

G PROTEIN-COUPLED RECEPTOR 35 HOLDS POTENTIAL AS A BEACON OF HOPE FOR TREATING CHONDROSARCOMA

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ABSTRACT

Introduction: A group of primary bone tumors called chondrosarcomas are diverse and characterized by neoplastic tissue of hyaline cartilage. They are the second most typical primary osseous cancer. Recurrence is indicative of a poor prognosis because traditional chondrosarcomas are resistant to treatment. Our knowledge of the pathobiology of conventional chondrosarcomas has greatly expanded owing to recent findings in these tumors' biology, genetics, and epigenetics studies. These findings also provide information on possible treatment targets. CHEK2 encodes a checkpoint kinase involved in DNA damage response and cell cycle regulation, and it is an important cancer susceptibility gene.

Methods: The Gene2 drug and DSEA assisted with in silico screening. The antitumor activities of the candidate drugs were extracted from DepMap via the PRISM viability assay on eight chondrosarcoma cell lines.

Results and Conclusion: Advances in studies show promise for discovering potential targeted treatments for cancer. Bisacodyl is a targeted G protein-coupled receptor 35. G protein-coupled receptors are widely known targets for cancer treatment. Here, we showed that bisacodyl can be a potential therapeutic agent in chondrosarcoma cell lines.

Keywords: bisacodyl, *CDS1*, *CHEK2*, chondrosarcoma, targeted treatment

INTRODUCTION

The term "chondrosarcoma" (CS) refers to a collection of diverse, usually slow-growing, primary malignant bone tumors distinguished by the development of hyaline

cartilaginous neoplastic tissue [1]. Chondrosarcoma affects approximately 3 in 1 million people annually. There are various subtypes of chondrosarcomas, including mesenchymal, clear cell, dedifferentiated (DDCS), and conventional (CCS) [2]. For every subtype, different genetic alterations were observed. The CCS and DDCS subtypes are characterized by mutations in isocitrate dehydrogenase (IDH) 1 or 2, but not in the other subtypes [2,3]. However, only the mesenchymal subtype shares a fusion gene with other subtypes [2]. In general, IDH1/2 mutations, Hedgehog pathway alterations, tumor suppressor pathways and PI3K–Akt–mTOR pathways [3] and Collagen Type II Alpha 1 Chain COL2A1 [4] mutations are commonly observed in CSs.

Patients with DDCS have a worse prognosis than those with other chondrosarcoma subtypes [3]. Therefore, a more potent systemic treatment for this malignancy is urgently required. Several cytotoxic medicines targeting sarcomas, including adriamycin and ifosfamide, have been shown in different retrospective studies to be effective when combined with surgical resection of DDCS. Nevertheless, the prognosis after such treatments is typically poor. For the treatment of chondrosarcoma, including DDCS, several prospective molecularly targeted medications have been suggested, including mTOR inhibitor [5] CDK4/6 inhibitor, multikinase inhibitor [6] and Hedgehog pathway inhibitor [7]. However, the clinical effectiveness of these medications has not been satisfactory. Therefore, strategies other than cytotoxic drugs and molecularly targeted medications are required.

The human equivalent of yeast checkpoint kinases Cds1 and Rad53 is encoded by the CHEK2 gene (MIM +604373). Upon activation, the encoded protein is recognised to inhibit CDC25C phosphatase, thereby preventing entry into mitosis, and has been demonstrated to stabilize the tumor suppressor protein p53, resulting in cell cycle arrest at the G1 phase. Moreover, this protein interacts with and phosphorylates BRCA1, facilitating BRCA1's role in

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restoring cell survival following DNA damage [8]. Gene Ontology (GO) annotations describe CHEK2's molecular functions as ATP binding, protein serine/threonine kinase activity, protein homodimerization activity, and transferase activity involving the transfer of phosphorus-containing groups [9]. Identifying promising targetable pathways and prognostic markers has resulted from advancements in the molecular knowledge of chondrosarcomas, despite the challenges posed by their rarity. Here, we applied drug repurposing using in silico tools and highlighted bisacodyl (CAS number: 603-50-9) as a potential compound for the CDS1 (CHEK2) gene in chondrosarcomas.

MATERIALS AND METHODS

We used in silico screening tools to identify potential therapeutic compounds that target CDS1 (CHEK2). Therefore, we used Gene2drug (<https://gene2drug.tigem.it/>) for in silico screening, and PRISM viability tests were applied to eight cell lines linked to chondrosarcoma (Table 1).

After compound selection, DepMap data were analyzed for antitumor activities of CHEK2 targets (<https://depmap.org/portal/>) [10].

Chondrosarcoma cell sensitivity to potential medications was sorted using DRUG Sensitivity (PRISM Repur-

posing Primary) (<http://depmap.org/portal/>) and CDS1 (CHEK2) expression.

Gene to Drug Analysis

The Gene2 drug database was used to identify compounds that target CDS1 (CHEK2). Based on PRISM viability assays on eight chondrosarcoma cell lines, DepMap data were analyzed for the antitumor activities of these drugs and their target CDS1 (CHEK2) [10-12].

Expression Public 23Q2 and drug sensitivity (PRISM repurposing primary screen) were used to sort chondrosarcoma cells for sensitivity to candidate drugs [10-12]. The P-value produced by Gene2drug determines the ranking of the compounds. The compounds implicated with $P < E-3$ are listed in Table 2. Here, we selected compounds implicated as those with $P < E-7$.

In silico examination of Bisacodyl

SwissADME was used to analyze bisacodyl in silico. This free web-based program makes it easier to evaluate small compounds' pharmacokinetics, drug-likeness (the likelihood of oral bioavailability), and medicinal chemistry friendliness (PAINS) [13]. The SwissTargetPrediction online tool also attempted target prediction [14].

RESULTS

Gene to Drug Analysis and the sensitivity of chondrosarcoma cells to candidate drugs

The lists of potential CDS1 (CHEK2)-targeted compounds were produced by Gene2drug [15]. The P value produced by Gene2drug determines the ranking of the compounds. The compounds implicated were those with $P < 1E-3$ (Table 2).

Chondrosarcoma cell sensitivity to potential medications was sorted using DRUG Sensitivity (PRISM Repurposing Primary) (<https://depmap.org/portal/>) and CDS1 (CHEK2) expression. A total of 593 drugs were analyzed

Table 1. The list of Chondrosarcoma cell lines

Name of the cell lines	Diseases Subtype
CAL78	Dedifferentiated Chondrosarcoma
CHSA0011	Chondrosarcoma
CHSA0108	Chondrosarcoma
CHSA8926	Chondrosarcoma
CS1	Chondrosarcoma
HEMCSS	Extraskelatal Myxoid Chondrosarcoma
OUMS27	Chondrosarcoma
SW1353	Chondrosarcoma

Table 2. CDS1 (CHEK2) expression public 23Q2 and drug sensitivity (PRISM repurposing primary screen)

Compound	Pearson	Spearman	Slope	Intercept	p-value (linregress)
aminophylline	0.127	0.131	3.78E-2	-4.29E-1	2.69E-3
levomepromazine	0.125	0.124	2.94E-2	-5.82E-2	3.26E-3
chloropyramine	0.117	0.127	7.68E-2	-2.00E+0	6.23E-3
chlorhexidine	0.150	0.133	8.26E-2	-8.63E-2	4.36E-4
parbendazole	0.123	0.124	1.21E-1	-2.26E+0	3.81E-3
bisacodyl	-0.217	-0.190	-7.49E-2	3.66E-1	2.76E-7
anisomycin	0.128	0.126	1.11E-1	-3.16E+0	2.71E-3
mepacrine	-0.141	-0.131	-4.70E-2	4.50E-1	1.02E-3
trifluridine	0.140	0.137	1.10E-1	-1.21E+0	1.31E-3

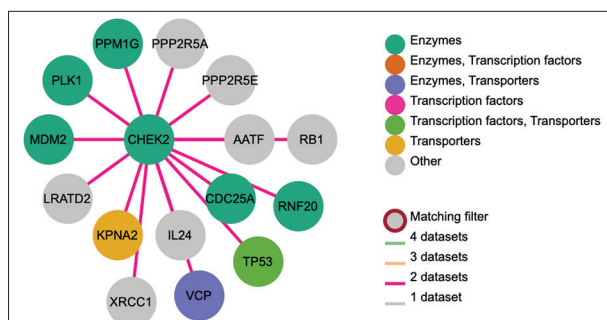


Figure 1. Protein interactions of CDS1 (CHEK2)
The figure illustrates protein interactions of CDS1 (CHEK2). The first-level consensus interactions for the nuclear protein CDS1 are colored according to subcellular location (left). Yellow circles show genes related to transporters and green circles show enzyme interactions of CDS1.

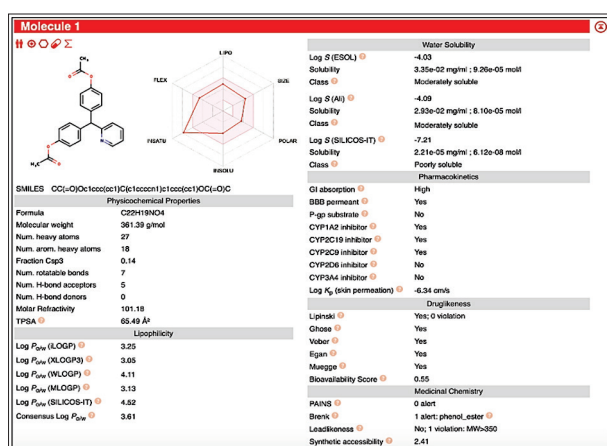


Figure 2. The bioavailability radars for Bisacodyl produced by the SwissADME web application. These radars underscore its physicochemical characteristics, supporting its potential for high oral bioavailability.

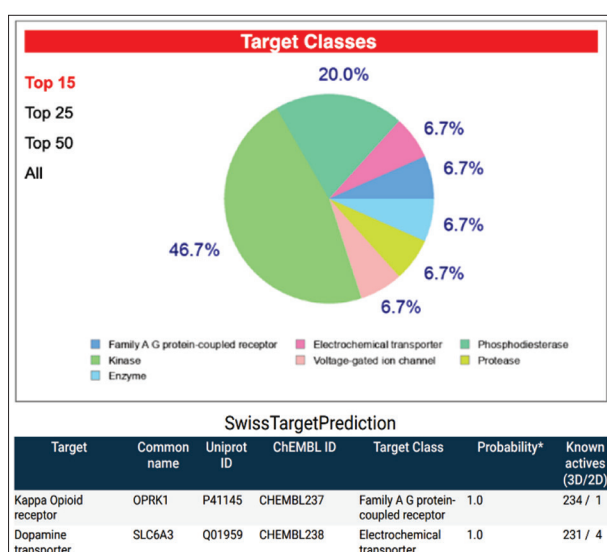


Figure 3. SwissTargetPrediction predicts the protein target for Bisacodyl. The figure shows the results of the target prediction method, highlighting the proteins with the highest probability ratings for bisacodyl.

using DRUG Sensitivity (PRISM Repurposing Primary) for chondrosarcoma. Nine compounds were significantly more sensitive to chondrosarcoma treatment ($P < E-3$). Here, we selected the compounds implicated with a $P < E-7$.

Protein-protein interaction

The Human Protein Atlas (<https://www.proteinatlas.org/ENSG00000163624-CDS1/interaction>) was used to demonstrate protein interactions of CDS1 (Figure 1).

In silico examination of Bisacodyl

The chosen drugs are represented pharmacologically by the bioavailability radars. Lipophilicity, size, polarity, solubility, saturation, and flexibility are all physicochemical properties essential for oral bioavailability and, as a result, may have activity against tumors outside the gastrointestinal tract. The pink-highlighted area in the graphs represents the ideal range for each property. All these characteristics were displayed by bisacodyl within the intended range, which helped to provide high oral bioavailability (Figure 2). To find alternate targets for bisacodyl, we used the online program SwissTargetPrediction. Bisacodyl produced noteworthy results with high probability ratings (Figure 3) [16].

DISCUSSION

Despite studies aimed at developing more effective cancer treatments, there is still an unmet medical need for numerous malignancies. There is an urgent need to establish novel lines of attack and discover new compounds that target the specificities and characteristics of chondrosarcomas. Advancements in new applications for existing drug repositioning are promising for identifying new cancer treatment targets.

Studies have demonstrated that dedifferentiated chondrosarcomas are associated with the upregulated expression of genes involved in G2/M checkpoints and E2F targets [17]. Therefore, we speculate that targeted treatment of these E2F targets could be used as a candidate treatment target. Therefore, we used in silico tools to identify the target-specific compounds in this study. The Gene2Drug database was used to identify bisacodyl (CAS number: 603-50-9) as a potential compound for the CDS1 (CHEK2) gene.

Bisacodyl (DrugBank Accession Number: DB09020) is an FDA-approved drug. According to the BindingDB website, the target of bisacodyl is a G protein-coupled receptor 35 (<https://www.bindingdb.org/rwd/bind/chemsearch/marvin/MolStructure.jsp?monomerid=61400#>). G protein-coupled receptors have been widely known as a target for cancer treatment [18,19]. Here, we showed that

bisacodyl can be a potential therapeutic agent in chondrosarcoma cell lines.

Bisacodyl is commonly prescribed for constipation and pre-surgical bowel cleansing. Numerous studies have been conducted in humans, rodents, and cell cultures to test their safety and absence of mutagenic effects [20]. Bisacodyl, by blocking InsP3-induced Ca^{2+} release, triggers the necrosis of these cells. Deregulation of InsP3R Ca^{2+} release has been reported to cause cell death [21,22]. Dong and colleagues demonstrated that bisacodyl/DDPM is a promising novel chemotherapeutic drug with legitimate properties that address the key causes of GBM therapy resistance and recurrence [23]. Littlepage et al. [24] demonstrated the therapeutic use of bisacodyl in patients with breast cancer. According to the United States patent application publication, bisacodyl and its analogues can be used as drugs to treat cancer [25].

Bisacodyl (CAS number: 603-50-9) was obtained for cells treated at pH 6.2. These results indicate that bisacodyl/DDPM-induced cell death occurs through a mechanism different from that of apoptosis. Researchers have observed reduced tumor growth and increased survival in a GLSC orthotopic xenograft mouse model treated with bisacodyl. Dong and colleagues demonstrated the cytotoxic activity of bisacodyl on glioblastoma cells [23].

Oncological treatments are expected to utilize customized techniques for each patient, integrating functional drug screenings using patient-derived models with molecular data obtained through new technologies (such as NGS and GWAS) to identify the most effective therapy for every case.

Tyrosine kinase inhibitors are the only targeted treatments for sarcomas; they are only valuable for gastrointestinal stromal tumors with PDGFRA or KIT mutations. Cytotoxic drugs (e.g., doxorubicin, ifosfamide, cisplatin) and/or radiation therapy continue to be the mainstay of systemic treatment for sarcomas [26].

Chondrosarcomas are a kind of sarcoma that are known to have fewer treatment choices. Treatments for metastatic or incurable tumors are currently non-existent, and these forms of cartilage-forming bone sarcomas are intrinsically resistant to traditional chemotherapy and radiation [27,28].

The first preclinical data supporting the use of enasidenib in the treatment of mIDH2 chondrosarcomas was presented by Rey et al. [29,30].

According to our in-silico study, CHEK2 (CDS1) may be useful as an adjunct diagnostic tool, and bisacodyl may be a therapeutic target in CS. The protein interactions shown in Figure 1 highlight the interactions of this gene with transporters and enzymes. Yellow color indicates genes that interact with transporters, and green color in-

icates enzyme interactions. The potential effects of these interactions on the biology of chondrosarcoma cells may play an important role in determining therapeutic targets. The effect of bisacodyl on CHEK2 supports its potential role in the functional pathways of this gene and increases the usability of this gene as a therapeutic target. Specifically, understanding the role of these protein interactions in the functional pathways of the CHEK2 gene may contribute to the discovery of new treatment agents. Here, we performed in silico screening using Gene2drug; however, its size limitation restricted our study. Our results hold promise for the CHEK2 gene and CHEK2-targeted bisacodyl treatment in chondrosarcoma.

Furthermore, these data support avatar models that can predict (pre)clinical responses in personalized medicine initiatives using lines obtained from chondrosarcoma patients. However, the limitations of this study are that it relies exclusively on in silico data and the limited number of cell lines analyzed. Additionally, analyses might not entirely reflect the intricate biological context, which includes variations specific to tissues, effects of the micro-environment, and interactions with other signaling pathways. Confirming the in-silico predictions may involve animal models, cell culture studies, and, if applicable, patient-derived samples. Combining these in-silico insights with current biological knowledge and performing further analyses that consider tissue-specific variables through collaboration with experimental biologists can yield a more thorough understanding. Bisacodyl could be further tested in future studies, such as employing preclinical models, exploring combination therapies, or utilizing in vitro testing and CRISPR-based approaches for target validation.

CONCLUSION

Finally, a better understanding of the molecular interplay in chondrosarcoma suggests that targeted therapies may play a role in the future. Chondrosarcoma's intrinsic and micro-environmental heterogeneity suggests the potential for developing resistance to any rationally planned, molecularly targeted therapy. Moreover, medications have been shown to induce genetic modifications in various malignancies. Our study suggests that bisacodyl is a potential CHEK2-targeted therapeutic agent for chondrosarcoma. Additionally, individual patient characteristics and the specific genetic makeup of the tumor can influence the choice of targeted therapy. As research progresses, new therapeutic targets may emerge, and personalised medical approaches may become more common in chondrosarcoma treatment.

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