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in this study, we used immunohistochemistry for quantification of the percentage of cells staining positive for sdma motif in a cohort of adult (n=18) and pediatric (n=6) gbm and oligoastrocytoma (oa, n=5). positive sdma staining was considered as presence of sdma motif, and we divided adult and pediatric gbm patients (n=23) according to the percentage of positive staining of cells. the patients molecular characterization, clinical features, and treatment are shown in additional file 2 : table s2. see additional file 3 : table s3 for the human snap-frozen gbm tumors used for metabolomic study. patients and methods: ninety-five pediatric patients with newly diagnosed gliomas admitted to peking university people's hospital between 2008 and 2014 were reviewed retrospectively. gbm was diagnosed according to the world health organization criteria [94]. the clinical characteristics (tumors location, histologic classification, who grade, patients age-ranges and gender) and outcomes are depicted in fig. 4 and additional file 4 : table s4. 62 of the 95 patients received a standard, who-based postoperative management protocol (referred in additional file 4 : table s4 as st+rt+cht+r). 13 patients received only st+rt+cht+r and 30 received st+rt+cht+vcr. primary endpoint was the association of age with the incidence of alkylation dna damage. secondary endpoints were the incidence of and severity of alkylation dna damage, prognostic value, and effects of dacarbazine on alkylation damage. human snap-frozen gbm tumors were kept at 80c (same set of tumors used for metabolomic study). without thawing, approximately 10mg of tumor tissue was transferred to a new tube for immunoblotting. lysates were made in 200l of radioimmunoprecipitation assay buffer supplemented with protease inhibitor (roche, #11836153001) and phosphatase inhibitor (roche, #04906837001) followed by sonication at 4c. protein quantitation was performed via bca assay (pierce, #23225), and lysates were equilibrated for running on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. the following antibodies were used for this study: sdma motif (cell signaling technology, #13222, lot: 6) in a 1:1000 dilution, mtpa (abcam, #ab126623, lot: gr97816-5, lot: yi070108c5, lot: gr90092-13) in a 1:1000 dilution, gapdh (sigma-aldrich, #g9545, lot: gr180112-03) in a 1:5000 dilution, iba1 (abcam, #ab178846, lot: yi030121v) in a 1:1000 dilution, and vinculin (cell signaling technology, #13901) in a 1:5000 dilution.



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for confirmation, we repeated the serum samples obtained from 5 patients of grade iv gbm (table 1) using the same protocol and also compared the results with those of grade iv gbm patients who were available for the long-term follow-up samples. all 5 patients had who grade iv gliomas with a median age of 45.5 years (range 29 to 55 years) at the time of diagnosis. the median time of follow-up was 104 months (range 69 to 128 months). a positive correlation between the levels of serum stm on days 15 and 29 and overall survival of these patients was detected. however, the overall survival curves of the 3 patients with grade iv gbm, stm levels on days 15 and 29 were significantly different, but the survival curve of the patient with normal stm level was similar to those of the other 2 patients with elevated stm levels at the beginning of chemotherapy (fig. 1g, supplementary fig. 5). patients with stm of less than 10ng/ml on day 15 had longer overall survival than those with stm values of greater than 10ng/ml, indicating that the patients with stm of less than 10ng/ml on day 15 could survive longer than those with stm of greater than 10ng/ml (fig. 5). the stm of 6 patients with grade iv glioma was detected at the beginning of the treatment, after initial surgery, and approximately the same levels of stm at 3, 6, 9, and 12months of chemotherapy. when the survival curves of these patients were compared, no significant difference was detected at any time point (fig. 1h). of note, 5 months after the surgery of grade iv glioma patients who had received radiotherapy alone, their stm levels were elevated (fig. 1h, supplementary fig. 6). 5ec8ef588b

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