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Pharmacological Profiling of Suppressive Drugs to Musculoskeletal Tumors

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Abstract

Treatment of aggressive musculoskeletal tumors remains clinically a conundrum, and requires the identification of novel drug that can be therapeutically exploited to improve patient outcome. The emerging genomic study suggested that there may be a core molecular determinant of progression of sarcoma, whereby we hypothesized that a commonly shared effective drugs by targeting the core of musculoskeletal tumors. Here, pharmacological profiling of suppressive drugs was shown using 29 types of patient-derived musculoskeletal tumor cell lines and 164 FDA-approved drugs. The cell line panel was composed of osteosarcoma, Ewing's sarcoma, undifferentiated pleomorphic sarcoma, chondrosarcoma, extraskeletal chondrosarcoma, synovial sarcoma, rhabdomyosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, clear cell sarcoma, and dermatofibrosarcoma protuberans. Based on cell viability, suppression ratio was determined on each tumor and anti-cancer agent. Then, clustering analysis was performed, and heatmap image were depicted. As a result, a nine drugs were found to be more suppressive drugs than doxorubicin, a standard drug to sarcomas, specifically homoharringtonine, mitoxantrone, and ponatinib potently suppressed the cell viability in a variety of sarcomas regardless of histological subtypes. In conclusion, the new outlook suggests delivering a commonly shared therapeutic umbrella rather than histotype-tailored regimen in musculoskeletal tumors.

Keywords: Sarcoma; Musculoskeletal tumor; Malignant

Introduction

Malignant musculoskeletal tumor originates in bone or soft tissues such as muscle, cartilage, connective tissues and metastatic foci from primary lesion to the skeleton(s) [1,2]. In some cases, they shows wider invasion to the surrounding soft tissues, or metastatic spreading to other parts of the body. To combat the aggressive behavior of malignant musculoskeletal tumors, effective chemotherapeutic drugs associated with tumor shrinkage and longer survival time have been identified, and currently standardized guidelines are available [3-6].

In patients with osteosarcoma, a combination of methotrexate, doxorubicin, cisplatin, and ifosfamide was utilized in clinical settings. As for Ewing's sarcoma, vincristine, doxorubicin, cyclophosphamide, ifosfamide, etoposide, and actinomycin-D were currently standard chemotherapeutic drugs. With regard to aggressive soft tissue sarcoma, doxorubicin and ifosfamide were known as key drugs. Recently, pazopanib, trabectedin, and eribulin has been shown the efficacy in clinical trials. In case of rhabdomyosarcoma, the applicable drugs were vincristine, actinomycin-D, cyclophosphamide, ifosfamide, etoposide, irinotecan, topotecan, and doxorubicin. Thus, although a variety of therapeutic agents are now offered, there is unmet medical needs to identify novel drugs to treat chemotherapy-resistant refractory sarcomas, or to target wider variety of histological subtypes in malignant musculoskeletal tumors. Indeed, a randomized phase III clinical trial did not show any superiority of histotype-tailored regimen to standard chemotherapy [7]. Thus, an essential chemotherapy to sarcoma remain a conundrum. The emerging genomic study suggested that there may be a core molecular determinant of progression of sarcoma [8], whereby we hypothesized that a commonly shared effective drugs by targeting the core of musculoskeletal tumors.

In this study, pharmacological profiling of suppressive drugs was shown using 29 types of patient-derived musculoskeletal tumor cell lines, and commonly effective drugs were identified.

Material and Method

Tissue culture

This study was approved by the ethics committee of National Cancer Center. A variety of musculoskeletal tumor tissue obtained at the time of surgical resection was subjected to the cell culture. The excised tumor tissue was minced with scissors, and seeded in a culture dish in a condition of 5% CO2 at 37^0 C . The cells were maintained in DMEM or RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 mg/ml).

Authentication of cell line

Genomic DNA was extracted from the original tissue and cultured cell using DNeasy blood and tissue kits (Qiagen). Then, we examined the analysis of Short Tandem Repeats (STRs) using STR multiplex assays Gene Print 10 (Promega, Madison, WI). The STR analysis included amplification and detection of 10 loci. The amplified products were tested by ABI 3500xL Genetic Analyzer and GeneMapper 5.0 and Peak scanner (Applied Biosystems, Waltham, MA).

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Drug screening

Bravo Automated Liquid Handling Platform was employed for high-throughput screening. The drug panel was composed of 166 FDA-approved anti-cancer agents. The compounds were dissolved in Dimethyl Sulfoxide (DMSO) and adjusted to a final drug concentration of 10 μM in culture medium. 1250 cells/well were seeded into 384-well culture plates. On the following day, each anticancer compounds or vehicle control were added. After 72 hours of incubation, cell viability was measured by using a Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc, Japan.), and calculated the suppression rates. Experiments were repeated at least twice.

Bioinformatics analysis

Heat map analysis was performed by R 3.5.3 software (The R Project for Statistical Computing) and its package software, Complex Heatmap version 1.20.0 [9].

Results

Identification of commonly shared suppressive drugs to sarcoma

In order to find commonly suppressive drug to musculoskeletal tumors, we employed 29 patient-derived sarcoma cell lines, including two cell lines of osteosarcoma, one cell line of Ewing's sarcoma, two cell lines of CIC-DUX4 sarcoma, six cell lines of undifferentiated pleomorphic sarcoma, one cell line of chondrosarcoma, two cell lines of extraskeletal chondrosarcoma, two cell lines of synovial sarcoma, two cell lines of rhabdomyosarcoma, two cell lines of leiomyosarcoma, five cell lines of malignant peripheral nerve sheath tumor, three cell lines of clear cell sarcoma, and one cell line of dermatofibrosarcoma protuberans. Based on cell viability, suppression ratio was determined on each tumor and anti-cancer agent. Then, the clustering analysis was performed, and heatmap image were depicted.

In the left side, 164 FDA-approved drugs were listed based on the bioinformatics clustering. The blue color indicates the suppressive effect on tumor proliferation, whereas the red color indicates no efficacy. The top group colored in blue was identified as commonly shared umbrella for inhibitory influences on sarcomas. Since doxorubicin is known as a standard key drug which often utilized in a variety of sarcomas, it was defined as reference line of inhibitory effect. As a result, top cluster of the heatmap included more suppressive drugs than doxorubicin. Specifically, the cluster was composed of homoharringtonine, mitoxantrone, ponatinib, romidepsin, belinostat, vorinostat, mitomycin C, mithramycin A and epirubicin. Among 29 types of sarcoma cells, one cell line of osteosarcoma (OS-#154) and both two cell lines of extraskeletal chondrosarcoma (CS-ExS-#66 and CS-ExS-#132) were shown to be chemotherapy-resistant phenotype. Nevertheless, homoharringtonine, mitoxantrone and ponatinib were shown to be commonly shared efficacy. These three agents were identified as potent suppressive drugs despite histological difference of sarcomas.

Discussion

This study presented the pharmacological profiling of sarcomas, which leads to be found three commonly shared suppressive drugs to musculoskeletal tumors.

First, homoharringtonine is also known as omacetaxine, a protein translation inhibitor derived from natural plant alkaloid, and have used for treatment of chronic myeloid leukemia. Homoharringtonine inhibits protein translation by preventing the initial elongation step of protein synthesis. In detail, homoharringtonine interacts with the ribosomes and prevents the correct positioning of amino acids of transported tRNAs [10]. Although clinical response has not been observed in solid tumors including malignant gliomas, melanoma, head and neck cancer, breast cancer, and colorectal cancer [11], the data depicted here implied an efficacy to sarcomas.

Secondly, mitoxantrone is one of the type II topoisomerase inhibitors, which intercalated with DNA and inhibition of protein synthesis, leading to inhibit the generation of topoisomerase II, which relaxes DNA coil at the time of transcription [12]. The mechanism of action is generally same as doxorubicin, which may be one of the reason mitoxantrone was selected as a considerable candidate in this study. Currently, based on a randomized clinical trial, mitoxantrone was used for relapsed acute lymphobastic leukaemia [13]. With regard to solid tumors, several clinical studies reported in patients with sarcoma. In metastatic or recurrent leiomyosarcoma, no complete or partial responses have been observed [14]. Similarly, mitoxantrone have shown a negative efficacy to advanced sarcomas in phase II clinical trial [15]. In case of recurrent abdominal sarcomas, surgical excision of all gross disease and postoperative intraperitoneal chemotherapy with mitoxantrone presented a feasible treatment outcome with minimal toxicity, which may provide a survival benefit for patients [16]. Although there are some discrepancy between previous clinical results and the data presented in this study, mitoxantrone may show a satisfactory result in a considered chemotherapeutic regimen.

Third, ponatinib is known as one of multi-tyrosine kinase inhibitor, targeting at KIT, PDGFRα, VGFER1, VGFER2, BCR-ABL, RET, FLT3, SRC, FGFR1, FGFR2, FGFR3, and FGFR4 [17]. The result is supported by pazopanib, another multi-tyrosine kinase inhibitor, which currently offered to soft tissue sarcoma, similarly targeting at KIT, PDGFRα, PDGFRβ, VEGFR1, VEGFR2, VEGFR3 [18]. In comparison between these two drugs, KIT, PDGFRα, VEGFR1, and VGEFR2 were common targets. In addition, ponatinib may have a superiority to pazopanib because of wider range spectrum of targets such as FGFR1, FGFR2, FGFR3, FGFR4, RET, SRC, and FLT3. Based on these additional targets necessary to proliferation, ponatinib showed a comprehensive inhibitory effect in the data of pharmacological profile regardless of a variety of histological types in musculoskeletal tumors. Indeed, ponatinib has been used for refractory chronic myeloid/acute lymphoid leukemia. The drug now repositioned to therapeutic drug for sarcoma.

In conclusion, the new outlook suggests delivering a commonly shared therapeutic umbrella rather than histotype-tailored regimen in musculoskeletal tumors.

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