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Tissue Expression of Manganese Superoxide Dismutase Is a Candidate Prognostic Marker for Glioblastoma

Chul-Kee Park[a] Ji Hye Jung[a] Min Jeong Moon[a] Young-Yim Kim[a]
Jin Hyun Kim[b] Sung-Hye Park[c] Chae-Yong Kim[a] Sun Ha Paek[a]
Dong Gyu Kim[a] Hee-Won Jung[a] Byung-Kyu Cho[a]

[a]Department of Neurosurgery, Seoul National University College of Medicine, Seoul, [b]Clinical Research Institute, Gyeongsang National University Hospital, Jinju, and [c]Department of Pathology, Seoul National University College of Medicine, Seoul, Korea

Key Words
Proteomics · Long-term survival · Glioblastoma · Prognostic factor · Manganese superoxide dismutase

Abstract
Background: Characterization of a rare subgroup of glioblastoma patients who survive for more than 3 years (long-term survival glioblastoma, LTSGBL, patients) may be helpful to identify prognostic factors. ***Materials and Methods:*** A molecular-profiling proteomic approach using two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were used to identify prognostic factors associated with glioblastoma by comparing frozen tumor tissue from LTSGBL patients with matched samples from short-term survival glioblastoma (STSGBL) patients. Western blot (WB) analysis, reverse-transcriptase polymerase chain reaction (RT-PCR) and immmunohistochemical (IHC) staining were used for confirmation. ***Results:*** Among most candidate spots identified by 2-DE, lack of overexpression of manganese superoxide dismutase (MnSOD) in LTSGBL samples was consistently observed using WB and RT-PCR. ***Conclusion:*** These results suggest that MnSOD expression level in tumor tissue is a candidate marker for the prognosis of glioblastoma patients.

Introduction

The prognosis of glioblastoma patients remains poor despite recent therapeutic advances involving multidisciplinary strategies. Nevertheless, 1–17% of glioblastoma patients survive for more than 3 years (long-term survival glioblastoma, LTSGBL, patients) [1]. Characterization of this subgroup of patients may help to elucidate the biological behavior of glioblastomas. Although molecular profiling has been used to identify biomarkers of LTSGBL [2–4], protein expression profiles have not been reported for LTSGBL tissue. In the present study, a proteomic approach involving two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify markers for LTSGBL by comparing the protein expression profile of LTSGBL tissue with that of short-

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Byung-Kyu Cho, MD, PhD
Department of Neurosurgery
Seoul National University College of Medicine
28 Yeongeon-dong, Jongno-gu, Seoul 110-744 (Korea)
Tel. +82 2 2072 3639, Fax +82 2 747 3648, E-Mail bkcho@snu.ac.kr

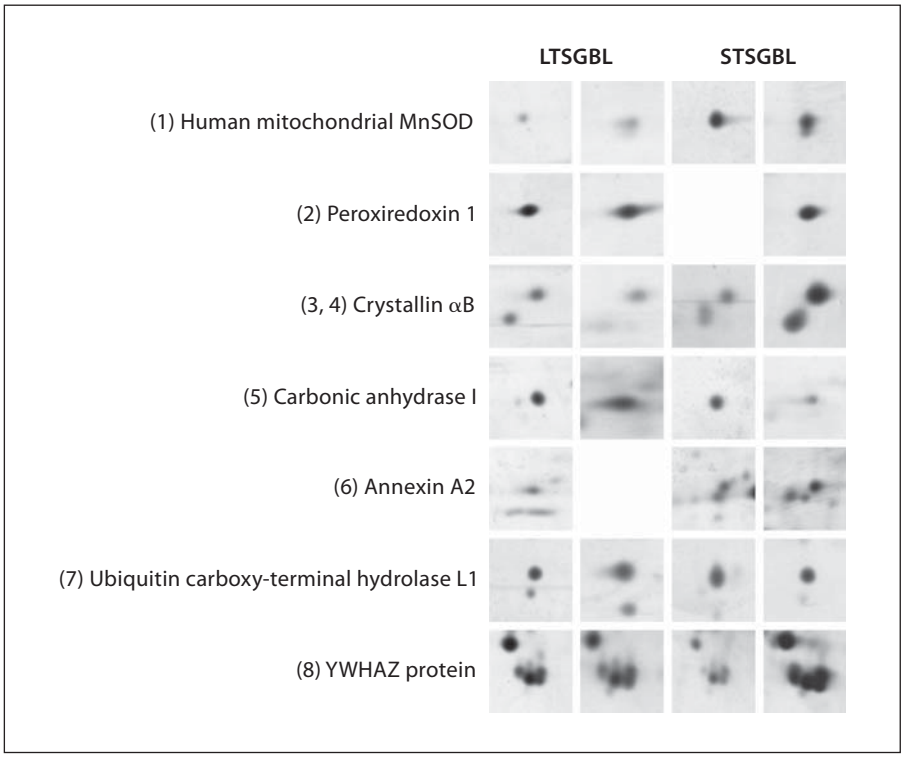

Fig. 1. Detailed comparison of individual spots of interest detected by 2-DE of 2 LTSGBLs and 2 STSGBLs. 1–8 represent spot No.

Table 1. Clinical data of glioblastoma patients enrolled in the study

	Sex	Age years	Location	Management	Survival period months
Exploratory study (2-DE, WB analysis, IHC staining, RT-PCR)					
Case 1	female	44	frontal	STR-RTx-CTx-GKS	99
Case 2	female	65	parieto-occipital	GTR-RTx	86
Case 3	female	47	frontotemporal	STR-CTx-RTx	13
Case 4	female	25	frontal	GTR-RTx	7
Confirmation study (WB analysis, RT-PCR)					
Case 5	male	46	temporal	STR-CTX-RTx-GKS-GTR-CTx	58
Case 6	female	38	frontal	STR-CTx-RTx-CTx-GKS	39
Case 7	male	24	temporal	STR-CTx-RTx	5
Case 8	male	46	frontal	STR-CTx-RTx	11

GTR = Gross total removal; STR = subtotal removal; RTx = radiation therapy; CTx = chemotherapy; GKS = gamma knife surgery.

term survival glioblastoma (STSGBL) tissue. Western blot (WB) analysis, the reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemical (IHC) staining were used to validate differentially expressed proteins.

Materials and Methods

Clinical data of the 8 glioblastoma patients whose tumor tissues were used in this study are summarized in table 1. Their snap-frozen tumor tissues obtained during surgery were used. Protein extraction, 2-DE, image analysis, and MALDI-TOF MS

Table 2. Proteins identified using MALDI-TOF MS

Spot No.	Protein ID	Accession No.	MASCOT score	Theoretical M_r (kDa)/pI	Sequence coverage, %
1	mutant human mitochondrial MnSOD	gi|2914417	110	22.3/6.9	38
2	peroxiredoxin 1	gi|55959887	90	19.1/6.4	53
3	crystallin αB	gi|4503057	122	20.1/6.8	47
4	crystallin αB	gi|4503057	122	20.1/6.8	47
5	carbonic anhydrase I	gi|4502517	128	28.9/6.6	45
6	annexin A2	gi|30962842	222	38.8/7.6	52
7	ubiquitin carboxy-terminal hydrolase L1	gi|4185720	78	23.4/5.3	34
8	YWHAZ protein	gi|49119653	137	30.1/4.7	42

were performed as previously reported [5]. The expression levels of the proteins of interest that were identified by 2-DE and MALDI-TOF MS were confirmed by WB analysis, RT-PCR and IHC. The antibodies used for WB analysis and IHC staining were anti-manganese superoxide dismutase (MnSOD; BD Transduction Lab, catalog No. 611581, San Jose, Calif., USA), peroxiredoxin 1 (Santa Cruz, catalog No. SC-33571, Santa Cruz, Calif., USA), annexin II (Santa Cruz, catalog No. SC-9061), YWHAZ(14-3-3) protein (Santa Cruz, catalog No. SC-1019). The oligonucleotide primers used for RT-PCR were GAPDH (sense, 5′-ACTTCAACAGCGACACCCACTC-3′ and antisense, 5′-AGGCCCCTCCCCTCTTCA-3′) and MnSOD (sense, 5′-TGTTGAGCCGGGCAGTGT-3′ and antisense, 5′-CTCCCAGTTGATTACATTC-3′).

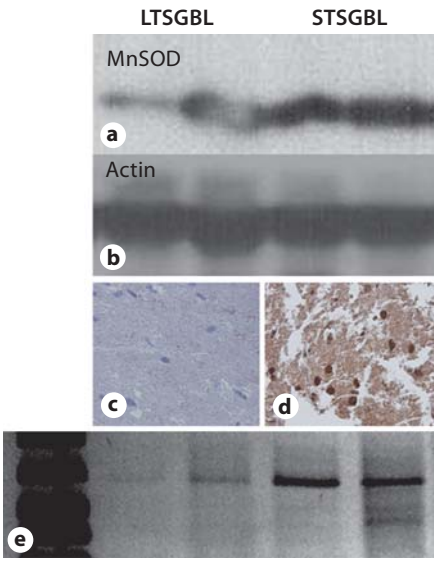

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Fig. 2. MnSOD expression (**a**) in tumor tissues showing consistent overexpression in STSGBL samples compared with LTSGBL samples in WB (**b**), IHC (**c**, **d**), and RT-PCR (631 bp) (**e**) analyses.

Results

The cross analysis of 4 sets of 2-DE images (2 LTSGBLs and 2 STSGBLs) revealed 8 candidate spots (fig. 1). These spots were consistently present in all samples but their expression levels differed between samples identified using MALDI-TOF MS and database searching (table 2). Of these molecules, MnSOD was the only one that was consistently expressed in 2-DE, WB, IHC, and RT-PCR analyses (fig. 2). MnSOD was overexpressed in STSGBL samples. To confirm this result, WB analysis and RT-PCR using an MnSOD probe were performed using a series of samples from patients other than those used in the first analysis (2 LTSGBL and 2 STSGBL patients). The results of the second series were the same as those of the first (data not shown).

Discussion

Although molecular profiling of this rare subset of LTSGBLs and its characterization is important for effective improvement of treatment of this devastating disease, few studies have been conducted [1]. In the present study, we used a proteomic molecular-profiling approach to identify markers associated with LTSGBLs and observed that a lack of overexpression of MnSOD may predict long-term survival of glioblastoma patients. MnSOD is an important antioxidant enzyme that protects cells from oxidative stress by catalyzing the conversion of the superoxide radical (O_2^-) to hydrogen peroxide and molecular oxygen in the mitochondria [6]. The results of previous in vitro studies using various cancer cell lines are conflicting, indicating that MnSOD is either a tumor suppressor or a tumor enhancer [7, 8]. However, the role of MnSOD as a tumor enhancer or a poor prognostic fac-

tor of human cancer is evident from the results of clinical studies [9]. Several mechanisms may underlie the prognostic value of MnSOD. Firstly, a high MnSOD level in tumor tissue is associated with resistance to radiation therapy or chemotherapy [10]. Secondly, overexpression of MnSOD is associated with inactivation of p53, which causes tumor cells to escape cell death, to survive and to proliferate under stress conditions [11].

In summary, molecular profiling of tissue samples of LTSGBL using a proteomic technique showed that a lack of overexpression of MnSOD protein and mRNA is consistent in LTSGBLs. And it was concluded that the level of MnSOD expression is a candidate prognostic factor in glioblastoma patients. Further studies with additional cases are warranted to confirm the predictive value of MnSOD expression and its role in the pathogenesis of malignant gliomas.

Acknowledgments

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