MEDICAL SCIENCE MONITOR

MOLECULAR BIOLOGY

e-ISSN 1643-3750
© Med Sci Monit, 2017; 23: 2042-2048
DOI: 10.12659/MSM.901191

Received: 2016.08.23
Accepted: 2016.09.13
Published: 2017.04.28

Identification of Key Genes Affecting Results of Hyperthermia in Osteosarcoma Based on Integrative ChIP-Seq/TargetScan Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Yuxia Shi*
ABCDEFG 1 Fan Yang*
ADF 2 Shuqing Wei
BD 1 Gang Xu

1 Department of Bone and Soft-Tissue Tumor, Shanxi Tumor Hospital, Taiyuan, Shanxi, P.R. China
2 Department of Geriatric, Shanxi Tumor Hospital, Taiyuan, Shanxi, P.R. China

* Yuxia Shi and Fan Yang contributed to this work equally

Corresponding Author: Shuqing Wei, e-mail: weishuqing1972@163.com
Source of support: Departmental sources

Background: The purpose of this study was to research the effects of hyperthermia on osteosarcoma (OS) by integrating the Chromatin Immunoprecipitation with the generation sequencing (ChIP-Seq) and TargetScan analysis of heat shock transcription factor 1 (HSF1).

Material/Methods: The HSF1 ChIP-seq dataset of GSE60984 was downloaded from the Gene Expressed Omnibus (GEO) database. The HSF1-binding sites were screened by MACS2 in OS cells after 10 and 20 min of hyperthermia, and they were annotated using the ChIPseeker package. The overlapped genes were selected out when HSF1-binding sites were located in the promoter region. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the overlaps. The miRNA-gene pairs of the overlaps were screened out via TargetScan, and the miRNA-gene-regulated network was constructed by Cytoscape software.

Results: 1880 and 1283 genes of promoter regions were obtained in the osteosarcoma cells after 10 and 20 min of hyperthermia, respectively, and 889 of them were overlapped. The overlapped genes were enriched in 122 GO terms and 3 KEGG pathways. There were 13 657 pairs involved in the miRNA-gene regulated network of the overlaps.

Conclusions: Some biomarkers were identified for OS treated with hyperthermia. Moreover, some GO terms (regulation of programmed cell death and regulation of cell death) and pathways (p53 signaling pathway, methane metabolism, and viral myocarditis) might be involved.

MeSH Keywords: Gene Expression • Hyperthermia, Induced • Osteosarcoma

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/901191

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Osteosarcoma (OS) is a common histological form of bone cancer, which is most prevalent in children and adolescents [1,2]. It was reported that OS was the eighth most common childhood cancer, accounting for about 20% of all primary bone cancers and 2.4% of all malignancies in pediatric patients [1]. Symptoms include pain at night, overt localized swelling, and bones that fracture easily. The causes may lie in OS stem cells and radiotherapy [3,4]. Various treatments, such as wide-excision surgery of tumors, radiotherapy, chemotherapy, and neoadjuvant chemotherapy, are usually adopted in OS [1]. Although significant improvements in the long-term outcome of OS patients have been made, it is still not satisfactory [5,6]. The best reported 10-year survival rate was 92%, and the protocol was an aggressive intra-arterial regimen that individualizes therapy based on arteriographic response [7]. The 3-year survival rates ranged from 50–75%, and 5-year survival rate ranged from 60–85% [8,9]. In addition, 30–40% children died of it, and 25–50% patients subsequently developed metastatic disease, which was the major cause of death [10]. Recently, some biomarkers for OS were selected, such as *CD20*, *ECM*, *ITGA,* and *PMP22* [11,12].

Hyperthermia therapy is a treatment approach in which a particular area or the whole body is heated above normal temperatures to achieve therapeutic effects. Hyperthermia has been used in cancer treatment since 1989, and currently it is used either for ablation purposes as an alternative to surgery, or, less frequently, in combination with chemotherapy or radiation therapy [13]. It was reported that hyperthermia can activate various systemic anti-tumor immune responses, and leads to methamphetamine-induced toxicity [13,14]. The principle may be as follows: heat alters membrane characteristics, leading to modification in cell morphology, intracellular sodium and calcium levels, and membrane potential [15–17]. Some studies reported that hyperthermia can induce apoptosis and reduce migration of OS. In this study, we aimed to screen gene expression changes of OS induced by hyperthermia via integrating the Chromatin Immunoprecipitation with generation sequencing (ChIP-Seq) and TargetScan analysis, and further explored the related potential biomarkers and mechanism.

Material and Methods

The ChIP-seq dataset

The ChIP-seq dataset of GSE60984 [18] was downloaded from the Gene Expressed Omnibus (GEO) database (*http://www.ncbi.nlm.nih.gov/geo/*). In this dataset, there were 3 samples of OS cell: one was treated with both anti-HSF1 antibody and heat shocked for 10 min, another was treated with anti-HSF1 antibody and was heat shocked for 20 min, and the last was treated with neither antibody nor hyperthermia (the control sample). The sequencing platform was Illumina Genome Analyzer IIx (*Homo sapiens*).

Data pre-processing

Raw fastq read sequences were demultiplexed, and cutadapt was used to remove adapter sequences from DNA high-throughput sequencing data. Afterwards, the expressed genes were aligned to a reference genome using Bowtie [19], an ultrafast genome aligning tool that was used for the mapping of the raw reads to enesembl (*http://www.ensembl.org/index.html*) hg19 genome with maximum of 2 mismatches in every reads and those who had only 1 match were reserved.

Screening and annotation of HSF1-binding sites

Model-based analysis of ChIP-seq (MACS) was a computational algorithm that identified genome-wide locations of transcription/chromatin factor binding or histone modification from ChIP-seq data [20]. The HSF1-binding sites (also known as peaks) were respectively obtained via MACS2 in the OS cells heat shocked for 10 and 20 min compared with the control samples, and recorded as Peek-10 and Peek-20, respectively. The threshold was $P<10^{-5}$ and the default ultrasonic interrupt length was 300 bp. ChIPseeker was an *R* package for annotating ChIP-seq data analysis [21]. Here, based on the ChIPseeker package, their related genes, distance to the closest transcription start sites (TSS), and genome features were assigned to the peaks. Moreover, some genes of Peek-10 and Peek-20 were separately selected out when HSF1-binding sites were located in the promoter regions, and their overlaps were found.

Functional and pathway enrichment analysis of overlaps

The Database for Annotation, Visualization and Integrated Discovery (DAVID) [22] (*https://david.ncifcrf.gov/*) is a widely used web-based tool for functional and pathway enrichment analysis. In this study it was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the above overlaps. Moreover, the GO terms and KEGG pathways were selected out with the criteria of P value <0.05.

Construction of the miRNA-gene network

TargetScan is a widely used database that predicts biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA [23,24]. The miRNA, which might regulate the overlaps, as well as the miRNA-gene pairs, were screened out with TargetScan. Furthermore, the miRNA-gene-regulated

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

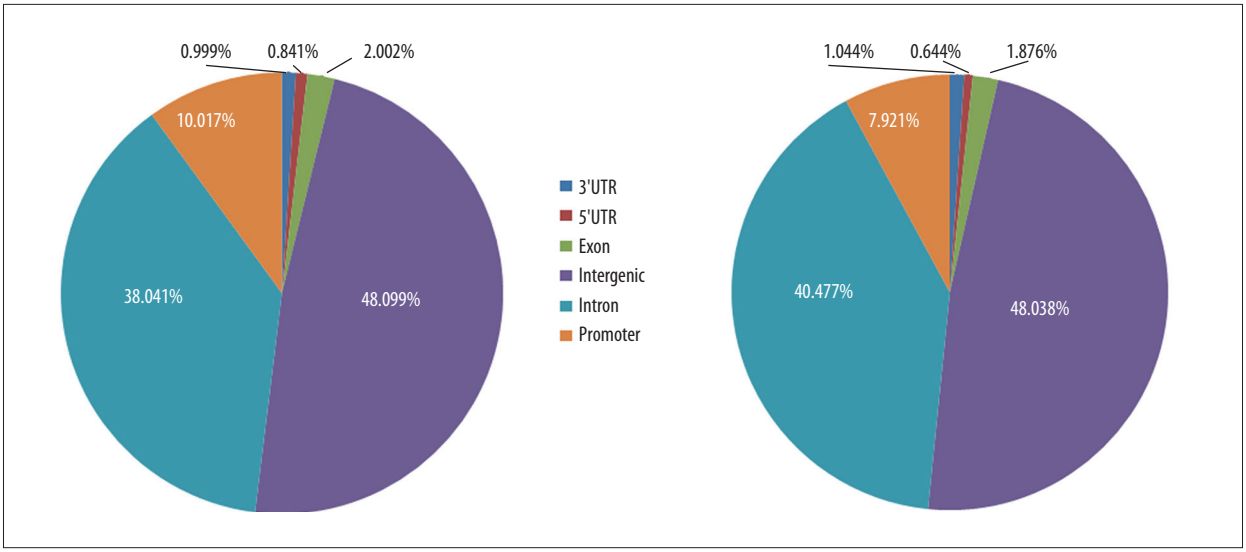

Figure 1. The genome-wide distributions of Peek-10 and Peek-20.

Table 1. 40 out of the overlapped genes in the promoter regions.

Gene			
SGMS2	SOD1	DNAJC7	SPIN3
CGGBP1	GNAS	SPOPL	ACER2
FTL	NCF4	FAM109B	C1GALT1C1
HNRNPH2	ACHE	SMAD1	IL32
JAK3	ADRA2C	OR13D1	KLK6
MEFV	ALDH3B1	GPX8	USP21
MLH1	OPRD1	ZNF761	FAM13A
PGK1	PPIC	TPST2	KAZN
PLOD1	PRIM2	CCDC147	NARG2
KRT5	RPL24	LPPR5	DMAP1

network was constructed based on the above miRNA-gene pairs. However, the selected miRNA-gene pairs might be overwhelming, and the miRNA-gene regulated network too complicated, so that it was not conducive to analyze and the accuracy was not high. Hence, module analysis of the network was conducted and the modules were visualized using Cytoscape software [25].

Results

HSF1-binding sites

A total of 19 626 and 18 015 HSF1-binding sites were obtained in the OS cells heat shocked for 10 and 20 min, respectively, compared with the control samples (recorded as Peek-10 and Peek-20). Their distributions in the genome-wide are shown in Figure 1, showing 10.017% Peek-10 and 7.921% Peek-20 located in the promoter regions. In other words, 1880 genes of Peek-10 and 1283 genes of Peek-20 were located in the promoter regions. Besides, 889 overlaps of them were found, and 40 of the overlaps are shown in Table 1.

Enriched GO terms and KEGG pathways of overlaps

A total of 122 GO terms were found to be enriched in the overlapped genes, and the top 10 most significant GO terms are exhibited in Figure 2. The overlaps were enriched in 3 KEGG pathways, namely p53 signaling pathway, methane metabolism and viral myocarditis.

The miRNA-gene network

The miRNA-gene regulated network of the overlaps was constructed, including 13 657 miRNA-gene-regulated pairs. The

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

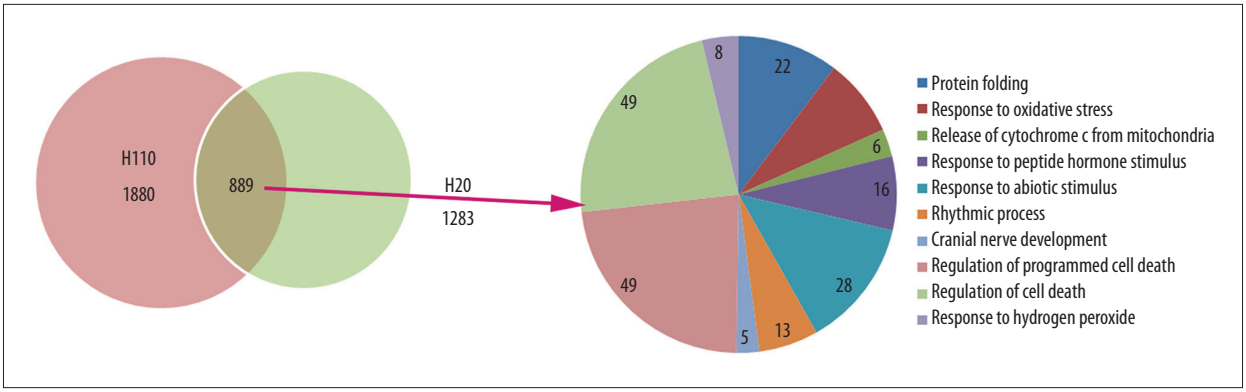

Figure 2. The top 10 most significant GO terms of the overlaps.

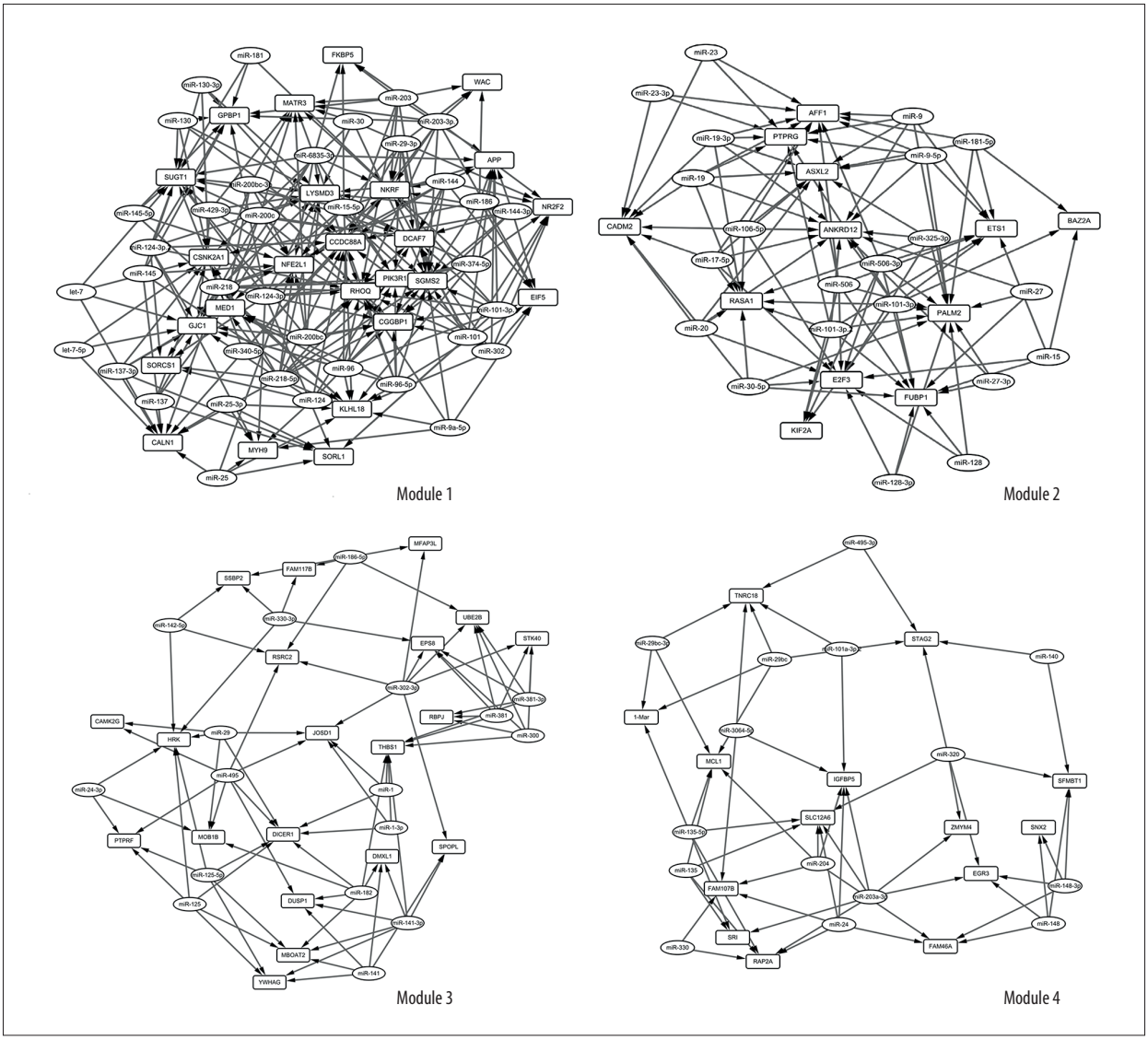

Figure 3. Modules of the miRNA-gene network.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 2. The 4 modules of the miRNA-gene network and some their attributes.

Cluster	Score	Nodes	Edges
Module 1	8.59	62	262
Module 2	6.625	33	106
Module 3	4.556	37	82
Module 4	4	29	56

network was divided into 4 modules, and they were recorded as Module 1, Module 2, Module 3, and Module 4. Modules and their attributes are shown in Figure 3 and Table 2. *SGMS2* and *CGGBP1* were included in Module 1 and were regulated by more miRNAs.

Discussion

Heat shock transcription factor 1 (HSF1) is localized to the cytoplasm as an inactive monomer, and heat shock can induce the activation of HSF1 [26]. The active HSF1 leads to synthesis and cytoprotection of heat shock proteins (HSPs) [27]. However, an increase in HSPs might render hyperthermia less effective [28,29]. In this study, the HSF1 binding sites in promoter regions were analyzed after 10 and 20 min of hyperthermia using the ChIP-Seq approach. After the functional enrichment analysis, more overlapped genes were enriched in processes of regulation of programmed cell death and regulation of cell death (Figure 2). Programmed cell death, especially the expressions of some receptors and ligands in the process, play critical roles in the progression and resistance to oncolytic adenovirus of OS [30–32]. Regulations the cell death processes of tumor cells and normal cells has become an important way to increase efficacy and reduce resistance to traditional treatments in OS [33–35]. Studies reported that hyperthermia induced apoptosis and enhanced cell death in human OS cells [36,37]. Therefore, processes of programmed cell death and cell death might be involved in the mechanism of hyperthermia in OS, and their rational regulation might contribute to a better curative effect.

The overlaps were found enriched in 3 KEGG pathways, p53 signaling pathway, methane metabolism, and viral myocarditis. The P53 signaling pathway was involved in many tumors, and OS is no exception. It was reported that the p53 signaling pathway participates in cell proliferation, metastasis, and angiogenesis of OS [38–40]. Furthermore, the p53 pathway is closely associated with cell cycle and apoptosis, which are involved in the enriched GO terms used in this study [41–43]. Matsumoto et al. [44] found that nitric oxide is an initiator of intercellular signal transduction after hyperthermia in mutant p53 cells of human glioblastoma. Few researchers have explored the functions of methane metabolism and viral myocarditis in OS. Our results indicate that the p53 signaling pathway, methane metabolism, and viral myocarditis might play critical roles in gene changes of OS regulated by HSF1. However, further in-depth research is needed, especially on the relationships between OS and pathways of methane metabolism and viral myocarditis.

After TargetScan analysis of the overlaps, the miRNA-gene-regulated network was constructed, including 13 657 miRNA-gene regulated pairs that might be unreliable. Consequently, the network was divided into 4 modules (Figure 3). Module 1 contained the most nodes and pairs, and *SGMS2* and *CGGBP1* were regulated by more miRNAs than other nodes. Therefore, *SGMS2* and *CGGBP1* are important nodes in the miRNA-gene-regulated network of the overlapped DEGs. *SGMS2* encoded sphingomyelin synthase 2 (SGMS2), which is a risk factor of liver steatosis and atherosclerosis [45,46]. Hailemariam et al. [47] found that SGMS2 is a modulator of NFκB activation. Moreover, NFκB signaling pathways is closely related to the proliferation and progression mechanism of many cancers, and targeting it has been used in cancer prevention and therapy [48–50]. *CGGBP1* encoded CGG triplet repeat-binding protein 1 (CGGBP1), which is a nuclear and midbody protein regulating abscission [51]. *CGGBP1* expression is important for cell cycle progression in various cancer cell lines [52]. Cell cycle directly affects cell proliferation and apoptosis, which is important in the progression and prognosis of OS. Although few studies have verified that *SGMS2* and *CGGBP1* are associated with effects of hyperthermia on OS, the present study indicates that they might be key genes of OS treated with heat shock.

The GSE60984 data set was provided by Janus et al. [18], who mainly researched potential genes regulated by TNF-α cytokine and heat shock based on ChIP-Seq analysis, and explored functions of NF-κB signaling pathways on OS in the process of hyperthermia. In this article, some different methods were used and some novel findings were obtained. For example, functional and pathways enrichment analysis were performed, and some different biological processes (e.g., programmed cell death and cell death) and more pathways (e.g., p53 signaling pathway, methane metabolism, and viral myocarditis) were found to be involved in the effects of hyperthermia on OS. Moreover,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

the miRNA-gene network was constructed and analyzed with TargetScan and Cytoscape, which contained more interactions and thus might be more reliable. We also screened out some different biomarkers (e.g., *SGMS2* and *CGGBP1*).

Conclusions

SGMS2 and *CGGBP1* might be biomarkers of heat shocked OS, and programmed cell death and cell death might be involved in the mechanism of hyperthermia in OS. The p53 signaling pathway, methane metabolism, and viral myocarditis might play critical roles in gene changes in OS regulated by HSF1.

Conflict of interest

None.

Acknowledgements

We would like to thank all the members of our research group for their enthusiastic participation in this study.

References:

1. Ottaviani G, Jaffe N: The epidemiology of osteosarcoma. Cancer Treat Res, 2009; 152: 3–13
2. Duong LM, Richardson LC: Descriptive epidemiology of malignant primary osteosarcoma using population-based registries, United States, 1999–2008. J Registry Manag, 2013; 40(2): 59–64
3. Osuna D, de Alava E: Molecular pathology of sarcomas. Rev Recent Clin Trials, 2009; 4(1): 12–26
4. Wang S, Pan Z, Shi H, Wang P: [The radiation-induced jaw osteosarcoma: CT imaging features.] Chinese Journal of Stomatology, 2014; 49(49): 5–8
5. Kim HJ, Chalmers PN, Morris CD: Pediatric osteogenic sarcoma. Curr Opin Pediatr, 2010; 22(1): 61–66
6. Rosen G, Suwansirikul S, Kwon C et al: High-dose methotrexate with citrovorum factor rescue and adriamycin in childhood osteogenic sarcoma. Cancer, 1974; 33(4): 1151–63
7. Wilkins RM, Cullen JW, Odom L et al: Superior survival in treatment of primary nonmetastatic pediatric osteosarcoma of the extremity. Ann Surg Oncol, 2003; 10(5): 498–507
8. Meyers PA, Schwartz CL, Krailo M et al: Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. J Clin Oncol, 2005; 23(9): 2004–11
9. Buecker PJ, Berenstein M, Gebhardt MC et al: Locking versus standard plates for allograft fixation after tumor resection in children and adolescents. J Pediatr Orthop, 2006; 26(5): 680–85
10. Link MP, Goorin AM, Miser AW et al: The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. New Engl J Med, 1986; 314(25): 1600–6
11. Wang DW, Yu SY, Cao Y et al: Identification ofCD20, ECM,andITGAas Biomarkers for Osteosarcoma by Integrating Transcriptome Analysis. Med Sci Monit, 2016; 22: 2075–85
12. Liu S, Chen Z: The functional role of PMP22 gene in the proliferation and invasion of osteosarcoma. Med Sci Monit, 2015; 21(7): 1976–82
13. Toraya-Brown S, Fiering S: Local tumour hyperthermia as immunotherapy for metastatic cancer. Int J Hyperthermia, 2014; 30(8): 531–39
14. Matsumoto RR, Seminerio MJ, Turner RC et al: Methamphetamine-induced toxicity: An updated review on issues related to hyperthermia. Pharmacol Ther, 2014; 144(1): 28–40
15. Stevenson MA, Calderwood SK, Hahn GM: Effect of hyperthermia (45 degrees C) on calcium flux in Chinese hamster ovary HA-1 fibroblasts and its potential role in cytotoxicity and heat resistance. Cancer Res, 1987; 47(14): 3712–17
16. Malhotra A, Heynen ML, Lepock JR: Role of extracellular calcium in the hyperthermic killing of CHL V79 cells. Radiat Res, 1988; 112(3): 478–89
17. Nishida T, Akagi K, Tanaka Y: Correlation between cell killing effect and cell membrane potential after heat treatment: Analysis using fluorescent dye and flow cytometry. Int J Hyperthermia, 2009; 13(13): 227–34
18. Janus P, Stokowy T, Jaksik R et al: Cross talk between cytokine and hyperthermia-induced pathways: identification of different subsets of NF-kappaB-dependent genes regulated by TNFalpha and heat shock. Mol Genet Genomics, 2015; 290(5): 1979–90
19. Langmead B: Aligning short sequencing reads with Bowtie. Curr Protoc Bioinformatics, 2010. Chapter 11: Unit 11 7
20. Feng J, Liu T, Qin B et al: Identifying ChIP-seq enrichment using MACS. Nat Protoc, 2012; 7(9): 1728–40
21. Yu G, Wang LG, He QY: ChIPseeker: An R/Bioconductor package for ChIP peak annotation, comparison and visualization. Bioinformatics, 2015; 31(14): 2382–83
22. Dennis G Jr., Sherman BT, Hosack DA et al: DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol, 2003; 4(5): P3
23. Agarwal V, Bell GW, Nam JW, Bartel DP et al: Predicting effective microRNA target sites in mammalian mRNAs. Elife, 2015; 4
24. Lewis BP, Burge CB, Bartel DP Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell, 2005; 120(1): 15–20
25. Shannon P, Markiel A, Ozier O et al: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res, 2003; 13(11): 2498–504
26. Morimoto RI: Regulation of the heat shock transcriptional response: Cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev, 1998; 12(12): 3788–96
27. Kus-Liśkiewicz M, Polańska J, Korfanty J et al: Impact of heat shock transcription factor 1 on global gene expression profiles in cells which induce either cytoprotective or pro-apoptotic response following hyperthermia. BMC Genomics, 2013; 14: 456
28. Parsell DA, Lindquist S: The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. Annu Rev Genet, 1993; 27(4): 437–96
29. Ohtsuka K, Hata M: Molecular chaperone function of mammalian Hsp70 and Hsp40 – a review. Int J Hyperthermia, 2009; 16(3): 231–45
30. Hasei J, Sasaki T, Tazawa H et al: Dual programmed cell death pathways induced by p53 transactivation overcome resistance to oncolytic adenovirus in human osteosarcoma cells. Mol Cancer Ther, 2013; 12(3): 314–25
31. Yang Y, Jin Y, Du W: Programmed cell death 2 functions as a tumor suppressor in osteosarcoma. Int J Clin Exp Pathol, 2015; 8(9): 10894–900
32. Zheng W, Xiao H, Liu H, Zhou Y: Expression of programmed death 1 is correlated with progression of osteosarcoma. APMIS, 2015; 123(2): 102–7
33. Kumar C, Vats K, Lohar SP et al: Camptothecin enhances cell death induced by (177)Lu-EDTMP in osteosarcoma cells. Cancer Biother Radiopharm, 2014; 29(8): 317–22
34. Yu Z, Geng J, Zhang M et al: Treatment of osteosarcoma with microwave thermal ablation to induce immunogenic cell death. Oncotarget, 2014; 5(15): 6526–39
35. Kawano M, Tanaka K, Itonaga I et al: Dendritic cells combined with doxorubicin induces immunogenic cell death and exhibits antitumor effects for osteosarcoma. Oncol Lett, 2016; 11(3): 2169–75
36. Hou CH, Lin FL, Hou SM, Liu JF: Hyperthermia induces apoptosis through endoplasmic reticulum and reactive oxygen species in human osteosarcoma cells. Int J Mol Sci, 2014; 15(10): 17380–95
37. Hori T, Kondo T, Lee H et al: Hyperthermia enhances the effect of β-lapachone to cause γH2AX formations and cell death in human osteosarcoma cells. Int J Hypertherm, 2011; 27(1): 53–62
38. Song R, Tian K, Wang W, Wang L et al: P53 suppresses cell proliferation, metastasis, and angiogenesis of osteosarcoma through inhibition of the PI3K/AKT/mTOR pathway. Int J Surg, 2015; 20: 80–87

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

39. Zhao S, Kurenbekova L, Gao Y et al: NKD2, a negative regulator of Wnt signaling, suppresses tumor growth and metastasis in osteosarcoma. Elife, 2015; 34(39): 5069–79
40. Walia MK, Ho PM, Taylor S et al: Activation of PTHrP-cAMP-CREB1 signaling following p53 loss is essential for osteosarcoma initiation and maintenance. Elife, 2016; 5. pii: e13446
41. Sun Y, Xia P, Zhang H et al: P53 is required for Doxorubicin-induced apoptosis via the TGF-beta signaling pathway in osteosarcoma-derived cells. Am J Cancer Res, 2015; 6(1): 114–25
42. Yang D, Wang P, Ren X: Apoptosis induced by chamaejasmine in human osteosarcoma cells through p53 pathway. Tumour Biol, 2015; 36(7): 5433–39
43. Wan D, Jiang C, Hua X et al: Cell cycle arrest and apoptosis induced by aspidin PB through the p53/p21 and mitochondria-dependent pathways in human osteosarcoma cells. Anticancer Drugs, 2015; 26(9): 931–41
44. Matsumoto H, Hayashi S, Hatashita M et al: Nitric oxide is an initiator of intercellular signal transduction for stress response after hyperthermia in mutant p53 cells of human glioblastoma. Cancer Res, 1999; 59(13): 3239–44
45. Li Y, Dong J, Ding T et al: Sphingomyelin synthase 2 activity and liver steatosis: An effect of ceramide-mediated peroxisome proliferator-activated receptor γ2 suppression. Arterioscler Thromb Vasc Biol, 2013; 33(7): 1513–20
46. Liu J, Huan CM: Macrophage sphingomyelin synthase 2 deficiency decreases atherosclerosis in mice. Circ Res, 2009; 105(3): 295–303
47. Hailemariam TK, Huan C, Liu J et al: Sphingomyelin synthase 2 deficiency attenuates NFkappaB activation. Arterioscler Thromb Vasc Biol, 2008; 28(8): 1519–26
48. Kavandi L, Lee LR, Bokhari AA et al: The Chinese herbs *Scutellaria baicalensis* and *Fritillaria cirrhosa* target NFkappaB to inhibit proliferation of ovarian and endometrial cancer cells. Mol Carcinog, 2015; 54(5): 368–78
49. Wang W, Nag SA, Zhang R: Targeting the NFkappaB signaling pathways for breast cancer prevention and therapy. Curr Med Chem, 2015; 22(2): 264–89
50. Zhang Y, Liu S, Wang H et al: Elevated NIBP/TRAPPC9 mediates tumorigenesis of cancer cells through NFkappaB signaling. Oncotarget, 2015; 6(8): 6160–78
51. Singh U, Westermark B: CGGBP1 is a nuclear and midbody protein regulating abscission. Exp Cell Res, 2011; 317(2): 143–50
52. Singh U, Roswall P, Uhrbom L, Westermark B: CGGBP1 regulates cell cycle in cancer cells. BMC Mol Biol, 2011; 12: 28

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]