base chordoma only, (2) spine chordoma only, and (3) DEGs common to both the skull base and spine chordoma samples.

In Vitro Cell Lines

Two separate chordoma cell lines were procured for tissue validation; UM-Chor1¹⁴ cells (obtained from ATCC #CRL-3270) cultured from clival chordoma samples and CH22¹⁵ cells (obtained from the Chordoma

TABLE 1. Significant Compounds (Adjusted *P*-value < .05) Identified by ksRepo in Each Category

DEG group	Compound	Adjusted <i>P</i> value ^a
Skull base	Acetaminophen	.00
	Folic acid	.00
	Lucanthone	.00
	Testosterone	.03
Spine	Acetaminophen	.00
	Zinc	.00
	Copper	.00
	Cyclosporine	.00
	Cytarabine	.00
	Cisplatin	.04
	Formaldehyde	.00
	Testosterone	.00
	Tretinoin	.00
	Valproic acid	.00
	Vitamin E	.00
Common to skull base + spine	Acetaminophen	.02
	Formaldehyde	.00

DEG: differentially expressed genes.

^a*P*-values adjusted using the Bonferroni correction.¹⁵

Foundation) from sacral chordoma samples. Our procedure for in Vitro validation of selected compounds is detailed in “in Vitro cell lines” in **Supplemental Digital Content 1**.

RESULTS

ksRepo Drug Candidates

The ksRepo computational screen of the 3 separate DEG groups and the CTD identified 13 unique compounds that displayed significant DEG interactions (Table 1). Of these, 3 were cytotoxic chemotherapies currently approved by the FDA for other malignancies: cisplatin, cytarabine, and lucanthone. Cisplatin was the first platinum-based therapy approved by the FDA for cancer treatment.¹⁶ Cytarabine is a cytosine analog chemotherapeutic that has been a standard component of acute myeloid leukemia therapy for decades.¹⁷ Finally, lucanthone is an autophagy and topoisomerase II inhibitor that has been shown to enhance the effectiveness of radiation¹⁸ and chemotherapy¹⁹ due to inhibition of the deoxyribonucleic acid (DNA)-repair pathway. Although not considered to be chemotherapeutic agents, valproic acid and tretinoin were considered for validation, as prior in Vitro studies have shown some antineoplastic activity in these molecules.^{20,21} Additionally, tretinoin is the standard therapy for acute promyelocytic leukemia.²² Cyclosporine has been shown to have some antileukemic properties²³ and to enhance the cytotoxic effects of other chemotherapies.^{24,25} Seven compounds were considered to be poor candidates for preclinical validation, as they were either toxic (formaldehyde) or had no known antineoplastic properties (zinc, copper, folic acid, testosterone, and

vitamin E). Six total compounds were ultimately selected for preclinical validation in chordoma tissue models.

Preclinical Validation of Select Compounds

Venetoclax, a Bcl-2 inhibitor and negative control, had minimal effect on UM-Chor1 and CH22 cell viability, as the *BCL-2* gene is not expressed in CH22 and UM-Chor1 chordoma cell lines. THZ1, an inhibitor of CDK7/12/13 actively reduced the viability of both lines, killing 70% of cells within 3 d. Of note, CH22 cells were more sensitive to THZ1 than UM-Chor1 cells, with 70% killing observed within 3 d, corresponding to previous in Vitro findings.²⁶

Neither CH22 nor UM-Chor1 were sensitive to valproic acid, an inhibitor of sodium channels and gamma-aminobutyric acid transaminase. Cisplatin, a platinum-based DNA alkylating agent, was shown to exhibit a similar antineoplastic effect in both cell lines with higher concentrations (in the micro molar range). Similarly, cyclosporine, an immunosuppressant inhibitor of calcineurin, exhibited equal cytotoxic effects in both chordoma cell cultures at slightly lower concentrations than cisplatin. Lucanthone, an intercalator of DNA and inhibitor of DNA repair enzymes, equally reduced the viability of CH22 and UM-Chor1 cells after 3 d but had no effect on these cells at lower concentrations. Tretinoin, a vitamin A derivative that has been shown to induce differentiation in neoplastic cells,²⁷ successfully reduced the viability of both chordoma cell lines, killing 70% of cells after 3 d. Additionally, tretinoin was observed to have differential sensitivity in the 2 cell lines. Although 70% of sacral-derived CH22 cells were eliminated in 3 d with a clear concentration relationship, there was little change in the viability of the skull-derived UM-Chor1 cell line until 3 d. Cytarabine, an inhibitor of DNA polymerase, was observed to be the most active against both CH22 and UM-Chor1 at lower concentrations. Concentration-response curves are delineated in Figure 3 and associated EC50 values are presented in Table 2. Phase contrast images of UM-Chor1 and CH22 cells following treatment with dimethyl sulfoxide (DMSO), tretinoin, and cytarabine are shown in Figure 4, with 3 d of treatment.

DISCUSSION

Background

Repurposing FDA-approved medications with computational techniques for new indications is quickly becoming a favorable approach to drug selection. This strategy offers a time and cost-effective alternative for evaluation of candidates for clinical trials, as it allows for secondary analysis of vetted therapies with optimized pharmacokinetics and acceptable toxicity profiles.²⁸ Until recently, repurposing of approved pharmacotherapies often resulted from serendipitous clinical or laboratory observations. Increasing volumes of molecular data amassed in publicly accessible repositories and contemporary advances in computational techniques have evolved a new frontier of drug discovery.²⁹

FIGURE 3. Concentration-response curves of each compound tested in Vitro over time in each chordoma cell line. The x-axis represents the logarithmic concentrations of the compound with serial dilutions. The y-axis represents the fraction of viable cells treated with the compound determined relative to the DMSO control. THZ1 and venetoclax represent positive and negative compound controls, respectively.

TABLE 2. EC50 Values for Each Compound and Cell Line			
Category	Compound	Cell Line	EC50
+ Control	THZ1	CH22	60 nM
	THZ1	UM-Chor1	500 nM
– Control	Venetoclax	CH22	NA
	Venetoclax	UM-Chor1	NA
Chemotherapeutics	Cisplatin	CH22	2.3 μM
	Cisplatin	UM-Chor1	3.5 μM
	Cyclosporine	CH22	234 nM
	Cyclosporine	UM-Chor1	344 nM
	Cytarabine	CH22	45 nM
	Cytarabine	UM-Chor1	650 nM
Other compounds	Lucanthone	CH22	3.7 μM
	Lucanthone	UM-Chor1	5.1 μM
	Tretinoin	CH22	30 nM
	Tretinoin	UM-Chor1	700 nM
	Valproic acid	CH22	NA
	Valproic acid	UM-Chor1	NA

Initially, computational methods were designed to identify similarities in chemical structures between compounds and ligands.³⁰ With more recent initiatives for open-access data, computational techniques for integrating and parsing multisource metadata from “big-data” archives are emerging. See **Supplemental Digital Content 2** for additional discussion on the background of computational drug repositioning.

In its original study, ksRepo was validated by successfully selecting several FDA-approved therapies for prostate cancer

from DEGs identified from 5 discrete prostate cancer datasets and the CTD.⁷ In 2018, Sagers et al¹⁰ applied the ksRepo platform to genomic expression data from vestibular schwannoma and identified eight FDA-approved compounds for preclinical validation. Of these, mifepristone was selected for preclinical validation and was observed to affect the proliferation of primary human vestibular schwannoma cells with no effect on healthy human Schwann cell controls.³¹

Like vestibular schwannoma and meningioma, chordoma has no FDA-approved chemotherapy options for treatment. Although indolent in early stages, chordoma often infiltrates adjacent tissue, complicating operative resection.³² Distant metastases are seen in the late stages of disease and high rates of recurrence despite aggressive combined surgical and radiation therapy impairs survival and quality of life. Additionally, it has been shown that chordoma has a “quiet” genome with few genetic drivers for which to potentially target with pharmacotherapies.³³ As such, repurposing FDA-approved pharmacotherapies using computational drug repositioning stands to benefit this patient population at reduced time and cost to conventional avenues for pharmacotherapy development.

Our study is the first to attempt to repurpose FDA-approved pharmacotherapies for chordoma using a computational technique. ksRepo, specifically, offered several advantages as a drug repositioning platform that benefitted our approach. First, the flexibility of the ranked gene input allowed us to provide a list of DEGs identified from RNA-Seq analysis of chordoma samples procured from our institution.^{11,12} Second, the ability to use the CTD for drug-gene interaction

comparisons which is continually updated and quality analyzed by professional biocurators. Additionally, the ksRepo algorithm has been applied in previous studies and provides a standardized computational approach to computational drug repositioning.^{7,10} By applying the ksRepo algorithm to 3 separate categories of DEGs (skull base, spine, and common DEGs only) identified by Bell et al,¹¹ we attempted to cast a wide net with in silico screening for drug candidates for preclinical validation. Although the majority of the compounds tested in Vitro exhibited concentration-dependent effects against chordoma proliferation, cytarabine and tretinoin exhibited the most potent effects on cell viability in both CH22 and UM-Chor1 cell lines. Interestingly, CH22, a sacral chordoma-derived cell line, was more sensitive to the effects of all compounds including the THZ1 control, tretinoin, and cytarabine, with higher concentrations

required for similar effects in clival chordoma-derived UM-Chor1 cells.

Future Implications

Due to the variability between cell lines and the fact they were derived from different anatomical regions (spine vs skull base), we recommend that both tretinoin and cytarabine be further evaluated as they had the greatest effect on both chordoma cell lines. Tretinoin increases the cytotoxic effects of chemotherapeutic agents, including cisplatin, in UM-Chor1 cell lines,³⁴ indicating a potential synergistic role with other chemotherapeutics. Notably, tretinoin has been shown to reduce proliferative capacity and brachyury levels by reduction of the brachyury gene, *TBXT* (*T*-gene) in UM-Chor1 chordoma cells.³⁵ This is particularly noteworthy as a 2019 study by Sharifnia et al²⁶ reported

that small-molecule targeting of brachyury transcription factor addition substantially reduces tumor growth and may represent a potential therapeutic strategy for chordoma.³⁶ Furthermore, tretinoin has been shown to induce the differentiation of several cell precursors, giving it a vital role in the development of the blood-brain barrier³⁷ and as a “differentiation” therapy in various malignancies, particularly acute promyelocytic leukemia.³⁸ Finally, tretinoin is generally well tolerated,³⁹ making it an ideal therapy to study in human trials.

Cytarabine has been used in the treatment of various leukemias,⁴⁰ although it has never been studied in the context of chordoma. Cytarabine is further advantaged by high interactions with the DNA repair enzyme poly(ADP-ribose) polymerase 1,^{41,42} which has been described as a potential therapeutic target for refractory chordoma.⁴³ Cytarabine has been associated with significant neurotoxicity, particularly with intrathecal administration.⁴⁴ Further evaluation of this compound as a candidate therapy will require careful dosing in order to minimize adverse effects and optimize chordoma cytotoxicity.

Although cytarabine and tretinoin were observed to have the most significant effect based on EC50 values and concentration-response curves, it should be noted that anti-chordoma activity was demonstrated by lucanthone, cisplatin, and cyclosporine. Lucanthone has not been studied in the management of chordoma although it has been shown to have some in Vitro activity against glioma.⁴⁵ Of the compounds studied, cisplatin and tretinoin are the only agents to have previously reported anti-chordoma activity in Vitro.^{34,35} Additionally, cytarabine is widely used in bony cancers, primarily osteosarcoma.⁴⁶ With new evidence indicating that advanced chordoma may be characterized by defective DNA repair pathways,⁴³ cisplatin (via induction of DNA strand breaks) and lucanthone (via topoisomerase II inhibition) may also represent potential chordoma therapies. Additionally, inhibition of cyclin-dependent kinases (CDK) has been shown to potently suppress the proliferation of chordoma cells.²⁶ Both cytarabine and tretinoin have been shown to increase the expression of CDKN1A,^{47,48} an endogenous, universal CDK inhibitor.⁴⁹

Limitations

Our study was limited by the lack of a tissue control for which to compare to chordoma cells for RNA-Seq analysis and with compound validation. This is a known obstacle to chemical testing within chordoma tissue models due to the lack of accessible tissue analogous to the embryological notochord. As a result, various tissues have been used as controls to chordoma cells in previous studies, from placenta to muscle.^{50,51} For RNA-Seq analysis, nasal turbinate tissue was used as a control,^{11,12} which is not entirely analogous to the notochord from which chordoma is derived. As a result, the determined DEGs could represent the differences between the chordoma cell gene expression and turbinate tissue rather than between notochord and chordoma cell expression. Despite this, we thought this tissue to be a reasonable

control based on our prior histologic and immunohistochemistry analysis of these turbinate samples.¹² We further attempted to mitigate this challenge using positive and negative chemical controls with established effects or noneffects on chordoma cell proliferation.²⁶ The strength of our conclusions is also hindered by the limitations inherent to the CH22 and UM-Chor1 cell lines. The sex of the patient (CH22 female, UM-Chor1 male) and primary location (CH22 sacral, UM-Chor1 clival) differ between the 2 cell lines and can confound the response to selected therapies. Additionally, the molecular features specific to these 2 cell lines are not fully understood, which may contribute to some of the observed differences in cytotoxicity for the same selected compound.

Finally, although ksRepo was developed to rectify the limitations of prior computational repositioning methodologies,⁷ the approach to drug repurposing with this platform is not without disadvantages. As mentioned by Sagers et al,¹⁰ this methodology will disregard biologic therapies engineered to target specific genes or proteins in favor of compounds with greater genome-wide expression differences. Thus, this method for drug identification is best applied in tumors with “quiet” genomes with few potential molecular targets, such as chordoma. Despite these limitations, our study is the first to offer in silico and in Vitro validation for repurposed FDA-approved compounds in chordoma.

CONCLUSION

Chordomas are aggressive primary bone malignancies that are challenging to manage with no FDA-approved therapies. Computational drug repositioning with the ksRepo platform identified compounds in silico for preclinical in Vitro validation on human chordoma cells. Treatment of chordoma cells with cytarabine and tretinoin independently reduced the viability as well as the THZ1 control, a tool compound shown to dramatically slow chordoma growth in murine models. Based on our findings, we recommend the evaluation of cytarabine and tretinoin in an expanded set of cell lines and in animal models of human chordoma.

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Supplemental digital content is available for this article at www.neurosurgery-online.com.

Supplemental Digital Content 1. Expands on the methods provided.

Supplemental Digital Content 2. Expands on the background of computational drug repositioning.

COMMENTS

Chordomas are aggressive tumors of bone that exhibit frequent local recurrence, the majority of which are treated with radical resection and adjuvant high dose radiation. Radicality of resection must also be balanced with morbidity of surrounding critical structures. There is currently a lack of clinically efficacious agents and lack of FDA approved targeted therapies. In this study, the authors have undertaken a bio-informatics approach to identify compounds for repurposing in human chordoma using a chordoma gene signature. This approach yielded cytarabine and tretinoin as promising agents in sacral and clival chordomas. Cytarabine was only effective in clival chordoma.

There is good evidence that chordomas from different locations and with different behaviors (from indolent incidental to dedifferentiated) have distinct genetic profiles. It will be important to understand which chordoma locations and tumor types were used in this study to generate these genetic profiles and how to properly apply them in future clinical trials. Secondly, it will be vital to use profiled cell lines that recapitulate these different disease states for any pre-clinical studies.

There are some limitations in the methods which suggest that further study is needed to verify these results. Half of the samples from the study were determined to be “unreliable”. In addition, the use of turbinates as non-matching controls potentially adds error. Matched controls such as blood (germline comparison) would be used to ensure that non-

neoplastic genetic material is factored out. Nevertheless, the authors should be applauded for their rigorous study of this challenging disease.

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Chordoma is known as a one in a million bone cancer, arising from the vestigial remnants of the notochord. 50% occur in the sacrum, 30% in the skull base. 10 year survival for a sacral chordoma has been reported as 46%.¹ If more than half of chordomas grow back, treatment options need to be offered to patients. The authors discuss the pharmacological intervention of cytarabine and tretinoin in chordomas and state that they need to expand their observations in human chordoma cell lines and pertinent animal models. Interestingly, both these drugs are FDA approved, and their repurposing into a rare tumor such as chordoma is a feasible approach. This is a challenging study for a rare tumor, and the authors used a list of DEGs identified from RNA-Seq analysis of chordoma samples at their institution. They were able to obtain a list of ranked genes, and they then applied an FDA-approved ksRepo Drug Repositioning Platform to obtain the drug targets. Hence the identification of cytarabine and tretinoin.

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