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Chemoradiotherapy Alters Protein Expression in Glioblastoma Multiforme

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Dear Editor,

Glioblastoma multiforme (GBM) is the most common malignant brain tumor and is essentially incurable, with fewer than 50% of patients surviving beyond 1 year. The specific intracellular proteins affected by chemoradiation therapy (CRT) in GBM are unknown. We used proteomic analysis to compare protein expression between untreated GBM and recurrent GBM treated with CRT.

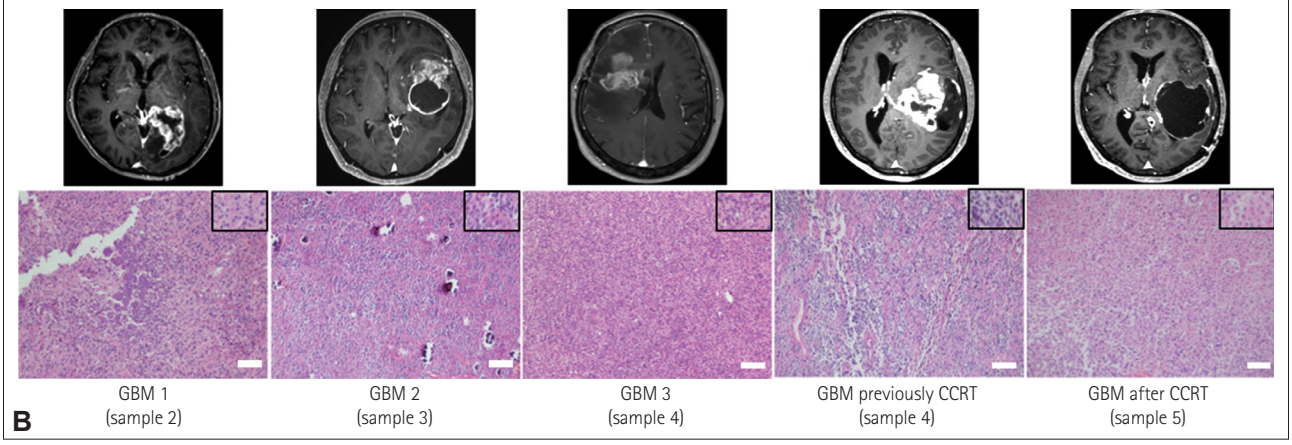
Whole-tissue lysates in the normal human brain were obtained from Novus Biologicals. Samples of brain tumor tissue were obtained from patients who underwent surgical resection of GBM in the departments of neurosurgery at the Department of Neurosurgery of Hanyang University Medical Center (Seoul and Guri), Korea. The Institutional Review Boards at both hospitals reviewed and approved the study protocol (IRB No. 2017-02-016 and IRB No. 2016-10-002). Two-dimensional polyacrylamide gel electrophoresis was applied to the brain tumor tissues. The gels were stained with Coomassie brilliant blue R-350 and then underwent matrix-assisted laser desorption ionization time-of-flight analysis and peptide mass fingerprinting using a MASCOT database developed by Matrix Science. A network of proteins was confirmed using the Laverne bioinformatics tool from Novus Biologicals.

Five tissue samples were analyzed: one from a human normal brain, three from GBM patients, and one from the second resection of a patient with recurrent GBM after concurrent CRT (CCRT). Fig. 1A provides detailed information about the samples. CCRT was applied using the standard protocol, comprising radiotherapy at 50.4 Gy in 28 fractions and concomitant adjuvant temozolomide. Fig. 1B shows the radiographic and histological findings of the samples, including for the first sample resected from the patient with GBM before CCRT shown in Fig. 1A. Fig. 1C–F shows the proteomics results for tissue samples from a normal human brain, three patients with untreated GBM, and a GBM patient treated with CCRT. The proteins whose expression levels differed significantly between GBM with and without CCRT were 14-3-3 protein gamma (YWHAG), ferritin light polypeptide (FTL), glyceraldehyde 3 phosphate dehydrogenase (GAPDH), and triosephosphate isomerase (TPI1). A bioinformatic analysis revealed associations between GBM and these four proteins in terms of the cell cycle and apoptotic processes (Fig. 1G).

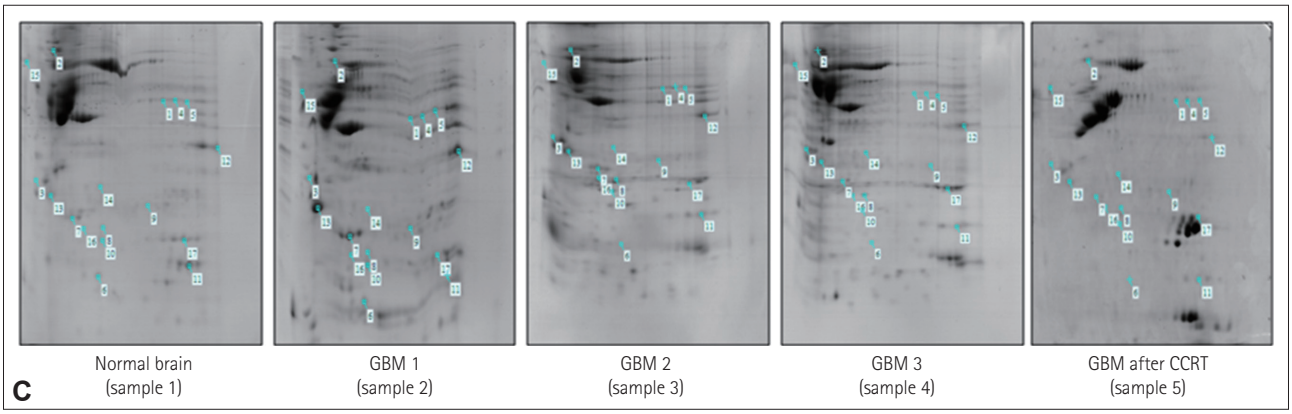
To the best of our knowledge, this study is the first to identify differences in protein expression between GBM with and without CCRT. The standard treatment for GBM inhibits the PI3K/AKT/mTOR pathway,[1] which might lead to increased apoptosis in GBM and the cytotoxic potential via cell-cycle arrest during the G2/M phase.[2] Previous studies have associated the PI3K pathway with YWHAG,[3] ferritin,[4] GAPDH,[5] and TPI1.[6] Therefore, we postulated that standard CCRT for GBM inhibited the PI3K pathway and subsequently affected YWHAG, FTL, GAPDH, and TPI1 expression in the present study.

Sample	Sex	Age, years	Tumor type	GFAP	Ki-67 index	p53	Past history
1	M	82	Normal brain (human adult)				
2	F	76	GBM	(+)	20%	60%	(-)
3	F	65	GBM	(+)	10%	40%	Thyroid cancer
4	F	77	GBM	(+)	30%	40%	(-)
5	M	57	GBM, necrosis after CCRT	(+)	<10%	(-)	CCRT [standard radiotherapy (50.4 Gy in 28 fractions) with concomitant and adjuvant temozolomide] for glioblastoma, 2 years before

A



B



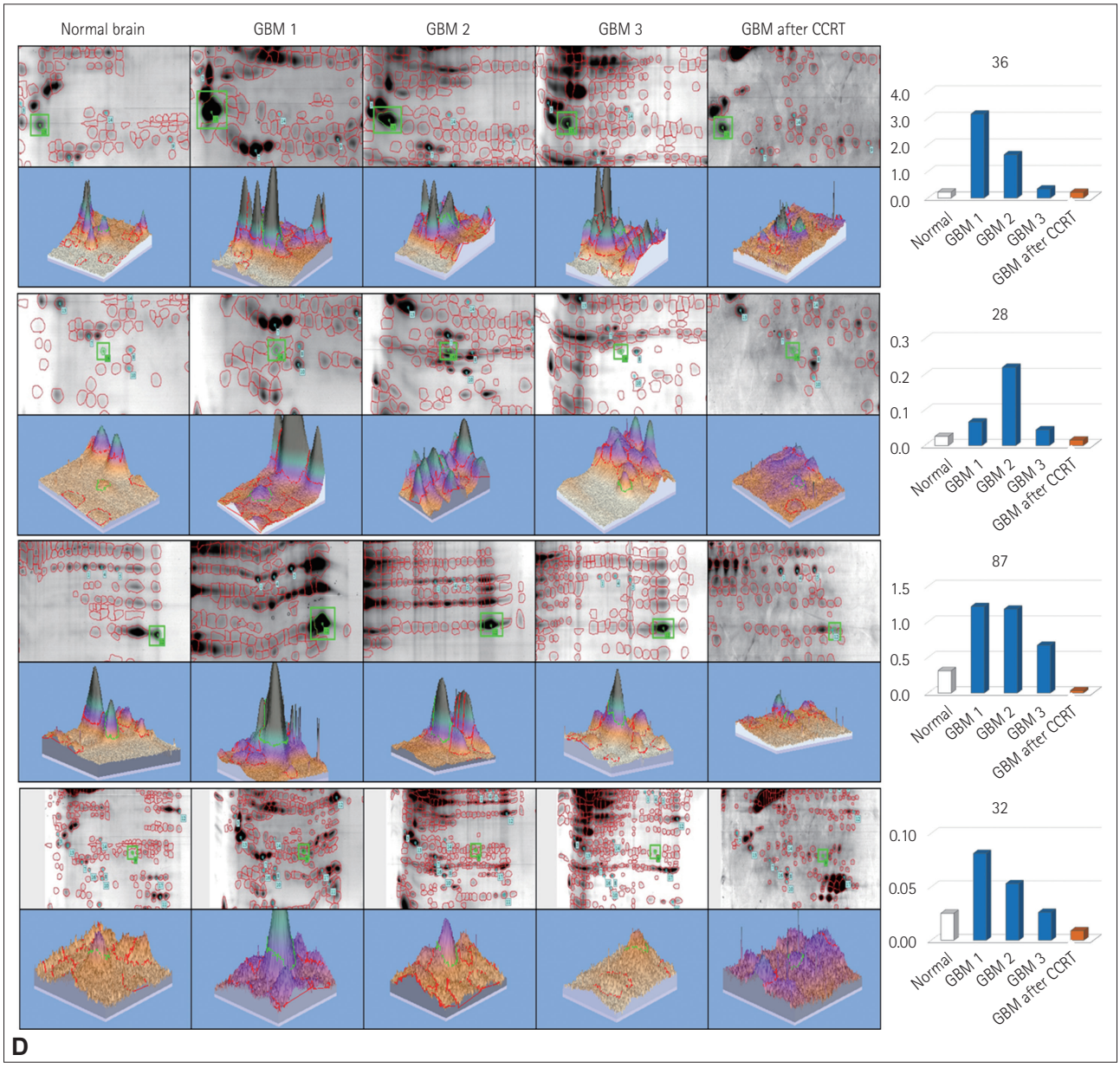
C

Fig. 1. MRI, pathological findings, and results from proteomic analyses of a normal brain and brains with GBM. A: Detailed information about the samples. B: Brain MRI and histological staining features of GBM (scale bar, 50 μm). C: Two-dimensional polyacrylamide gel electrophoresis gels stained with Coomassie brilliant blue R-350. Protein spots in tissue samples from a normal brain, three patients with untreated GBM, and a GBM patients treated with CRT with common landmarks. CCRT: concurrent CRT, CRT: chemoradiation therapy, FTL: ferritin light polypeptide, GAPDH: glyceraldehyde 3 phosphate dehydrogenase, GBM: glioblastoma multiforme, GFAP: glial fibrillary acidic protein, TPI1: triosephosphate isomerase, YMHAH: 14-3-3 protein gamma.

Our study had an important limitation, in that the tumor tissue after CCRT was obtained 2 years after the therapy and not from the same patients as the other GBM tissues before CCRT. This limitation might have affect our results, but their novelty should prompt future investigations.

Author Contributions

Conceptualization: Seong-Ho Koh, Hyun-Hee Park, Mina Hwang, Myung-Hoon Han. Data curation: Seong-Ho Koh, Hyun-Hee Park, Mina Hwang, Myung-Hoon Han. Formal analysis: Seong-Ho Koh, Hyun-Hee Park, Mina Hwang, Myung-Hoon Han. Funding acquisition: Seong-Ho Koh, Hyun-Hee Park, Myung-Hoon Han. Investigation: Hyun-Hee Park, Mina Hwang. Methodology: Mina Hwang, Myung-Hoon Han. Project administration: Seong-Ho Koh, Hyun-Hee Park. Resources: Seong-Ho Koh, Hyun-Hee Park. Software: Seong-Ho Koh, Hyun-Hee Park, Mina Hwang, Myung-Hoon Han. Supervision: Hojin Choi, Kyu-Yong Lee, Young Joo Lee, Jae Min Kim, Jin Hwan Cheong, Je Il Ryu, Yong Ko. Validation: Hojin Choi, Kyu-Yong Lee, Young Joo Lee, Jae Min Kim, Jin Hwan Cheong, Je Il Ryu, Yong Ko. Visualization: Mina Hwang, Myung-Hoon Han. Writing—original draft: Mina Hwang, Myung-Hoon Han. Writing—review & editing: Seong-Ho Koh, Hyun-Hee Park, Hojin Choi, Kyu-Yong Lee, Young Joo Lee, Jae Min Kim, Jin Hwan Cheong, Je Il Ryu, Yong Ko.



Match ID	Normal brain	GBM 1	GBM 2	GBM 3	GBM after CCRT	Normal brain:GBM average range ratio	GBM average:GBM after CCRT range ratio
36	0.222	3.161	1.632	0.340	0.214	7.697	7.985
28	0.026	0.066	0.220	0.044	0.015	4.313	7.240
87	0.313	1.213	1.178	0.670	0.033	3.263	31.340
32	0.025	0.081	0.053	0.026	0.009	2.115	5.943

E

Match ID	Protein
36	YMHAH
28	FTL
87	GAPDH
32	TPI1

F

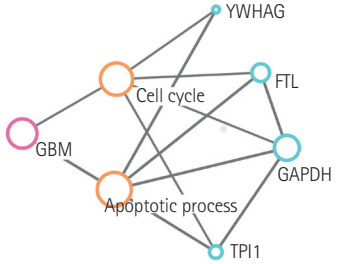


Fig. 1. MRI, pathological findings, and results from proteomic analyses of a normal brain and brains with GBM. D: Protein spots exhibiting changes in expression level of greater than twofold between GBM cells treated with CRT and untreated GBM cells. Details (E) and identification (F) of four selected proteins. G: A protein–protein interaction network constructed between GBM and the selected four proteins using bioinformatics. CCRT: concurrent CRT, CRT: chemoradiation therapy, FTL: ferritin light polypeptide, GAPDH: glyceraldehyde 3 phosphate dehydrogenase, GBM: glioblastoma multiforme, GFAP: glial fibrillary acidic protein, TPI1: triosephosphate isomerase, YMHAH: 14-3-3 protein gamma.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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