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LETTER TO THE EDITOR

Low expressions of ASS1 and OTC in glioblastoma suggest the potential clinical use of recombinant human arginase (rhArg)

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To the Editor,

Glioblastoma multiforme's (GBM) inherent aggressiveness and the lack of effective treatments call for new treatment strategies. As a hallmark of cancer, metabolic reprogramming including a higher dependency on exogenous supply of arginine has aroused interests within the oncology community as a potentially targetable pathway. Recent studies reported very low or absent expressions of some urea cycle enzymes involved in arginine de novo synthesis rendering them susceptible to arginine deprivation. Khoury and colleagues have recently published an article in your journal demonstrating the cytotoxicity of rhArg in GBM cells [1]. Our group accordingly investigated the potential use of arginine degrading enzyme PEG-BCT-100, a PEGlyated form of recombinant human arginase (rhArg), in the treatment of GBM through in vitro studies. We have also investigated its translational potential for clinical investigation through immunohistochemistry analyses of urea cycle enzymes in GBM patients' samples.

Firstly, we established the expression profile of urea cycle enzymes, including argininosuccinate synthase 1 (ASS1), argininosuccinate lyase (ASL) and ornithine carbamoyltransferase (OTC), in six GBM cell lines (A172, M059J, M059K, T98G, U373 and U-87 MG) by Western blot. ASL was basally expressed at different levels among cell lines. None of the six cell lines had ASS1 expression, and only M059J, M059K and U373 were weakly OTC positive (Fig. 1a). Sensitivity of GBM cell lines towards PEG-BCT-100 were then evaluated using MTT cytotoxicity assay. Cells were treated with PEG-BCT-100 for 48 h and maximum 70–80 % growth inhibition was observed. The EC_{50} of A172, M059J, M059K, T98G, U373 and U-87 MG were 0.4436, 0.2979, 0.5224, 0.1333, 0.3048 and 0.3795 IU/ml (Fig. 1b), respectively. Prior studies in hepatocellular carcinoma (HCC) cell lines which were deemed sensitive to PEG-BCT-100 have shown similar EC_{50} values [2] and clinical trials of PEG-BCT-100 are underway.

To examine the occurrence of ASS1/OTC deficiency in GBM patients, immunohistochemistry (IHC) analyses of samples from 70 GBM patients were conducted. All samples were analyzed for OTC expression, and 28 of the 70 samples were randomly chosen for analysis of ASS1 expression. The performances of antibodies were affirmed using normal mouse liver (known to be ASS1 and OTC positive) in parallel experiment. None of the examined samples were ASS1 or OTC positive (Fig. 1c–e).

Discussion

A number of recent studies and our data suggest the possibility of arginine depletion based enzymotherapy as a potentially new treatment approach in GBM management. Khoury and colleagues [1] have previously shown that cytotoxicity of arginine starvation is less significant in normal glial cells as opposed to GBM cells [1]. Specifically, there have been no obvious neurological adverse events in prior clinical trials of PEG-BCT-100 in other disease indications. Sensitivity of cell lines however appeared to be independent of the expression level of urea cycle enzymes, namely ASS1

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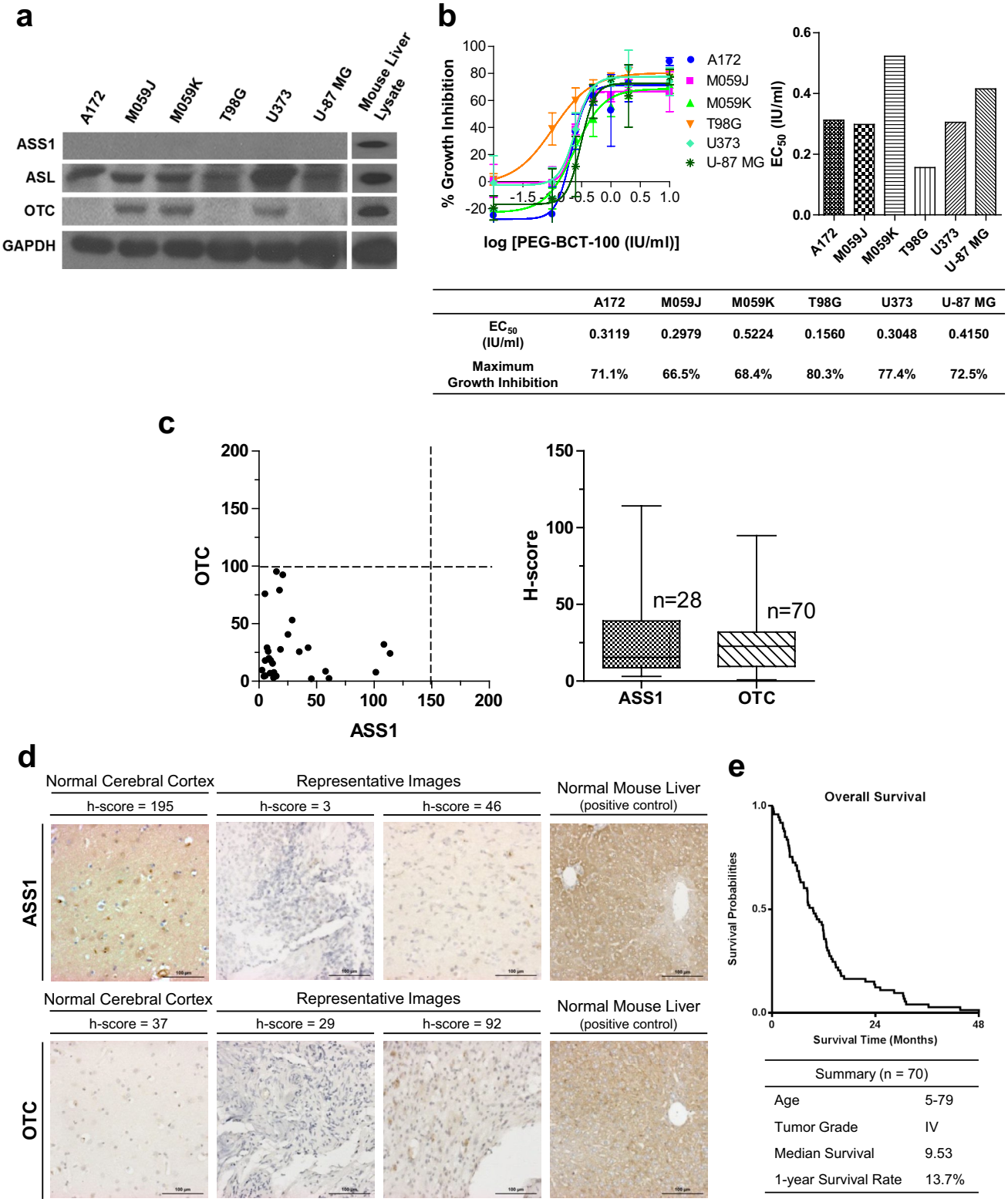

	A172	M059J	M059K	T98G	U373	U-87 MG
EC_{50} (IU/ml)	0.3119	0.2979	0.5224	0.1560	0.3048	0.4150
Maximum Growth Inhibition	71.1%	66.5%	68.4%	80.3%	77.4%	72.5%

Summary (n = 70)	
Age	5-79
Tumor Grade	IV
Median Survival	9.53
1-year Survival Rate	13.7%

Fig. 1 **a** Protein expressions of ASS1, ASL, OTC in GBM cell lines were detected by immunoblotting. All cell lines were ASS1 negative but ASL positive. **b** Representative dose response curve on GBM cell lines and their corresponding EC_{50} at 48 h of incubation of PEG-BCT-100. **c** H-score distribution of ASS1 and OTC expressions in 70 glioblastoma patients were detected by immunohistochemistry (IHC) and all GBM patients did not expressed or expressed very low levels of ASS1 and OTC. **d** IHC staining of patients' biopsy with different expression levels of ASS1 and OTC. **e** Overall survival and summary statistics of 70 glioblastoma patients

and OTC. Nevertheless, as the comparisons were made between cell lines, the difference in growth rates among them may also contribute to any discrepancy. Our data supports the potential of use of rhArg treatment in patients with tumors that have either ASS1 or OTC negativity.

Expression levels of ASS1 and OTC in adult GBM have not been reported previously. Only a few groups have examined the methylation profile and mRNA expression of ASS1 gene in primary GBM cultures, and the data were quite consistent with downregulation of ASS1 in half of the samples [3]. For that reason, we have examined the expression levels of ASS1 and OTC in GBM samples using immunohistochemistry. Either none or very low ASS1 and OTC levels were observed in all samples. As there were no samples, which were either ASS1 or OTC positive for us to use as a comparator, at present, no conclusion could be drawn from the ASS1/OTC expressions in relation to survival. Additional studies are required to validate the predominance of ASS1 and OTC deficiencies in GBM.

In conclusion, arginine deprivation through the use of a rhArg is a promising treatment option in GBM. Through our pre-clinical experiments, ASS1 and OTC appear to have the potential to be predictive markers for response. Also, on the grounds that arginine depletion takes place in the micro-environment, arginine-degrading enzymes need not penetrate the blood–brain barrier to in order to be active in intra-cerebral malignancies. This specific quality further increases the call for further investigations of rhArg in GBM.

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