



FINAL REPORT

Project Title: Targeting kinases to reverse multidrug resistance in human sarcoma

Project Number: SFA09-02

1. Date project was initiated: _____5/1/2008_____

2. Period covered by this report: From ___6/1/2009_____ To ___5/31/2010_____

3. Publications, Abstracts, and Presentations:

- a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry must include the author(s), article title, journal [book, editors(s), publisher, volume number, page number(s), and date.]

(1) Lay Press:

(2) Peer-Reviewed Scientific Journals:

1. Oleanane triterpenoid CDDO-Me induces apoptosis in multidrug resistant osteosarcoma cells through inhibition of Stat3 pathway.
Ryu K, Susa M, Choy E, Yang C, Hornicek FJ, Mankin HJ, Duan Z.
BMC Cancer. 2010 May 10;10:187.
2. Lentiviral shRNA screen of human kinases identifies PLK1 as a potential therapeutic target for osteosarcoma.
Duan Z, Ji D, Weinstein EJ, Liu X, Susa M, Choy E, Yang C, Mankin H, Hornicek FJ.
Cancer Lett. 2010 Jul 28;293(2):220-9. Epub 2010 Feb 9.
3. Activation of signal transducer and activator of transcription 3 (Stat3) pathway in osteosarcoma cells and overexpression of phosphorylated-Stat3 correlates with poor prognosis.
Ryu K, Choy E, Yang C, Susa M, Hornicek FJ, Mankin H, Duan Z.
J Orthop Res. 2010 Jul;28(7):971-8.
4. The kinase Mirk is a potential therapeutic target in osteosarcoma.
Yang C, Ji D, Weinstein EJ, Choy E, Hornicek FJ, Wood KB, Liu X, Mankin H, Duan Z.
Carcinogenesis. 2010 Apr;31(4):552-8. Epub 2009 Dec 30.
5. Inhibition of ABCB1 (MDR1) expression by an siRNA nanoparticulate delivery system to overcome drug resistance in osteosarcoma.
Susa M, Iyer AK, Ryu K, Choy E, Hornicek FJ, Mankin H, Milane L, Amiji MM, Duan Z.
PLoS One. 2010 May 24;5(5):e10764.

(3) Invited Articles:

1. Nanoparticles: A Promising Modality in the Treatment of Sarcomas.
Susa M, Milane L, Amiji MM, Hornicek FJ, Duan Z.
Pharm Res. 2010 May 27. [Epub ahead of print]
2. Signal Transducer and Activator of Transcription 3 Signaling Pathway: A Potential Target in Sarcoma Treatment.
Michiro Susa, Francis J. Hornicek, Xianzhe Liu and Zhenfeng Duan
Current Enzyme Inhibition, 2010, 6

(4) Abstracts:

1. Duan Z, et al. Identification of DYRK1B as a potential therapeutic target in osteosarcoma. 14th Annual Connective Tissue Oncology Society Meeting. 2009
2. Yang C and Duan Z, et al. A novel target for treatment of chordoma: Stat3. 14th Annual Connective Tissue Oncology Society Meeting. 2009
3. Duan Z et al: Comparison of miRNA expression between human osteoblasts and osteosarcoma cells. Annual Meeting of American Association for Cancer Research. 2010
4. Susa M and Duan Z. et al. Inhibition of ABCB1 (MDR1) Expression by an siRNA Nanoparticulate Delivery System to Overcome Drug Resistance in Osteosarcoma. Annual Meeting of American Association for Cancer Research. 2010

b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (*) if presentation produced a manuscript.

1. RNAi and shRNA workshop, St.Louis, April 27
2. Connective Tissue Oncology Society 15th meeting, Miami, Nov 9-12*
3. 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, Mar 6-9*

4. Provide a brief list of keywords: (limit to 20 words)
osteosarcoma, kinase, multidrug resistance, MDR1, Stat3, Mirk, PLK1, nanoparticle

5. Summarize the progress during the period of this report and its impact on your plans for the remainder of the project. Include a summary of the progress toward the achievement of the originally stated aims and list the significant results:

We have made significantly progress on the aims stated in this project. Aim 1 in this project will look the functional role of kinases in supporting the drug resistant phenotype in sarcoma cell lines. Using lentiviral short hairpin RNA (shRNA) collections targeting different kinase genes 96-well plates, individual knockdown experiments were performed. Specific genes whose knockdown was found to be associated with decreased cellular proliferation and induced apoptosis included PLK1 Mirk, and ROCK1. Western blot analysis confirmed that these genes include PLK1 is highly expressed and activated in several osteosarcoma cell lines as well as in resected tumor samples. Immunohistochemistry analysis showed that patients with high PLK1 tumor expression levels correlated with significantly shorter survival than patients with lower levels of tumor PLK1 expression. These results demonstrate the capability and feasibility of a high-throughput screen with a large collection of lentiviral kinases and its effectiveness in identifying potential drug targets. Currently, we are validating the potential function roles of these kinase genes in support drug resistance in osteosarcoma cell lines. We also made progress on the Aim 2 of this project: To determine the effect of inhibiting kinases (identified in specific Aim 1) with respect to reversing

drug resistance in a xenograft mouse model of sarcoma. The protocol (A pre-clinical model to validate PLK1 as a potential therapeutic target in osteosarcoma treatment) for animal use in this project has been approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (SRAC) under the protocol number 2009N000229.

6. In layperson's terms, summarize the progress during the period of this report.

Explain any medical significance or implications of your results to date:

The human genome contains at least 600-protein kinases that play critical roles in human diseases. A functional understanding of the role of the kinases in osteosarcoma is not well understood, and a study of these proteins and their functions will contribute to the discovery and development of new therapeutics. Kinases play an important role in cancer cell growth and survival; however, the role of kinases in osteosarcoma pathogenesis and drug resistance is largely uncharacterized. We found osteosarcoma cells display high levels of several kinases (such as PLK1, Mirk) expression. PLK1 expression knocked down inhibit cell growth and induce apoptosis in osteosarcoma cells. Immunohistochemistry analysis showed that osteosarcoma patients with high PLK1 tumor expression levels were associated with significantly shorter survival than osteosarcoma patients with low level of tumor PLK1 expression. We believe that this study provides a new insight in the osteosarcoma pathogenesis and potential drug resistance, which suggests that PLK1 and other kinases could be novel candidates for targeted therapy in the treatment of osteosarcoma.

Zhenfeng Du

Principal Investigator (signature)

7/14/10

Date

X
Harry E. Rubens

Department Chair (signature)

7/14/10

Date