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Research paper

Comparative proteomics analysis of human osteosarcoma by 2D DIGE with MALDI-TOF/TOF MS

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ABSTRACT

Osteosarcoma (OS) is the most common primary malignant tumor of bone and the third most common cancer in childhood and adolescence. However, controversy concerning the ideal combination of chemotherapy agents ensued throughout the last quarter of the 20th century because of conflicting and often nonrandomized data. Collaborative efforts to increase understanding of the biology of osteosarcoma and the use of preclinical models to test novel protein targets will be critical to identify the path toward improving outcomes for patients. We attempted to identify potential protein markers or therapy targets of osteosarcoma and give a glance at tumorigenesis of osteosarcoma. A sensitive and accurate method was employed in comparative proteomic analysis between benign tumor and osteosarcoma. Tumor tissues obtained by open biopsy before induction chemotherapy were investigated With 2D DIGE and MALDI-TOF/TOF MS, 22 differentially expressed proteins were identified after database searching, including 8 up-regulated and 14 down-regulated proteins. We also validated the expression levels of interesting proteins(have higher Ratios(tumor/normal)) by Western blotting assay. Annotating by bioinformatic tools, we found structural and signal transduction associated proteins were in large percentage among altered level proteins. In particular, some low abundant proteins involving translation and transcription, such as EEF2(Elongation Factor 2), LUM Lumican 23 kDa Protein) and GTF2A2(Transcription Initiation Factor Iia Gamma Chain.), were firstly reported by our study comparing to previous observations. Our findings suggest that these differential proteins may be potential biomarkers for diagnosis or molecules for understanding of osteosarcoma tumorigenesis, coming with biologic, preclinical, and clinical trial efforts being described to improve outcomes for patients.

1. Introduction

Osteosarcoma (OS) is the most common primary malignant bone sarcoma, which usually occurring in children and adolescents [1]. Actually, OS comprises approximately 1/5 of all bone tumors, which is the fifth most common type of cancer in young people [2–4]. At present, despite modern treatment protocols that combine chemotherapy and surgery, the optimal schedule of therapy is still being investigated because of high recurrence and drug-resistance [5].

Osteosarcoma is pathologically defined by production of osteoid [6], but it is broadly characterized by genetic complexity caused by chromosomal alternations [7]. Perhaps, the most attractive data potentially indicating pathogenesis of osteosarcoma is germ-line genetic alternations. It was reported that germ-line mutations in Rb gene is correlated to OS [8,9]. In fact, a large number of animal models are developed for osteosarcoma, including P53 knock out mouse model [10], transgenic c-fos mouse model [11] and parathyroid hormone injection mouse model [12]. But it is still unclear which model could accurately recapitulate the human disease.

There is a real need to identify signaling molecules and mechanisms that promote OS progression and metastasis, particularly for the development of novel and more effective treatment strategies.

Since the advent of genomics technology, proteomics provides a powerful tool to discover new biomarkers for early diagnosis [13] . It

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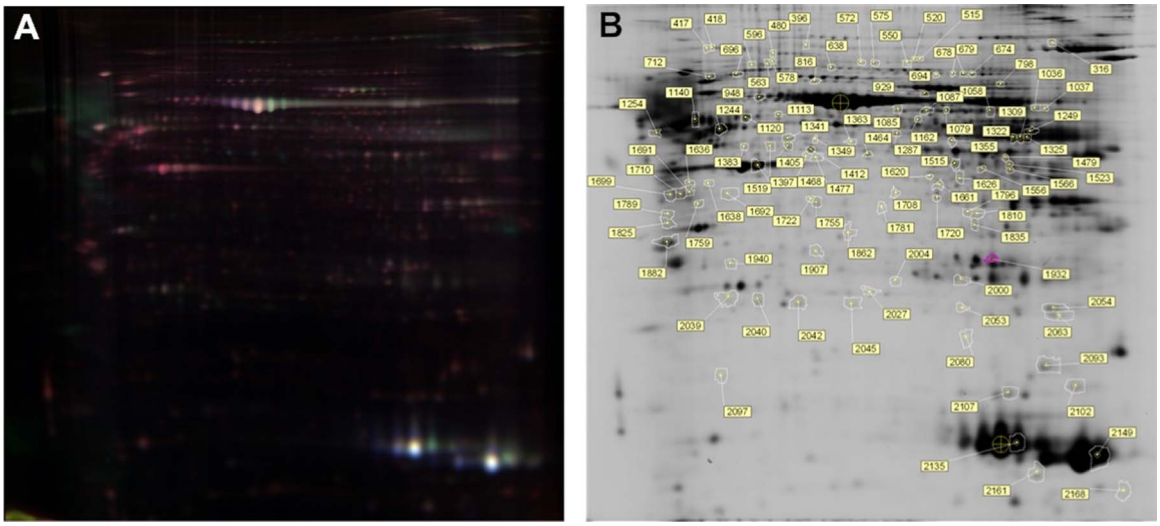

Fig. 1. the comparison of 2D-DIGE of normal and osteosarcomas tissue.

Table 1.
Differential proteins identified by 2D-DIGE.

Up-regulated proteins			
IPIAccession	Gene_name	Ratio(tumor/normal)	Definition
IPI00440493	ATP5A1	1.52	ATP synthase subunit alpha, Mitochondrial precursor.
IPI00298497	FGB	2.17	Fibrinogen beta chain precursor.
IPI00025363	GFAP	2.24	Isoform 1 of glial fibrillary acidic protein, Astrocyte .
IPI00410714	HBA1	2.63	Hemoglobin subunit alpha.
IPI00853068	HBA2	1.61	Hemoglobin Alpha-2.
IPI00654755	HBB	3.09	Hemoglobin subunit beta.
IPI00794403	LUM	1.53	LUM 23 kDa protein.
IPI00006114	SERPINF1	3.58	Pigment epithelium-derived factor precursor.
Down-regulated proteins			
IPIAccession	Gene_name	Ratio(tumor/normal)	Definition
IPI00894365	ACTB	−1.53	cDNA FLJ52842, highly similar to actin, Cytoplasmic 1.
IPI00186290	EEF2	−1.88	Elongation factor 2.
IPI00465248	ENO1	−1.57	Enolase 1.
IPI00011454	GANAB	−1.75	Isoform 2 Of neutral alpha-glucosidase Ab precursor.
IPI00004353	GTF2A2	−1.7	Transcription initiation factor lia gamma chain.
IPI00003865	HSPA8	−2.37	Isoform 1 of heat shock cognate 71 kDa protein.
IPI00784154	HSP60	−1.68	60 kDa heat shock protein, Mitochondrial precursor.
IPI00220327	KRT1	−1.57	Keratin, Type II cytoskeletal 1.
IPI00789173	LDHB	−2.07	LDHB_HUMAN L-lactate dehydrogenase B chain
IPI00514204	LMNA	−2.3	Lamin A/C
IPI00872814	MOES	−2.13	Moesin
IPI00479185	TPM3	−1.95	Tropomyosin 3 Isoform 4
IPI00027107	TUFM	−1.58	Tu translation elongation factor, Mitochondrial
IPI00418471	VIM	−2.12	Vimentin

has been reported that there are vast of genes and proteins expression changes, involved in biochemical pathways, occurred in tumorigenesis [14,15]. So far, several proteomics researches of osteosarcoma have been established base on serum, cell lines and tissues treated by chemotherapy [16–18]. According to these studies, the expression of SAA, Fibrinogen, AHA1, SLP-2 and EZR was significantly changed. The most extensively studied prognostic marker is *p*-glycoprotein encoded by multi-drug resistance (MDR1) gene [19], but its efficacy remains controversial [20]. These proteins may be considered as potential molecular targets for therapy of osteosarcoma. However, there are few reports directly concerning primary bone sarcoma tissues with high-through and sensitive methods. Hence, it needs urgently to uncover robust and specific markers for understanding the biological behavior of osteosarcoma.

In this study, clinical tissues from osteosarcoma were analyzed by 2D DIGE and MALDI-TOF/TOF MS. With sensitive labeling method, some low expression proteins were identified, which play important roles in signal transduction pathways. Many different kinds of structural proteins were also detected firstly in our study comparing to previous research. More importantly, combination of our result and previous proteome data, discovery of specific bio-marker for clinical diagnosis is promising.

2. Materials and methods

2.1. Patients and tissues specimens

Primary tumor samples, including benign tumors and osteosarcomas, were obtained when patients underwent surgery for tumor resection. According to World Health Organization (WHO) histologic classification, 6 patients were diagnosed with an osteoblastic variant of osteosarcoma and two had a chondroblastic variant of osteosarcoma. The benign bone tumors collected included two osteoblastoma, two chondroblastoma, and one giant cell tumor of bone collected from patients ranging from 9 to 41 years old.