We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 10

OMICS Approach for Identifying Predictive Biomarkers in Osteosarcoma

Tadashi Kondo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67449

Abstract

Osteosarcoma has a complex genetic background, and the response to treatments varies among patients. Induction chemotherapy has substantially improved the clinical outcome of osteosarcoma. Currently, there is no practical predictive modality in clinical settings, and therefore, uniform chemotherapy is applied for all patients. However, since the response to induction chemotherapy considerably influences the prognosis, the therapeutic strategy should be optimized for each patient before initiating treatments. Therefore, identification and establishment of predictive biomarkers for induction chemotherapy have been a long-standing goal in osteosarcoma research. Because of the complex genetic traits associated with osteosarcoma, adoption of an omics approach for global gene expression is attractive in the search for predictive biomarkers, and omics technologies have recently been applied to the development of predictive biomarkers in malignancies, including osteosarcoma. Global studies have been performed at the genome, transcriptome, and proteome levels in osteosarcoma, and various candidate biomarkers have been reported using clinical specimens. Further investigation of the clinical utilities of these identified predictive biomarkers will be merited through validation and verification studies.

Keywords: osteosarcoma, biomarker, chemotherapy, omics, genome, transcriptome, proteome

1. Introduction

Osteosarcoma is a rare mesenchymal malignancy, accounting for less than 1% of all adult cancers [1]. In contrast, osteosarcoma represents the most frequent type of malignant tumor in children and adolescents, and its incidence has increased over time in younger cases [1, 2]. Mesenchymal malignancies are classified according to the unique molecular background:

INTECH
open science | open minds

© 2017 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

those characterized by unique transfusion genes and simple karyotypes, and those without unique genetic characters but complex genetic backgrounds [3]. Osteosarcoma is a typical example of the latter case and is associated with highly complex karyotypes and frequent chromosomal copy number changes [4–11]. The advent of chemotherapy has improved the clinical outcome of patients with nonmetastatic osteosarcoma [12–16]. However, despite the considerable progress in cancer research, no novel therapeutic strategies for osteosarcomas have been established since the 1980s, and the cure rate of osteosarcoma patients has thus reached a plateau [15]. Effective molecular targeting drugs that may inhibit specific molecular aberrations common in certain cancer types are not currently available for osteosarcoma, which is likely attributed to the complex molecular backgrounds. Consequently, uniform induction chemotherapy is performed for all patients with osteosarcoma.

The identification of predictive biomarkers has been a long-standing goal in osteosarcoma research. The response to induction chemotherapy is evaluated by histopathological examination of tumor necrosis. When a tumor responds to the induction chemotherapy, a better prognosis can be expected [17–19]. Studies of the molecular events contributing to the different responses to induction chemotherapy have been undertaken using clinical samples. As osteosarcoma is not associated with typical genetic alterations, a global approach to investigating the molecular backgrounds may be the most promising strategy. With the advent of modern technologies, omics approaches have become increasingly popular in translational research and are starting to be applied in the studies of predictive biomarkers in various types of cancers, including osteosarcoma.

In this article, we review the researches on potential biomarkers for osteosarcoma with the intention to establish predictive biomarkers for induction chemotherapy. Providing an overview of the current status of knowledge on these predictive biomarkers will offer a perspective for further development of osteosarcoma treatments as well as new ideas of what is needed to achieve a better clinical outcome for osteosarcoma patients.

2. Omics studies for predictive biomarkers in osteosarcoma

2.1. Genomic studies

Predictive biomarkers based on genomic knowledge have proven to be clinically beneficial for various types of malignancies. Schwaederle et al. reported the results of a meta-analysis of 570 Phase II studies, including 32,000 patients with various types of malignancies [20]. They reported that the use of predictive genomic biomarkers resulted in a higher treatment response rate and prolonged progression-free survival as well as overall survival. These results should motivate and facilitate the development and use of predictive genomic biomarkers in osteosarcoma.

Smida et al. reported the DNA copy number alterations and allelic imbalances associated with the response to induction chemotherapy using single nucleotide polymorphism (SNP) arrays [21]. They investigated the biopsy samples from 44 patients with osteosarcoma who

have a record of the subsequent response to chemotherapy. They reported that patients with a significantly higher frequency of loss of heterozygosity (LOH) often had a poor response to chemotherapy compared to patients with a lower LOH frequency. They also showed that specific chromosomal regions in chromosomes 10 and 11 were associated with the poor response to chemotherapy. Further investigations of these molecules will likely lead to the establishment of novel predictive modalities in osteosarcomas.

Hagleitner et al. investigated 384 SNPs among 54 selected genes in 177 osteosarcoma patients, with the aim of identifying genetic variants associated with survival. The 54 genes included representative candidate genes involved in the cisplatin and doxorubicin pathway, according to the literature and Pharmacogenomics Knowledge Base (https://www.pharmgkb.org/). In addition to SNPs associated with progression-free survival, they found 14 SNPs that were significantly associated with the poor response to chemotherapy [22]. The clinical utility of these 14 SNPs has not yet been evaluated, and thus, further investigation is needed for validation.

2.2. Transcriptomic studies

Technologies for comprehensive analyses of mRNA and microRNA expression became available more than a decade ago and have since been widely used for biomarker development in various malignancies [23–28].

Ochi et al. examined the mRNA expression profiles of biopsied tumor tissues from osteosarcoma patients using a cDNA microarray consisting of 23,040 genes [29]. They compared six responders and seven nonresponders to induction chemotherapy and identified 60 genes whose expression patterns were associated with favorable or poor response to neoadjuvant chemotherapy. Man et al. identified a novel molecular signature of chemoresistance by comparing the profiles of 7 responders and 13 nonresponders using surgically resected tissues after chemotherapy [30]. They hypothesized that the surgically resected tumor tissues of nonresponders were enriched for resistant cells. The identified tissues consisted of 45 unique genes, and their predictive performance was confirmed in 14 biopsied tumor tissues obtained before chemotherapy. Moreover, the expression levels measured by cDNA microarray were confirmed for seven genes using quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Although these three studies had the common goal of identifying predictive biomarkers and used biopsy tumor tissues of osteosarcoma patients, the genes involved in the chemoresistance signatures were quite different. This difference may be attributed to the different age distributions, small sample sizes, different chemotherapy regimens, and different methods used for expression analysis among the studies. Therefore, to establish the clinical utilities of the candidate predictive biomarkers, extensive validation studies as well as functional studies of the identified genes will be required.

Kubota et al. examined the microRNA expression profiles of open-biopsied tumor tissues from osteosarcoma patients using a microarray [31]. They compared four responders and four nonresponders to induction chemotherapy and identified six microRNAs whose expression patterns were associated with favorable or poor response to neoadjuvant chemotherapy. They confirmed the significant association of miR-125b and miR-100 with poor response to

chemotherapy by RT-PCR. The association between poor prognosis and the abundance of miR-125b and miR-100 was confirmed in 20 additional osteosarcoma patients. Overexpression of these microRNAs in three osteosarcoma cell lines resulted in the enhanced cell proliferation, invasiveness, and resistance to chemotherapeutic drugs. As the area under the receiver operating curve for these microRNAs were approximately 0.9 ($p < 0.01$), their clinical application is worth challenging. Kubota et al. reported mRNAs whose expression was commonly affected by the transfection of miR-125b and miR-100 in osteosarcomas [31]. Those included sirtuin (silent mating type information regulation 2 homolog 5, SIRT5), which was previously associated with the resistance against therapeutic reagents in nonsmall cell lung cancer [32]. Thus, the expression profiles of miRNAs may be linked to those of mRNAs and provide a clue to understand the multilayer omics data in osteosarcomas.

2.3. Proteomics studies

Proteomics is another promising approach to biomarker discovery because the proteome is the functional translation of the genome, directly regulating the phenotypes of tumors. The modalities of proteomics have been considerably developed and applied to several cancer biomarker studies. Proteomics provides unique data that cannot be obtained with other technologies. This level of analysis is important, given the frequent reports of the discordance between protein and mRNA expression in global expression studies. In particular, proteomics is the only omics modality that can identify the protein status and characteristics such as post-translational modifications, intra- and extracellular localization, complex formation, activity, and degradation. Therefore, adoption of a proteomics approach shows good promise for biomarker development in osteosarcoma.

Arai et al. reported the proteins corresponding to the response to chemotherapy reagents in 11 osteosarcoma cell lines using two-dimensional differential gel electrophoresis (2D-DIGE) [33]. They found a differential response to the drugs between monolayer and spheroid cultured cells. Among the 4762 protein species observed, they reported the upregulation of cathepsin D in spheroid cells that showed resistance to a chemotherapy reagent. Cathepsin D has been implicated in chemoresistance, and its clinical utilities were suggested in various malignancies [33]. Saini et al. also compared monolayer and spheroid culture cells using proteomic, transcriptomic, and immunophenotyping approaches and identified CBX3 and ABCA5 as possible biomarkers for tumor stem cells that showed heterogeneous response to anti-cancer drugs [34]. Moreover, they reported that spheres and monolayers showed different responses to the approved cancer drugs. The applications of spheroidal cells may offer a great opportunity to evaluate the drug effects in preclinical studies, and adoption of an omics approach will be a powerful tool to further develop the biomarkers to predict the response to treatments.

Li et al. reported plasma proteins that may have good predictive performance for osteosarcoma patients [35]. They investigated the proteome of plasma collected from 54 osteosarcoma patients comparing before ($n = 27$) and after ($n = 27$) induction chemotherapy. They developed two classifiers for responsiveness and revealed that both showed 85% accuracy for prediction of response. They also examined the biological backgrounds of serum amyloid protein A and transthyretin, which were included in the classifiers. An extensive list of plasma proteins in

osteosarcoma patients was established using a proteomics approach [36], which should serve as a useful dataset for the plasma proteomics of osteosarcoma.

To characterize the proteome backgrounds associated with resistance to induction chemotherapy, Kikuta et al. examined the differential protein expression between patients who showed a favorable response to induction chemotherapy and those who did not [37]. Among the several thousand protein species observed by 2D-DIGE, they focused on peroxiredoxin 2 (PRDX2), an enzyme that catalyzes the free radicals produced by catalase and protects cells from oxidative stress. The patients with primary tumor tissues showing high PRDX2 expression ultimately developed resistance to induction chemotherapy, and vice versa. The results were validated in additional cases of osteosarcoma using the specific antibody for PRDX2. Kikuta et al. examined osteosarcoma patients who received combination therapy with isofomide, cisplatin, and doxorubicin, and in a subsequent study, Kubota et al. used 2D-DIGE to examine the protein expression in the osteosarcoma patients who received different versions of induction chemotherapy: methotorexate, cisplatin, or doxorubicin [38]. Both studies concordantly identified the high expression of PRDX2 in the nonresponders. The functional significance of the high expression of PRDX2 was also investigated in an in vitro experiment; the gene silencing of endogenous PRDX increased the sensitivity of cells to chemotherapy reagents. These results were reproduced using additional osteosarcoma cases receiving the same induction chemotherapy.

3. Future treatments for osteosarcoma patients with poor chemo-response potential

One of the most critical questions in the study of predictive biomarkers is what options to offer patients who are predicted to show an unfavorable response to induction chemotherapy. Due to the lack of predictive biomarkers for chemotherapy response in osteosarcoma, there has been no clinical trial conducted to identify patients with poor response. However, many lines of evidence suggest several possible therapeutic strategies for osteosarcoma patients. Meyers et al. reported that the addition of muramyl tripeptide to chemotherapy improved the overall survival of osteosarcoma patients [39]. Lewis et al. reported the possible application of interleukin-11 receptor alpha as a functional target in osteosarcoma [40]. In addition, there are also several possible immunotherapies for osteosarcoma. Cripe et al. reported the possible application of treatment with a vaccinia virus, pexastimogene devacirepvec, through viral lysis and induction of granulocyte macrophage colony-stimulating factor-driven tumor-specific immunity in osteosarcoma [41]. Tsukahara et al. demonstrated the possibility of peptide therapy in osteosarcoma [42]. Lussier et al. reported that combination immunotherapy with alpha-CTLA-4 and alpha-PD-L1 antibody might be effective for treating metastatic osteosarcoma [43]. Moreover, therapy using a genetically modified T cell line has been under evaluation for osteosarcoma patients [44]. Because these therapeutic approaches may have different modes of action compared with the conventional chemotherapeutic reagents, combinatory treatment of these novel drugs and conventional chemo-agents will promise new therapeutic strategy against osteosarcoma. Clinical trials of the novel treatment strategies are strongly desired to establish predictive biomarkers especially for the patients with potential of resistance to conventional chemotherapy.

4. Perspective of predictive biomarker identification with an omics approach

The advent of omics technology has made it feasible to observe tens of thousands of genes and proteins simultaneously with relative ease. Technologies for omics studies are continuously been developed, with expected improvements in terms of accuracy, comprehensiveness, and cost-effectiveness, which can be applied to osteosarcoma research. Next-generation sequencing (NGS) technologies have made it possible to produce even more detailed omics data. For example, NGS-based methods can be used to obtain the expression data of individual splice variants of mRNAs, which are not possible using conventional DNA microarray systems. Therefore, refined data of mRNA expression can be obtained by applying the novel technologies to previously examined sample sets. Moreover, proteomics was traditionally used to examine only protein expression levels, which do not necessary correlate with protein activity. The recent advent of high-throughput technology allows for the measurement of the actual activities of various specific kinases as well as nuclear receptors across hundreds of samples in only a few hours. All these omics technologies will be useful for advancing the study of predictive biomarkers in osteosarcoma.

One of the major current limitations of current studies for predictive biomarkers in osteosarcoma would be the small number and amount of clinical materials available. Considering the low prevalence of osteosarcoma, it is going to be very difficult to achieve the sufficient sample collection by single institutes and individual efforts alone. Therefore, nation-wide and/or international collaboration studies will be required.

The perspective provided herein on searching for predictive biomarkers in osteosarcoma, a rare malignancy with complex genetic traits, could be applicable to other major malignancies like lung cancers. Indeed, a recent NGS-based analysis revealed that the majority of lung cancers can be grouped into various minor subtypes according to the clinically important genetic aberrations. We believe that innovations developed in rare malignancies like osteosarcoma will not only benefit the patients with those diseases but also make a great impact on the researches for majority of malignancies.

Acknowledgements

This research was supported by the Practical Research for Innovative Cancer Control Program (15ck0106089h0002) from the Japan Agency for Medical Research and Development (AMED), and the National Cancer Center Research Core Facility and National Cancer Center Research and Development Fund (26-A-9).

Conflict of interest

The authors declare that they have no conflicts of interest.

Author details

Tadashi Kondo

Address all correspondence to: takondo@ncc.go.jp

Division of Rare Cancer Research, National Cancer Center Research Institute, Tokyo, Japan

References

[1] Mirabello, L., Troisi, R. J. & Savage, S. A. (2009) Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program, *Cancer.* **115**, 1531–43.

[2] Savage, S. A., Mirabello, L. Using epidemiology and genomics to understand osteosarcoma etiology. *Sarcoma.* 2011:**2011**:548151. PubMed PMID: 21437228; PubMed Central PMCID: PMC3061299. doi:10.1155/2011/548151

[3] Taylor, B. S., Barretina, J., Maki, R. G., Antonescu, C. R., Singer, S. & Ladanyi, M. (2011) Advances in sarcoma genomics and new therapeutic targets, *Nat Rev Cancer.* **11**, 541–57.

[4] Bridge, J. A., Nelson, M., McComb, E., McGuire, M. H., Rosenthal, H., Vergara, G., Maale, G. E., Spanier, S. & Neff, J. R. (1997) Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature, *Cancer Genet Cytogenet.* **95**, 74–87.

[5] Boehm, A. K., Neff, J. R., Squire, J. A., Bayani, J., Nelson, M., Bridge, J. A. Cytogenetic Findings in 36 Osteosarcoma Specimens and a Review of the Literature. Pediatric Pathology & Molecular Medicine. 2000 Issue 5;**19**, 359–376.

[6] Zielenska, M., Bayani, J., Pandita, A., Toledo, S., Marrano, P., Andrade, J., Petrilli, A., Thorner, P., Sorensen, P. & Squire, J. A. (2001) Comparative genomic hybridization analysis identifies gains of 1p35 approximately p36 and chromosome 19 in osteosarcoma, *Cancer Genet Cytogenet.* **130**, 14–21.

[7] Ozaki, T., Schaefer, K. L., Wai, D., Buerger, H., Flege, S., Lindner, N., Kevric, M., Diallo, R., Bankfalvi, A., Brinkschmidt, C., Juergens, H., Winkelmann, W., Dockhorn-Dworniczak, B., Bielack, S. S. & Poremba, C. (2002) Genetic imbalances revealed by comparative genomic hybridization in osteosarcomas, *Int J Cancer.* **102**, 355–65.

[8] Bayani, J., Zielenska, M., Pandita, A., Al-Romaih, K., Karaskova, J., Harrison, K., Bridge, J. A., Sorensen, P., Thorner, P. & Squire, J. A. (2003) Spectral karyotyping identifies recurrent complex rearrangements of chromosomes 8, 17, and 20 in osteosarcomas, *Genes Chromosomes Cancer.* **36**, 7–16.

[9] Al-Romaih, K., Bayani, J., Vorobyova, J., Karaskova, J., Park, P. C., Zielenska, M. & Squire, J. A. (2003) Chromosomal instability in osteosarcoma and its association with centrosome abnormalities, *Cancer Genet Cytogenet.* **144**, 91–9.

[10] Squire, J. A., Pei, J., Marrano, P., Beheshti, B., Bayani, J., Lim, G., Moldovan, L. & Zielenska, M. (2003) High-resolution mapping of amplifications and deletions in pediatric osteosarcoma by use of CGH analysis of cDNA microarrays, *Genes Chromosomes Cancer.* **38**, 215–25.

[11] Man, T. K., Lu, X. Y., Jaeweon, K., Perlaky, L., Harris, C. P., Shah, S., Ladanyi, M., Gorlick, R., Lau, C. C. & Rao, P. H. (2004) Genome-wide array comparative genomic hybridization analysis reveals distinct amplifications in osteosarcoma, *BMC Cancer.* **4**, 45.

[12] Carter, S. K. (1980) The dilemma of adjuvant chemotherapy for osteogenic sarcoma, *Cancer Clin Trials.* **3**, 29–36.

[13] Provisor, A. J., Ettinger, L. J., Nachman, J. B., Krailo, M. D., Makley, J. T., Yunis, E. J., Huvos, A. G., Betcher, D. L., Baum, E. S., Kisker, C. T. & Miser, J. S. (1997) Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group, *J Clin Oncol.* **15**, 76–84.

[14] Bielack, S. S., Kempf-Bielack, B., Delling, G., Exner, G. U., Flege, S., Helmke, K., Kotz, R., Salzer-Kuntschik, M., Werner, M., Winkelmann, W., Zoubek, A., Jurgens, H. & Winkler, K. (2002) Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols, *J Clin Oncol.* **20**, 776–90.

[15] Hagleitner, M. M., de Bont, E. S. & te Loo, D. M. (2012) Survival trends and long-term toxicity in pediatric patients with osteosarcoma, *Sarcoma.* **2012**, 636405.

[16] Whelan, J. S., Jinks, R. C., McTiernan, A., Sydes, M. R., Hook, J. M., Trani, L., Uscinska, B., Bramwell, V., Lewis, I. J., Nooij, M. A., van Glabbeke, M., Grimer, R. J., Hogendoorn, P. C., Taminiau, A. H. & Gelderblom, H. (2012) Survival from high-grade localised extremity osteosarcoma: combined results and prognostic factors from three European Osteosarcoma Intergroup randomised controlled trials, *Ann Oncol.* **23**, 1607–16.

[17] Rosen, G., Caparros, B., Groshen, S., Nirenberg, A., Cacavio, A., Marcove, R. C., Lane, J. M. & Huvos, A. G. (1984) Primary osteogenic sarcoma of the femur: a model for the use of preoperative chemotherapy in high risk malignant tumors, *Cancer Invest.* **2**, 181–92.

[18] Bacci, G., Avella, M., Brach Del Prevert, A., Capanna, R., Fiorentini, G., Malaguti, C., Picci, P., Rosito, P. & Campanacci, M. (1988) Neoadjuvant chemotherapy for osteosarcoma of the extremities. Good response of the primary tumor after preoperative chemotherapy with high-dose methotrexate followed by cisplatinum and adriamycin. Preliminary results, *Chemioterapia.* **7**, 138–42.

[19] Winkler, K., Beron, G., Delling, G., Heise, U., Kabisch, H., Purfürst, C., Berger, J., Ritter, J., Jurgens, H., Gerein, V. & et al. (1988) Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response, *J Clin Oncol.* **6**, 329–37.

[20] Schwaederle, M., Zhao, M., Lee, J. J., Eggermont, A. M., Schilsky, R. L., Mendelsohn, J., Lazar, V. & Kurzrock, R. (2015) Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials, *J Clin Oncol.* **33**, 3817–25.

[21] Smida, J., Baumhoer, D., Rosemann, M., Walch, A., Bielack, S., Poremba, C., Remberger, K., Korsching, E., Scheurlen, W., Dierkes, C., Burdach, S., Jundt, G., Atkinson, M. J. & Nathrath, M. (2010) Genomic alterations and allelic imbalances are strong prognostic predictors in osteosarcoma, *Clin Cancer Res.* **16**, 4256–67.

[22] Hagleitner, M. M., Coenen, M. J., Gelderblom, H., Makkinje, R. R., Vos, H. I., de Bont, E. S., van der Graaf, W. T., Schreuder, H. W., Flucke, U., van Leeuwen, F. N., Hoogerbrugge, P. M., Guchelaar, H. J. & te Loo, D. M. (2015) A first step toward personalized medicine in osteosarcoma: pharmacogenetics as predictive marker of outcome after chemotherapy-based treatment, *Clin Cancer Res.* **21**, 3436–41.

[23] Masica, D. L. & Karchin, R. (2013) Collections of simultaneously altered genes as biomarkers of cancer cell drug response, *Cancer Res.* **73**, 1699–708.

[24] Puyo, S., Houede, N., Kauffmann, A., Richaud, P., Robert, J. & Pourquier, P. (2012) Gene expression signature predicting high-grade prostate cancer responses to oxaliplatin, *Mol Pharmacol.* **82**, 1205–16.

[25] Kim, M. K., Osada, T., Barry, W. T., Yang, X. Y., Freedman, J. A., Tsamis, K. A., Datto, M., Clary, B. M., Clay, T., Morse, M. A., Febbo, P. G., Lyerly, H. K. & Hsu, D. S. (2012) Characterization of an oxaliplatin sensitivity predictor in a preclinical murine model of colorectal cancer, *Mol Cancer Ther.* **11**, 1500–9.

[26] Dao, P., Wang, K., Collins, C., Ester, M., Lapuk, A. & Sahinalp, S. C. (2011) Optimally discriminative subnetwork markers predict response to chemotherapy, *Bioinformatics.* **27**, i205–13.

[27] Nagji, A. S., Cho, S. H., Liu, Y., Lee, J. K. & Jones, D. R. (2010) Multigene expression-based predictors for sensitivity to Vorinostat and Velcade in non-small cell lung cancer, *Mol Cancer Ther.* **9**, 2834–43.

[28] Dry, J. R., Pavey, S., Pratilas, C. A., Harbron, C., Runswick, S., Hodgson, D., Chresta, C., McCormack, R., Byrne, N., Cockerill, M., Graham, A., Beran, G., Cassidy, A., Haggerty, C., Brown, H., Ellison, G., Dering, J., Taylor, B. S., Stark, M., Bonazzi, V., Ravishankar, S., Packer, L., Xing, F., Solit, D. B., Finn, R. S., Rosen, N., Hayward, N. K., French, T. & Smith, P. D. (2010) Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244), *Cancer Res.* **70**, 2264–73.

[29] Ochi, K., Daigo, Y., Katagiri, T., Nagayama, S., Tsunoda, T., Myoui, A., Naka, N., Araki, N., Kudawara, I., Ieguchi, M., Toyama, Y., Toguchida, J., Yoshikawa, H. & Nakamura, Y. (2004) Prediction of response to neoadjuvant chemotherapy for osteosarcoma by gene-expression profiles, *Int J Oncol.* **24**, 647–55.

[30] Man, T. K., Chintagumpala, M., Visvanathan, J., Shen, J., Perlaky, L., Hicks, J., Johnson, M., Davino, N., Murray, J., Helman,L., Meyer, W., Triche, T., Wong, K. K. & Lau, C. C. (2005) Expression profiles of osteosarcoma that can predict response to chemotherapy, *Cancer Res.* **65**, 8142–50.

[31] Kubota D, Kosaka N, Fujiwara T, Yoshida A, Arai Y, Qiao Z, Takeshita F, Ochiya T, Kawai A, Kondo T. miR-125b and miR-100 Are Predictive Biomarkers of Response to Induction Chemotherapy in Osteosarcoma. Sarcoma. 2016;2016:1390571. PubMed PMID: 27990096; PubMed Central PMCID: PMC5136640.

[32] Lu, W., Zuo, Y., Feng, Y. & Zhang, M. (2014) SIRT5 facilitates cancer cell growth and drug resistance in non-small cell lung cancer, *Tumour Biol.* **35**, 10699–705.

[33] Arai, K., Sakamoto, R., Kubota, D. & Kondo, T. (2013) Proteomic approach toward molecular backgrounds of drug resistance of osteosarcoma cells in spheroid culture system, *Proteomics.* **13**, 2351–60.

[34] Saini, V., Hose, C. D., Monks, A., Nagashima, K., Han, B., Newton, D. L., Millione, A., Shah, J., Hollingshead, M. G., Hite, K. M., Burkett, M. W., Delosh, R. M., Silvers, T. E., Scudiero, D. A. & Shoemaker, R. H. (2012) Identification of CBX3 and ABCA5 as putative biomarkers for tumor stem cells in osteosarcoma, *PLoS One.* **7**, e41401.

[35] Li, Y., Dang, T. A., Shen, J., Hicks, J., Chintagumpala, M., Lau, C. C. & Man, T. K. (2011) Plasma proteome predicts chemotherapy response in osteosarcoma patients, *Oncol Rep.* **25**, 303–14.

[36] Li, Y., Dang, T. A. & Man, T. K. (2012) Plasma proteomic profiling of pediatric osteosarcoma, *Methods Mol Biol.* **818**, 81–96.

[37] Kikuta, K., Tochigi, N., Saito, S., Shimoda, T., Morioka, H., Toyama, Y., Hosono, A., Suehara, Y., Beppu, Y., Kawai, A., Hirohashi, S. & Kondo, T. (2010) Peroxiredoxin 2 as a chemotherapy responsiveness biomarker candidate in osteosarcoma revealed by proteomics, *Proteomics Clin Appl.* **4**, 560–7.

[38] Kubota, D., Mukaihara, K., Yoshida, A., Tsuda, H., Kawai, A. & Kondo, T. (2013) Proteomics study of open biopsy samples identifies peroxiredoxin 2 as a predictive biomarker of response to induction chemotherapy in osteosarcoma, *J Proteomics.* **91**, 393–404.

[39] Meyers, P. A., Schwartz, C. L., Krailo, M. D., Healey, J. H., Bernstein, M. L., Betcher, D., Ferguson, W. S., Gebhardt, M. C., Goorin, A. M., Harris, M., Kleinerman, E., Link, M. P., Nadel, H., Nieder, M., Siegal, G. P., Weiner, M. A., Wells, R. J., Womer, R. B., Grier, H. E. & Children's Oncology, G. (2008) Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group, *J Clin Oncol.* **26**, 633–8.

[40] Lewis, V. O., Ozawa, M. G., Deavers, M. T., Wang, G., Shintani, T., Arap, W. & Pasqualini, R. (2009) The interleukin-11 receptor alpha as a candidate ligand-directed target in osteosarcoma: consistent data from cell lines, orthotopic models, and human tumor samples, *Cancer Res.* **69**, 1995–9.

[41] Cripe, T. P., Ngo, M. C., Geller, J. I., Louis, C. U., Currier, M. A., Racadio, J. M., Towbin, A. J., Rooney, C. M., Pelusio, A., Moon, A., Hwang, T. H., Burke, J. M., Bell, J. C., Kirn, D. H. & Breitbach, C. J. (2015) Phase 1 study of intratumoral Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus, in pediatric cancer patients, *Mol Ther.* **23**, 602–8.

[42] Tsukahara, T., Emori, M., Murata, K., Hirano, T., Muroi, N., Kyono, M., Toji, S., Watanabe, K., Torigoe, T., Kochin, V., Asanuma, H., Matsumiya, H., Yamashita, K., Himi, T., Ichimiya, S., Wada, T., Yamashita, T., Hasegawa, T. & Sato, N. (2014) Specific targeting of a naturally presented osteosarcoma antigen, papillomavirus binding factor peptide, using an artificial monoclonal antibody, *J Biol Chem.* **289**, 22035–47.

[43] Lussier, D. M., Johnson, J. L., Hingorani, P. & Blattman, J. N. (2015) Combination immunotherapy with alpha-CTLA-4 and alpha-PD-L1 antibody blockade prevents immune escape and leads to complete control of metastatic osteosarcoma, *J Immunother Cancer.* **3**, 21.

[44] DeRenzo, C. & Gottschalk, S. (2014) Genetically modified T-cell therapy for osteosarcoma, *Adv Exp Med Biol.* **804**, 323–40.

IntechOpen

IntechOpen

IntechOpen