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Specific activity of glycosidases in brain tumors and their expression in primary explants culture

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Abstract

Glycosidases such as β-galactosidase, β-glucosidase and β-hexosaminidase were assayed by monitoring the release of methyl umbelliferone spectro-flourimetrically in meningiomas (n=96), gliomas (n=101) and in their derived cell lines (n=17) and (n=10) respectively. Glycosidases showed significant specific activity in case of brain tumors as compared to normal brain. β-galactosidase activity (363.3907±83.68 nmoles/min./mg in meningiomas and 259.025±94.9137 nmoles/min/mg in gliomas) is less compared to β-Hexosaminidase (965±1192.2931 nmoles/min./mg in meningiomas and 3292.398±1251.171 nmoles/min./mg in gliomas) but β-glucosidase activity did not show significant changes in tumors to that of normal brain. The Glycosidases activity was also done in cell cultures of meningothelial meningioma, fibrous meningioma, transitional, A typical, anaplastic astrocytoma, anaplastic oligodendroglioma G-III and GBM IV which showed monolayers. The cell culture passages expressed more activity (in first) in majority of meningiomas and gliomas for β-galactosidase and β-hexosaminidase enzymes. However β-hexosaminidase showed higher activity in respective passages of all glioma cell cultures when compared with parent tumors, but not for β-glucosidase. Therefore, β-galactosidase and β-hexosaminidase exhibited similar activity in brain tumors and in their cell cultures. This confirms their identical enzymatic function in metabolism of tumors related to the stability in confluency of monolayer cells of primary explants.

Key words: β -Galactosidase, β-Glucosidase, β-Hexosaminidases, meningiomas, gliomas.

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Introduction

Glycosidases are hydrolytic enzymes responsible for catalyzing the hydrolysis of carbohydrates (degradation moieties of substrates), glycoconjugates such as mucopolysaccharides, glycolipids, glycosphingolipids and glycoproteins. Earlier reports suggested that cell glycoconjugates are associated with adhesion and migration in normal cellular function, metastasis and invasion in tumor cells (Hakomori 2002). The mechanism of cellular release of these hydrolytic enzymes probably involves tumor lysosomal exocytosis. Increased tumor glycosidase levels may promote increased tumor cell shedding from primary tumors, local invasion and perhaps be responsible directly or indirectly for structural changes in tumor cell surface glycoconjugates (Ralph 1985). In this paper, the activity of three glycosidases are discussed with refer to the types and grade of the tumors and in their derived cell culture.

The β-galactosidase (E.C.3.2.1.23.) is an extracellular glycoprotein and an eukaryotic hydrolase localized in the lysosome (Suzuki et al. 1995). It cleaves β-linked terminal galactosyl residues from a wide range of naturally occurring substrates, such as gangliosides, glycoproteins and glycosaminoglycans, as well as a number of artificial substrates. The SA-β-galactosidase assay on a variety of cells and tissues to demonstrate the onset of replicative senescence in culture (e.g. Reznikoff et al. 1996; Tsukamoto et al. 1998; Matsunaga et al. 1999) and in vivo (Sigal et al. 1999; Mishima et al. 1999). While the other glycosidase is β-Glucosidases (E.C. 3.2.1.21) are members of glycosyl hydrolase families 1 and 3 (Henrissat, B et al. 1997). This β-glucosidase was used to break amygdalin down to release cyanide, thereby killing the cancer cells and arresting tumor growth, leads to cell death (Newmark et al. 1981 and Kochi 1985).

In mammals, β-hexosaminidase (Hex, E.C. 3.2.1.52) is responsible for the hydrolysis of the acetylated hexose, *N*-acetylgalactosamine. Systematic alteration may indicate that β-galactosidase, acid phosphatase and hexosaminidase are the key enzymes, essential for the maintenance of the metabolic advantages that neoplasm possess in comparison with the normal tissues (Ramsey RB et al. 1980). The ratio of hexosaminidase activity to beta-glucuronidase activity was significantly lower for metastatic than for primary tumors or normal white matter. When histological observations do not clearly establish if a brain tumor is primary or metastatic, this ratio may help. Alteration of hydrolytic enzyme activities as demonstrated may be indicative of "ket enzymes" that are essential for maintaining the metabolic advantages of tumors.

These data gives the information for the cytochemical investigations in brain tumor research and for diagnosis. Current study also suggests about the importance of biochemical assays not only related to note the malignancy nature of the tumor cell types but also related to confluency and stability of the cells in the culture for these glycosidases activity. So the current investigation for glycosidases in brain tumors was done to find out the difference in specific activity among three glycosidases related to the type, grade of tumors and cell confluency in the cell culture.

Materials

Collection of brain tissue sample

Normal postmortem human brain tissue (n = 82) (Prabha M. et al. 2013) and the surgically removed human brain tumor specimens (n= 214) comprised the materials for the study. The normal specimens were collected from the department of Neuropathology, NIMHANS which has got national brain tissue repository wherein fresh brain tissue samples were obtained at autopsy after informed consent is available for research purposes. Brain tumor specimens comprising both meningiomas (n=96) and gliomas (n=101) of different types (supplementary material) were procured from Department of Neurosurgery, NIMHANS, Bangalore, India.

Tissue specimens were collected in a sterile condition after surgical removal at the operation theater, Dept. of neurosurgery and transported to lab within 30-60 minutes, immediately the specimens were kept in refrigerator (-20^{0}C) after dividing into two parts for the cell culture and for biochemical studies. The diagnosis of respective Brain tumor types was collected from Department of Neuropathology, NIMHANS.

Methods

Processing of tissue specimens

The weight of the respective brain tissue sample was noted. Brain tumor specimens were handled aseptically and divided into two portions which was used for biochemical studies and immediately for cell culture.

Preparation of tissue extraction from specimens for biochemical studies

Brain specimens, both normal postmortem brain tissue and human brain tumors, were cleared of the blood clot and meninges, if any and washed in saline. Specimens were subjected to homogenization in Evlenger Potter type glass homogenizer in tissue extraction buffer (TBS pH=7.4 containing 1% (v/v) triton-X 100, 0.5mM phenyl methyl sulphonyl fluoride in ethanol) with the tissue to buffer ratio of 1:31 w/v (wet weight). All the steps in extraction procedure was observed and denaturation was prevented by maintaining at low temp (5^o-10^{o}C) for homogenization, centrifugation with extraction buffer. Homogenates were centrifuged at 16,000 g for 15 minutes at 5^{o}C. The supernatants was collected for biochemical work.

Lowry's method

Protein estimation was done by using Bovine Serum Albumin as standard protein (1 mg/ml) by Lowry's method (Lowry et al. 1951; Hartree 1972) and total assay volume was 1.5 ml.

Precipitation of tissue extract

To 20 µl of tissue extract in a glass centrifuge tube, add 0.2 ml of ice-cold acetone and allow precipitating in icebox. Centrifuge at 10,000 rpm for 15 minutes at 4°C. Discard the supernatant and dissolve the precipitate with 125 µl of 0.01 N NaOH. The protein estimation was done by Lowry method by reading the absorbance at 660 nm wavelengths against appropriate blank without protein.

Enzyme Activity Glycosidase(s)

Enzyme activity of the glycosidases in tissue extracts were assayed by spectroflourometric methods. The following glycosidases namely β-galactosidase, β-glucosidase and β-hexosaminidase(s) were assayed by monitoring the release of product namely methyl umbelliferone spectro-flourimetrically at an excitation and emission wavelengths of 360 nm and 450 nm respectively, wherein a common standard curve derived from 4-methyl umbelliferone was employed to quantify the umbelliferone released. Specific activity was expressed as nanomoles of methyl umbelliferone hydrolyzed per minute per mg of protein.

Specific substrates of 4-methyl umbelliferone derivatives (Sigma, USA) were used as substrate(s) under defined assay conditions for specific enzymes such as 4-methyl umbelliferyl β-D-Galactoside (Sigma, USA) for β-Galactosidase (Falck B 1962), 4-methyl umbelliferyl β-D-Glucoside (Sigma, USA) for β- Glucosidase (Peters et al. 1976) and 4-methyl umbelliferyl N-acetyl-β-D-Glucosaminide (Sigma, USA) for β-Hexosaminidase (O'Brien et al. 1970; Leaback & Walker 1961) respectively.

Electrophoresis

Discontinuous Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) by Laemmli (1970) method was done for protein analysis.

Sample preparation

The protein sample was dissolved in SDS-sample buffer by boiling for one minute. Samples were allowed to cool for room temperature before they were loaded on the gel. The samples were electrophoreses at a constant voltage of 50 Volts for 10 min, followed by 150 Volts for 1 h (approximately).
The run was stopped when the marker dye reached 1–2 mm above the lower edge of the plate. The gel was carefully transferred to the staining solution with Coomassie Brilliant Blue R-250 at room temperature for 1 hr and de-stained with glacial acetic acid till the background became clear.

Cell culture

Primary culture (Manfred & Hildegard 1998).

The Brain tumor cells were derived from primary brain tumors tissues. The tissue suspension was transferred to petri dishes supplemented with addition volume of MEM containing FCS (Sigma, USA). Petri dishes were transferred to CO_2 incubator set at 37^{0}C. Media was changed after 3 days.

Subculture

The cells were rinsed with TVG and detached from the substratum with MEM containing 10 % (v/v) FCS. After the cells were detached, half the portion of cell suspension was transferred to new petri dishes for further growth then transferred to CO_2 incubator.

Remaining portion of the cell suspension was processed for biochemical studies. The Brain tumor cell lines were utilized up to passages of 1 and 7 in meningiomas and 1 to 4 in Gliomas respectively.

Extraction of enzymes from the cell culture (monolayer cells)

The cells were washed with phosphate buffered saline (1X PBS) pH=7.4 and the cells were kept in IX PBS for 1-2 minutes. The cells were detached and homogenized with tris buffered saline (TBS- 50 mM Tris HCl and 145 mM NaCl pH 7.4) with 1% triton X 100 (1:31w/v) and 0.5mM PMSF in ethanol for 2-3 minutes. Cells with TBS were centrifuged at 10,000 rpm for 30 minutes at 4^{o} C. The supernatant was collected and subjected for biochemical studies. Protein and enzyme assays in respective subsequent passages of cell culture were followed as per the earlier procedures for the parent brain tumors. Specific activity was expressed as nm of 4-methyl umbelliferone hydrolyzed per minute per mg of cellular protein.

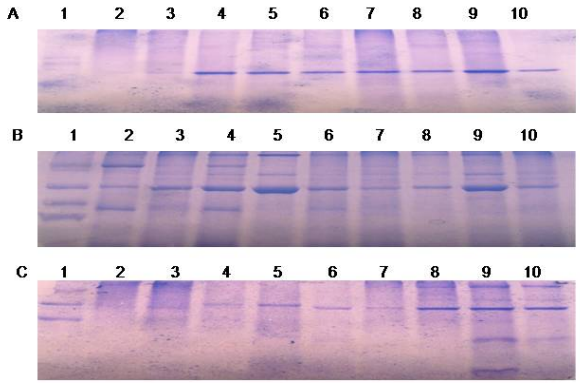

Figure 1: Protein Band patterns of (SDS-PAGE) Gel electrophoresis in normal human brain and brain tumors (M-Male, F-Female,y-year)
Gel A: 1. Marker; 2. Parietal (white); 3. Parietal (gray); 4. Atypical meningioma G-II tempo parietal 50y/M; 5. Meningothelial meningioma G-I sellar 45y/F; 6. Atypical meningioma WHO G-II left frontal parasagital 35y/M; 7. Transitional meningioma WHO G-I right frontal 36y/F; 8. Transitional meningioma olfactory groove 35y/F 9. Anaplastic oligodendroglioma G-III right frontal; 10. Meningothelial meningioma basifrontal 43y/M.
Gel B: 1. Marker; 2. Anaplastic gemistocyte astrocytoma G-III ® temporal parietal; 3. Atypical meningioma WHO G-II 52y/M; 4. Anaplastic oligodendroglioma G-III ® frontal; 5. Glioblastoma posterior frontal 29y/F; 6. Anaplastic oligodendroglioma 40y/M; 7. Glioblastoma with high mitotic activity (L) frontal 38y/M; 8. GBM G-IV parieto-temporal (L) frontal 56y/F; 9. Spindle cell sarcoma meningioma left temporal; 10. Medulloblastoma 5y/M.
Gel C: 1. Marker; 2. Frontal (white) 50y/M; 3. Frontal (gray) 50y/M; 4. Atypical meningioma left tempo parietal 48y/M; 5. Transitional meningioma olfactory groove 35y/F; 6. Meningothelial meningioma basifrontal 43y/M; 7. Malignant oligoastrocytoma G-III left inferior frontal and temporal 55y/M; 8. Diffuse anaplastic oligodendroglioma G-III 50y/M; 9. Metastatic carcinoma with necrosis left frontal probable sites gut, pancreas and prostrate 55y/M; 10. Ewing's sarcoma skull base 30y/F.

Statistical Analysis

The mean and standard deviation was calculated for three glycosidases with respect to different grade and types of brain tumors by using't' test calculator (Quick Calcs, Graphpad Software). The p-value was calculated for glycosidases in brain tumors by comparing with mean value of glycosidases of normal human brain (Prabha et al., 2013).

Results and Discussions

Brain tissue protein

The protein was estimated to all types of brain tumors and compared with normal human brain. The protein content was higher in brain tumors when compared to gray matter of normal human brain (Prabha et al., 2013). Meningiomas showed average of 40-50 mg protein and gliomas has got 50-60 mg/g of wet tissue respectively.

Gel doc images for coomassive blue stained protein band pattern

The protein band patterns of gel electrophoresis (SDS-PAGE) were done for both normal human brain and brain tumors. The tissue extracts of white and grey matter of frontal and parietal normal human brain showed light band of ~60 kD protein in Fig 1 gel A and C but due to different rate of flow, the bands showed little variation in their molecular weight in respective set of protein gels. The same protein (~60kD) was expressed more in case of almost all tumors in gel A but little less intense band in meningothelial meningioma basifrontal 43/M (10 th lane gel A). Another gel B showed very much intense band of ~60 kD protein in anaplastic oligodendroglioma G-III ® frontal (fourth lane), glioblastoma posterior frontal (gel.B fifth lane) and in spindle cell sarcoma meningioma left temporal (ninth lane of gel.B). Western blot was not done for these samples to identify the protein since there were so many samples to be analyzed. Therefore based on the marker, the protein molecular weight was considered and any standard marker of protein may be taken for this study. The protein bands is to show more intense protein in tumors and in that particular band of ~60 kd showed more intense band compared to normal brain protein.

The third set of protein Fig 1 gel C showed light intense band of ~100 kD and heavy intense band of ~60 kD in diffuse anaplastic oligodendroglioma G-III (eighth lane) and in ewing's sarcoma skull base (tenth lane). Metastatic carcinoma with necrosis left frontal probable sites gut, pancreas and prostrate (ninth lane) showed the same band with extra two light bands. The amounts of protein content in mg from spectrophotometric results were related to the intensity of band patterns in gel showing almost the same in all samples. Therefore still work has to be carried out with western blot to analyze the interesting unknown protein of MW 60 kD. By knowing total protein content the specific activity of enzymes can be calculated which varies in brain tumors.

Assay of glycosidases

The glycosidases assay was done using the supernatant of respective brain samples, which are measured with the fluorescence of the 4-methylumbelliferone freed by hydrolysis permitted the measurement of β--galactosidase, β-glucosidase, β-hexosaminidases (total hexosaminidase). Earlier reports for glycosidases were also determined in the supernatant of normal, benign, premalignant, and cancerous human tissue (De Martinez et al. 1984). In the present study, glycosidases are measured by their specific activity i.e., the total activity to the total amount of protein that is expressed in nm/min/mg of protein. Average hydrolytic enzyme activities of normal brain, meningiomas and gliomas are mentioned in Table 1. All glycosidases of meningiomas showed higher specific activity when compared to normal brain and gliomas, while gliomas showed higher activity for β-galactosidase and β-hexosaminidase than normal brain but not for β-glucosidase, infact it showed lower activity than normal brain. The activity of β-glucosidase was less in malignant tumors than normal brain because the enzymes have migrated through blood circulation since they are localized in

membranes of the cell and also there is utilization of more glucose by glial cells (Heinrich W et al. 1997), hence the glucosidase activity is less. Where as meningioma cells are benign in nature in which glycosidases are membrane bound glycoproteins, hence they are not released and migrated from the cell. Therefore it is one of the hypothesis that meningiomas has got higher glycosidase activity. The results of all three glycosidases (lysosomal enzymes) are discussed below one by one which are related to parent brain tumor types and respective derived passages of meningiomas and gliomas respectively (Table 1, 2A, 2B, 3A and 3B).

β-Galactosidase

Meningiomas

All meningiomas showed higher specific activity of β-galactosidase than normal brain (Table 1 and 2A). By conventional criteria, this difference is considered to be extremely statistically significant. Transitional meningioma showed highest activity of 465.729±75.5231 by 2.689 fold (The two-tailed P value is less than 0.0001), angiomatous meningioma has shown 220.541±93.272 nmoles/min./mg.

Table 1: Average Glycosidases activities of normal brain, Meningiomas and gliomas

Enzymes	β-Galactosidase	β-Glucosidase	β-Hexosaminidase
Normal brain (n)	(115)	(111)	(108)
Mean ± SD	173.16 ± 41.11	168.17 ± 30.16	2869.189 ± 440.26
Range	(120.28 – 250.21)	(134.48 – 234.62)	(2195.43 – 3818.09)
Meningiomas (n)	(49)	(54)	(53)
Mean ± SD	363.3907±83.68	208.26±38.3602	5872.965±1192.2931
Range	(220.54-465.72)	(154.2316-259.532)	(4258.56-8274.03)
Gliomas (n)	(55)	(50)	(58)
Mean ± SD	259.025±94.9137	108.1644±46.8743	3292.398±1251.171
Range	(145.325-382.868)	(33.619-178.6084)	(2037.15-4943.38)

n→Number of samples; Mean ± SD→ Mean and Standard Deviation; values are expressed in Specific activity→nm/min/mg of protein; Range→ Range of enzyme activity minimum to maximum (nmoles/min/mg)

The two-tailed P-value equals 0.0594 that was lower among meningiomas, but higher than normal brain 173.16±41.11 nmoles/min./mg of protein. By conventional criteria, this difference is considered to be not quite statistically significant. This may be due to the angiomatous meningioma morphologically different from other meningiomas by the variation in vascular arrangement and the features of the cells (Lee Y et al, 1991 and Burger 2002).

The β-galactosidase activity was higher in passages of eight meningioma cell lines, (Table 3A). While meningothelial right front parietal, transitional right frontal, transitional left tempo parietal, fibroblastic, meningioma right C.P.angle cell lines showed higher specific activity in respective parent tumor.

Gliomas

Majority of gliomas showed higher β-galactosidase activity. Anaplastic oligoastrocytoma G-III exhibited 382.868±110.1976 by 2.21 higher fold than normal brain. The two-tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant. Malignant oligoastrocytoma showed lowest activity of 145.325±13.21 nmoles/min./mg. The two-tailed P value equals 0.3426. By conventional criteria, this difference is considered to be not statistically significant. Glioblastoma multiforme GBM (n=17) have showed higher activity of 337.61±86.9318 nmoles/min./mg of protein by 1.94 fold than normal brain. The two-tailed P value is less than 0.0001.

Majority of the glioma cell lines have shown higher β-galactosidase activity in passages than their parent tumor, while ewing's sarcoma skull base metastasis has got increased enzyme activity in passage-3. However, Glioblastoma multiforme GBM progression from low to high-grade, anaplastic oligodendroglioma diffuse cortical spread, oligodendroglioma frontal showed lower activity in passages.

The present study has got similar results with earlier observation that β-galactosidase was also investigated in cell cultures of brain tumors where the activity was found to be increased, as with other hydrolases, irrespective of the type of tumor (Matakas 1969). Meningiomas cell lines showed highest enzyme activity than gliomas in both tumors and in their derived cell lines. The increased enzyme activity exhibited in passages-1 of the majority of meningiomas and gliomas than their respective parent tumors but it varies with other tumor cell lines (Table 3A and 3 B).

β-Glucosidase

Meningiomas

Among meningiomas, anaplastic meningioma showed highest activity of 259.532±77.35 nmoles/min./mg of protein by 1.543 higher fold. The two-tailed P value is less than 0.0001 and other meningiomas showed similar activity when compared to normal brain 168.17±30.16 nmoles/min./mg of protein and lowest activity in angiomatous meningioma of 154.23±62.24 nmoles/min./mg of protein. The two-tailed P value equals 0.444. By conventional criteria, this difference is considered to be not statistically significant (Table 2A).

Majority of the meningioma cell lines showed lower enzyme activity in passages with respect to parent tumor (Table 3A). Transitional meningioma G-I L tempoparietal parent tumor showed similar activity to that passage 1 to 3 enzyme activity.

Gliomas

All six types of gliomas (n=42) showed lower enzyme activity when compared to normal brain 168.17±30.16 nmoles/min./mg of protein (Table 2B). The malignant astrocytoma G–III showed lowest of 33.619 (±7.4) nmoles/min./mg of protein by 5.022 fold lower activity, the two-tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant. Where as 17 samples of GBM showed highest activity of 178.608±51.023 nmoles/min./mg of protein among gliomas and higher by 1.062 fold than normal brain. The two-tailed P value equals 0.2343. By conventional criteria, this difference is considered to be not statistically significant.

All glioma cell lines have higher specific activity in parent tumor than their passages except for anaplastic astrocytoma ® tempo parietal area passage-1, 2 which has got higher specific activity of 242.59, 212.46 than parent tumor 196.02 nmoles/min./mg of protein but this difference is not statistically significant (Table 3B).

This enzyme activity is not clear with refer to grade, types and confluency of the tumor cells. β-glucosidase (E.C. 3.2.1.21) that degrades glycosphingolipids that releases glucose. An earlier study says that astrocytes are able to build up the glycogen store, which is supposed to be the energy reserve for the central nervous system. Glycogen particles have also been found in glioma tumor cells, thus glycogen bound glucose molecules, as well as blood glucose, should serve as a substrate for glycolysis in both normal astrocytes and tumor cell related to glioblastoma multiforme with higher β-glucosidase activities which are in need of glucose (Heinrich Wiesinger et al. 1997), even though these results are true for our present study in GBM, for other tumors still the reason for lower β-glucosidase activity has yet be known.

Comparison of β-Galactosidase and β-Glucosidase in brain tumors

The present study of glycosidases in brain tumors suggest that each of these enzymes activity varies among the type of tumors to irrespective of their identification within the broad classification whether they are meningiomas or gliomas. Further, it rather difficult to decipher the precise role of these enzymes especially β-glucosidase, if any, with respect to the benign or metastatic nature of the tumor.

However both of these enzymes can be interpreted with refer to brain samples. Earlier studies of sweet almond emulsin and most other β-glucosidase preparations contain β-galactosidase, so they are relative in some properties (O.P. Malhotra and P.M. Dey 1967). The 115 samples of normal brain in 11 regions were shown 173.16 ± 41.11 nmoles/min./mg of β-galactosidase and 111 samples has got 168.17 ± 30.16 nmoles/min./mg of β-glucosidase (Prabha et al. 2013) which showed almost similar activity (Table 1). Even few brain tumor samples such as malignant oligoastrocytoma (n=2) has shown 145.325±13.21 of β-galactosidase and β-glucosidase has got 135.892±30.5123 and not much difference in activity of both enzymes (Table 2B). One more sample of atypical meningioma G-II tempoparietal shown 277.685 nmoles/min./mg of β-galactosidase and 263.305 nmoles/min./mg of β-glucosidase. GBM IV R frontal region has shown 258.016 nmoles/min./mg of β-galactosidase and 244.525 nmoles/min./mg of β-glucosidase. Among meningiomas, angiomatous meningioma showed lower activity for both β-galactosidase (220.541±93.272) and β-glucosidase (154.23±62.24) even though the specific activity varies.

Hofmann (1934a) opposes this; nothing that the differences between the ratio β-galactosidase and β-glucosidase in enzyme preparations from different sources are so great that one enzyme cannot be responsible for the hydrolysis of both types of glycosides. This is also true with our study of enzyme activity incase of many brain tumors especially in meningiomas, which also have shown significant difference among both enzyme activities. These results are quiet controversial. However β-galactosidase activity was significantly more compared to β-glucosidase for many brain tumors but not in normal brain. β-galactosidase has also showed higher activity in respective passages of cell culture which is similar to β-hexosaminidase especially in passage-1 however the specific activity was lower.

β-Hexosaminidase

Hexosaminidase activity were found to be elevated in most of the brain tumors when compared to normal brain (Table 2A, 2B and 3A, 3B).

Meningiomas

All eleven types of meningiomas (n=45) showed significant higher enzyme activity by 2 fold, in which anaplastic meningioma showed highest activity of 8274.03±1920.28 nmoles/min./mg of protein by 2.883 fold than normal brain 2869.189±440.26 nmoles/ min./mg of protein (Table 2A). All meningiomas showed the two-tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant.

All meningioma cell lines showed higher activity in passages when compared to parent tumor except two fibrous meningiomas (Table 3A). Fibrous MCF base passage-1 to 7 showed variation in the activity as compared to parent tumor of 3690.05 nmoles/min./mg of protein. Fibrous right parasagital passage-1, 2 were also showed lower activity of 2143.9 and 1926.38 when compared to parent tumor 6994.42 nmoles/min./mg of protein. However the reason behind this is yet to be known.

Gliomas

Among gliomas, malignant oligoastrocytoma G-III showed lowest of 2037.15±543.5641 nmoles/min./mg of protein than normal brain 2869.189±440.26. The two-tailed P value equals 0.0025. By conventional criteria, this difference is considered to be very statistically significant. GBM has got highest of 4943.38±1540.556 nmoles/min./mg of protein by 1.7229 fold than normal brain (Table 2B). The two-tailed P value is less than 0.0001. By conventional criteria, this difference is considered to be extremely statistically significant.

One of the most interesting result is that all the gliomas and metastasis showed higher enzyme activity in passages particularly in the first with respect to parent tumor later decreases with subsequent passages but not in metastasis of passage-3 (Table 3B). Glioblastoma multiforme ® frontal passage-1, 2 have higher specific activity of 5821.23 and 7874.85 than parent tumor 4054.06 nmoles/min./mg of protein. So the increased activity and growth of cells was also maintained constantly from parent tumor to passage-1 and 2. Ewing's sarcoma skull base passage-1, 2, 3 were also showed higher specific activity of 5255.37, 4186.23, and 5554.46 respectively when compared to parent tumor 3359.43 nmoles/min./mg of protein. It may be due to mitotic potential of tumors and/or of their adaptability to the growth medium and culture environment.

β-Hexosaminidase (EC 3.2.1.52) showed higher activity for all meningiomas and majority of gliomas except anaplastic astrocytoma and malignant oligoastrocytoma that showed lower enzyme activity when compared to normal brain. This is also similar to earlier studies of parent tumors that showed total activities of hexosaminidase, β-glucuronidase and β-galactosidase which were significantly higher in tumors than in normal cerebral white matter. Hexosaminidase was also found to be increased in human gliomas (Ramsey RB et al.1980). It also increased when an early neoplastic changes in CNS tissue were apparent however no reports for cell culture.

In cell culture, the present observation of β-hexosaminidase showed higher activity in all meningioma passages, particularly in passage-1 but not in fibrous meningiomas of both MCF base and right parasagital, in which the enzyme activity was more in parent tumor. The tumor cell culture of all meningiomas including anaplastic and also gliomas such as GBM, malignant astrocytoma G-IV showed higher levels of enzyme specific activities may be due to more number of cells in specified area. It is very important to note that all

passages of gliomas showed higher enzyme activity particularly in the first passages than parent tumor and normal brain. This shows that the β-hexosaminidase is membrane bound enzyme, hence it is less in parent tumor because it has migrated through blood circulation.

meningioma cell lines (except fibrous meningioma) and all glioma derived cell lines showed higher β-hexosaminidase activity. Even majority of meningioma and glioma derived cell lines also showed higher β-galactosidase activity in respective passages of tumor cell culture. Apart from these results of glycosidases in cell culture, one

Table 2A Specific activity of Glycosidases in different grades of Meningiomas

Brain Samples	β-Galactosidase	β-Glucosidase	β-Hexosaminidase
Normal brain (n)	115	111	108
Mean ±SD	173.16±41.11	168.17±30.16	2869.189±440.26
Range	120.28 – 250.21	134.48 – 234.62	2195.43 – 3818.09
Meningiomas: Meningiomas G-I			
Fibrous (n)	13	15	13
Mean±SD	431.219±56.203	219.9±69.4718	6351.41±1497.67
Range	335.677-529.948	148.847-346.045	4083.08-8319.865
Transitional (n)	7	8	9
Mean±SD	465.729±75.5231	224.88±84.714	6166.43±957.2818
Range	327.085-544.945	135.45-381.73	5036.345-8093.018
Microcystic (n)	2	3	2
Mean±SD	421.992±71.3725	225.334±83.045	6145.036±201.8383
Range	371.524-472.46	157.28-317.86	6002.315-6287.757
Meningothelial (n)	6	7	8
Mean±SD	273.65±62.8979	182.05±35.29	5148.565±1087.701
Range	220.41-383.050	130.34-211.47	3209.645-7085.13
Angiomatous (n)	3	3	3
Mean±SD	220.541±93.272	154.23±62.24	5483.869±1310.685
Range	127.65-314.195	83.08-198.60	4363.2871-6925.136
Secretory (n)	2	2	2
Mean±SD	355.54±65.2977	159.897±57.32	4258.56±1053.065
Range	309.37-401.715	119.36-200.43	3513.93-5003.19
Meningiomas G-II			
A typical (n)	11	13	13
Mean±SD	338.209±96.393	240.25±68.003	5155.81±1457.73
Range	230.505-474.810	159.52-366.70	3300.3-7621.37
MeningiomasG-III			
Anaplastic (n)	5	3	3
Mean±SD	400.24±105.359	259.532±77.35	8274.03±1920.28
Range	243.93-538.0675	171.159-314.95	6068.86-9577.63

Table 2B: Specific activity of Glycosidases in Gliomas

Brain Samples	β-Galactosidase	β-Glucosidase	β-Hexosaminidase
Normal brain (n)	115	111	108
Mean±SD	173.16±41.11	168.17±30.16	2869.189±440.26
Range	120.28 – 250.21	134.48 – 234.62	2195.43 – 3818.09
Gliomas			
Gliomas G-III			
Anaplastic Astroocytoma (n)	13	10	13
Mean±SD	146.043±41.11	71.75±17.989	2050.23±589.578
Range	110.81-217.48	52.515-108.81	1030.4-3196.85)
Anaplastic Oligodendroglioma(n)	11	9	10
Mean ±SD	281.002±68.52	130.60±33.6821	3482.27±1024.03
Range	183.02—381.04	89.81—180.18	2578.97—5701.89
Malignant Astrocytoma G-III (n)	6	5	6
Mean±SD	203.77±44.19	33.619±7.4053	2290.80±540.027
Range	165.12—269.66	24.914—42.685	1770.51—3300.56
Malignant Oligoastrocytoma (n)	2	2	3
Mean±SD	145.325±13.21	135.892±30.5123	2037.15±543.5641
Range	135.98—154.67	114.31—157.46	1481.75—2568.05
Anaplastic Oligoastrocytoma (n)	4	5	5
Mean±SD	382.868±110.1976	100.25±9.587	3368.382±510.58
Range	247.97—-481.845	86.324—112.025	2693.006-3905.133
Gliomas G-IV			
Malignant Astrocytoma G-IV (n)	2	2	2
Mean±SD	316.55±103.177	106.41±16.23	4874.54±1239.356
Range	243.6—389.515	94.935—117.89	3998.19—5750.905
Glioblastoma /(GBM)			
(n)	17	17	19
Mean ±SD	337.61±86.9318	178.608±51.023	4943.38±1540.556
Range	233.641—510.3	127.89—290.545	2887.295--7989.29

n→Number of samples; Mean±SD→ Mean and standard deviation of enzyme activity expressed in 'nmoles/min/mg'; Range→ Range of enzyme activity minimum to maximum (nmoles/min/mg)

more observation is necessary to mention that since β-galactosidase is well-known marker for cell senescence in culture, (Devarakonda et al. 1999 and David J Kurtz et al. 2000) β-hexosaminidase assay can also be used as cell senescence marker with advanced standardization of biochemical and molecular biology studies.

Comparison of β-Galactosidase and β-Hexosaminidase in brain tumors

Earlier studies of the total enzyme activities for hexosaminidase and β--galactosidase were determined in the supernatant of both

Table 3 A Specific activity of Glycosidases in respective passages of Meningioma cell lines

Sl No	Brain tumors	Age Pa tu Passages	Protein in mg	β-Galactosidase	β-Glucosidase	β-Hexosaminidase
	Meningiomas G- I					
1	Meningothelial menin gioma parasagital posterior frontal	61y/M	1.8248	383.05	158.38	8562.18
		Pass-1	0.724	722.44	56.264	27034.1
		Pass-2	0.5247	454.95	146.56	17627.1
		Pass-5	0.3408			
2	Meningothelial meningioma WHO G-I olfactory groove	65y/F	1.244	240.12	209.55	5196.15
		Pass-1	0.959	730.95	420.72	12307.2
		Pass-2	0.449	427.37	112.62	9740.64
		Pass-3	0.8599			
		Pass-5	0.779			
3	Meningothelial meningioma basifrontal	43y/M	0.877	60.184	211.47	988.701
		Pass-1	0.605	853.61	208.27	4267.53
		Pass-2	0.6555	536.68	283.69	3993.82
		Pass-3	0.57	121.03	111.39	1879.06
		Pass-4	0.658	355.77	268.6	2841.21
4	Meningothelial meningioma G-I R fronto parietal	M	1.972	248.3	130.34	5316.27
		Pass-1	0.865	79.488	48.53	14026.1
		Pass-2	0.755	90.215	44.83	7224.65
5	Fibrous menin gioma G-I MCF base	30y/F	1.843	166.83	114.56	3690.05
		Pass-1	1.77	344.62	279.58	2156.9
		Pass-2	0.981	249.58	194.68	2225.31
		Pass-3	0.732	780.44	516.17	5863.05
		Pass-4	0.7149	370.48	361.07	5129.88
		Pass-5	0.525	626.29	410.17	5632.63
		Pass-6	0.5136	208.74	209.69	3857.78
		Pass-7	0.4819	162.91	160.41	2985.75
6	Fibrous meningioma R parasagital	45y/F	1.617	335.67	424.3	6994.42
		Pass-1	1.613	361	152	2143.9
		Pass-2	0.439			1926.38
7	Transitional menigioma G-I R frontal	36y/F	0.9595	266.53	325.44	8093.02
		Pass-1	1.3635	195.78	14.292	11074.2
		Pass-2	0.7715	616.58	74.501	10409.6
		Pass-3	0.4069	415.94	424.96	30518.6
		Pass-4	0.4421	751.82	238.11	8016.37
		Pass-5	0.3335	339.76	343.33	8491.68
		Pass-6	0.464	574.87	228.1	
8	Transitional meningioma G-I L tempoparietal	20y/M	1.87	327.09	182.95	6259.5
		Pass-1	0.9	255.96	182.31	20497.5
		Pass-2	0.565	86.632	180.35	12197.4
		Pass-3	0.26	290.79	171.14	10909.1
		Pass-4	0.195	207.14	64.135	6162.83
9	Fibroblastic meningioma G-I	45y/F	1.886	464.41	270.73	8260.71
		pass-1	0.7575	321.58	134.48	11894.6
10	Meningioma R C P angle	F	2.068	137.34	102.17	4341.57
		Pass-1	0.8705	49.013	89.35	5922.62
		Pass-2	0.472	37.13	215.28	8032.26
		Pass-3	0.4083	28.805	149.21	5338.99
	Meningiomas G-II					
11	A Typical transitional	26y/M	1.535	238.02	366.71	4589.32
		Pass-1	0.745	1010.8	414.14	25207.8
12	A Typical men ingioma G-II post L frontal/ fibroblastic men G-I post frontal	42y/F	1.7677	453.37	170.04	4041.61
		Pass-1	0.554	1267.7	275.6	21938.2
		Pass-2	0.729	820.56	241.76	14468.3
		Pass-3	0.4865			
13	Atypical meningioma G-II tempoparietal	50y/M	1.77	277.69	263.31	5903.8
		Pass-1	0.6106	174.17	198.89	9799.38
		Pass-2	0.3025	681.04	42.635	10426.9
		Pass-3	0.4812	770.89	120.53	7907.3
		Pass-4	0.268	477.68	156.36	7103.97
		Pass-5	0.269	538	315.56	6640.63
14	Meningioma G-III Anaplastic meningioma L recurrent frontal parasagital	60y/M	1.8147	426.59	314.95	4996.46
		Pass-1	0.6604			

Pa tu→Parent tumor; Pass→Passages; M→Male; F→Female; mg→milligram; values are expressed in Specific activity→nm/min/mg of protein; y→years

Table 3B Specific activity of Glycosidases in respective passages of Glioma cell lines

Sl No	Brain tumors	Age/gender Pa tu passages	Protein in mg	β-Galactosidase	β-Glucosidase	β-Hexosaminidase
	Gliomas G-II					
1	Oligodendroglioma G-II frontal	60y/F Pass-1 Pass-3	0.851 0.8 0.42	135.98 389.73 278.16	295.79 76.095 116.14	2568.05 11287.3 8125.52
	Gliomas G-III					
2	Protoplasmic astrocytoma G-II to G-III	27y/F Pass-1	0.861 0.4405	126.96 270.22	148.68 46.819	3966.22 6090.35
3	Anaplastic astrocytoma G-III R tempoparietal	F Pass-2 Pass-3	1.38 0.597 0.399	119.65 324.09 313.78	196.02 242.59 212.46	5561.15 10153.2 10989.9
4	Anaplastic astrocytoma gemistocystic corpus collusum	40y/M p-1	2.125 0.92			
5	Anaplastic oligodendroglioma	32y/M Pass-1 Pass-2 Pass-4 Pass-5	1.589 0.4085 2.081 0.7606 0.704	381.04 215.43 14.594	105.43 48.195	2372.02 9328.71 480.99
6	Anaplastic oligodendro glioma G-III R frontal	40y/M Pass-1 Pass-2	1.1951 0.377 0.406	272.31 673.31 421.9	218.17 70.235 66.192	4047.87 10039.2 8432.58
	Gliomas G-IV					
7	GBM IV R Frontal	34y/M Pass-1 Pass-2	1.6214 0.5449 0.3505	258.02 305.09 207.67	244.53 23.311 34.255	4054.06 5821.23 7874.85
8	GBM IV Corpus collusum	33y/M Pass-2 Pass-3 Pass-4	1.5117 0.453 0.5615 0.293	236.28	127.95	2990.6
9	GBM progression from low to high grade	50y/F pass-2	2.3195 0.525	428.98 234.51	433.45 322.29	6962.88 8477.52
10	Metastasis Ewings sarcoma skull base	30y/F Pass-1 Pass-2 Pass-3	1.274 0.432 0.2875 0.363	380.37 9.9345 45.954 247.79	431.57 184.24 56.16 299.98	3359.43 5255.37 4186.23 5554.46

Pa tu→Parent tumor; Pass→Passages; M→Male; F→Female; mg→milligram; values are expressed in Specific activity→nm/min/mg of protein; y→years

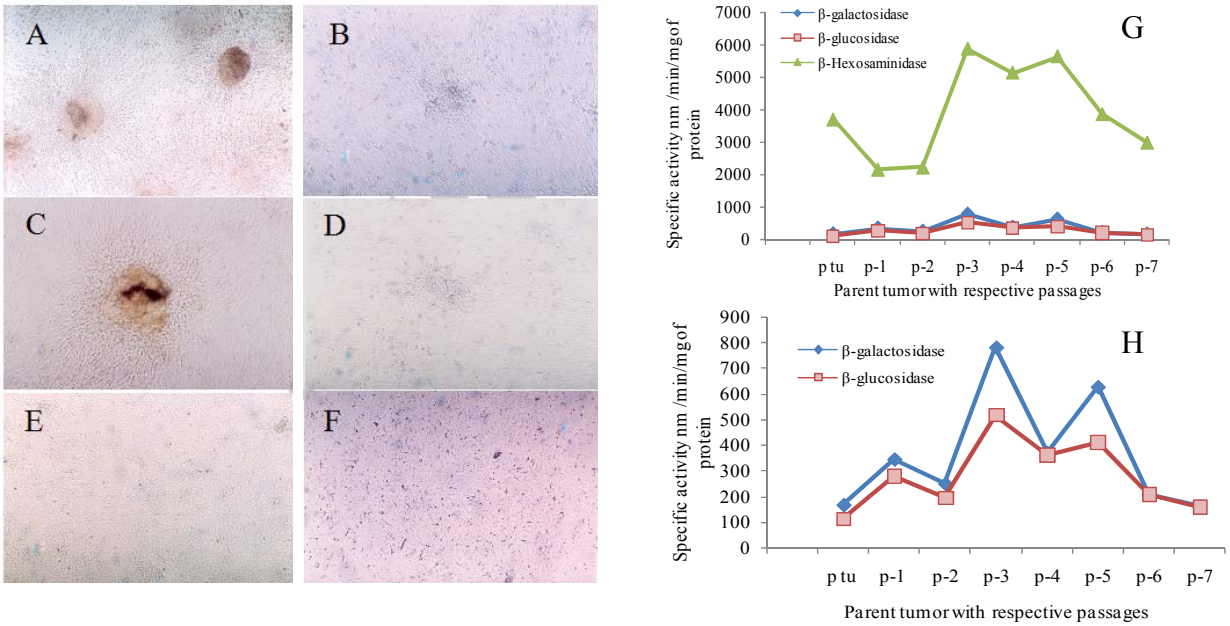

Figure 2: Fibrous meningioma MCF base Grade-I with panels of different passage monolayers **A.** Primary explant monolayer (4X), **B.** Passage-1 (4X) **C.** Passage-2 (10X), **D**. Passage-3 (4X), **E.** Passage-4 (4X), **F.**Passage-5 (4X) and their expression of glycosidases **G.** β-Hexosaminidase activity **H.** β-galactosidase and β-glucosidase with respective seven passages.

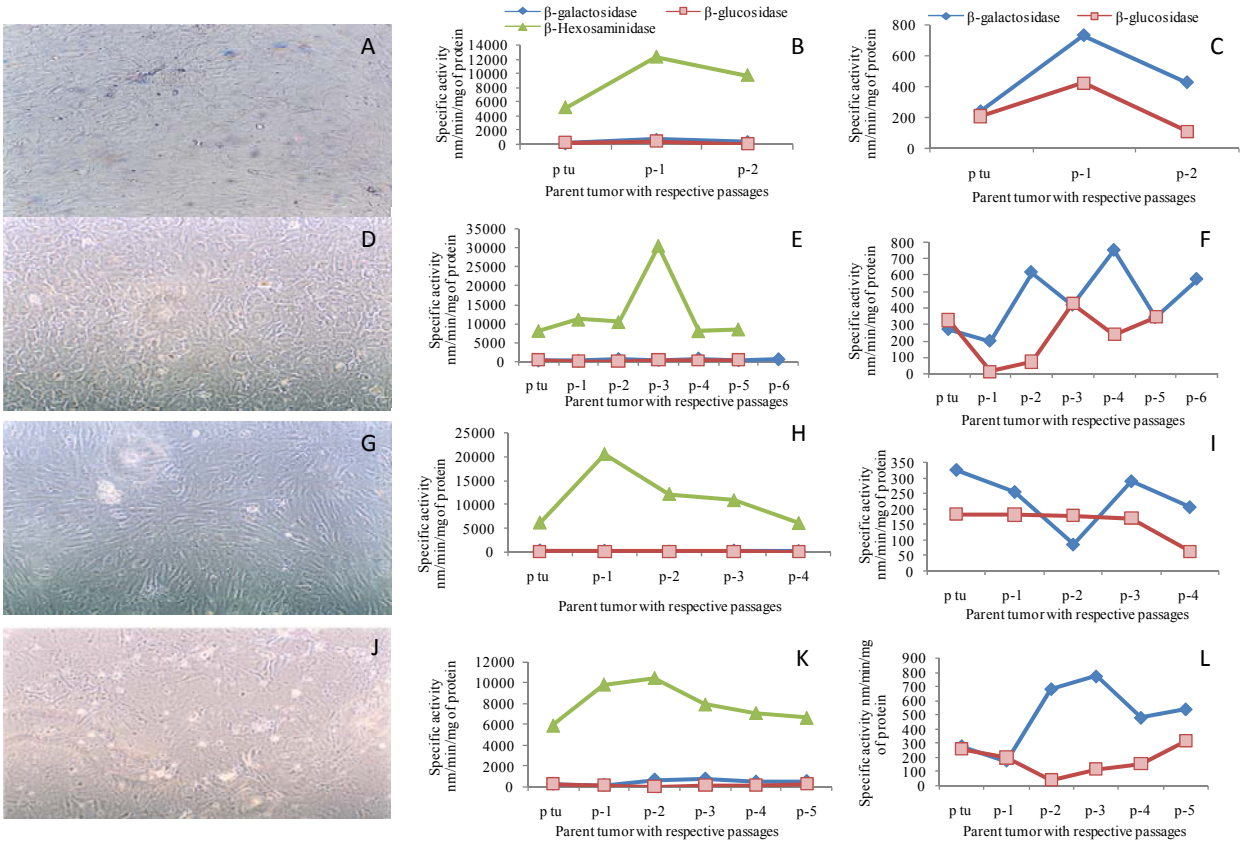

Figure 3: Panel A,D,G,J →Meningioma tumor monolayers of respective passage A-> Meningothelial meningioma G-I olfactory groove Monolayer of 13 days primary explant (10X) D-> Transitional meningioma G-I ® frontal of passage –3 (10 X) G-> Transitional meningioma G-I (L) tempo parietal of Passage –3 (10 X) J-> Atypical meningioma G-II tempo parietal of passage-5 (10 X) with expression of Glycosidases B,E,H,K → β-Hexosaminidase activity C,F,I,L→ β-galactosidase and β-glucosidase activity with respective passages. The legends of E, H, K and F, I, L are same as B and C respectively.

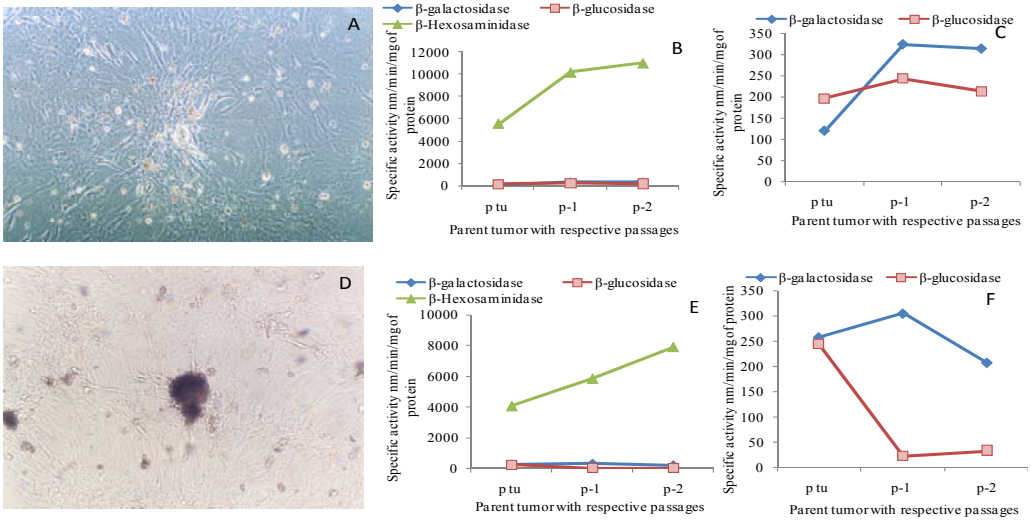

Figure 4: Panel A, D→Glioma tumor monolayers of respective passage A->Anaplastic Astrocytoma G-III ® tempo parietal of Passage-1 (10 X), D-> Glioblastoma multiforme WHO G-IV right frontal 17 days primary culture (10X)with their panels B, E→ Hexosaminidase activity, panels C, F→β-Galactosidase and β-Glucosidase activity of respective passages.

malignant tumors and premalignant lesions, which were significantly greater than normal tissues (De Martinez NR et al. 1999) but not in cell culture. So our study also showed that the elevated activity of β-hexosaminidase and β-galactosidase in all meningiomas and in majority of gliomas, while in cell culture all meningioma cell lines (except fibrous meningioma) and all glioma derived cell lines showed higher β-hexosaminidase activity. Even majority of meningioma and glioma derived cell lines also showed higher β-galactosidase activity in respective passages of tumor cell culture. Apart from these results of glycosidases in cell culture, one more observation is necessary to mention that since β-galactosidase is well-known marker for cell senescence in culture, (Devarakonda et al. 1999 and David J Kurtz et al. 2000) β-hexosaminidase assay can also be used as cell senescence marker with advanced standardization of biochemical and molecular biology studies.

The relation of enzyme activity with Brain tumor monolayer Meningiomas

It is already known that among glycosidases, β–hexosaminidase (Table 2A, 3A, Fig 2, 3) has got higher activity as compared to other two glycosidases, so the β-galactosidase and β-glucosidase activity was put in the graph (Fig 2, 3) to show the units of enzyme activities. Fibrous meningioma MCF base G-I monolayers of primary explant and five different passages were shown in the Figure 2 (Panel A, B, C, D, E, F). The primary explant of fibrous meningioma after 20 days, which showed the monolayer with dark dotted round cells. In other passages the longitudinal cells which are dispersed radially under the inverted light microscope images. In graph X-axis showed parent tumor with respective passages and Y-axis showed respective enzyme activity. However error cannot be shown since many culture of the same tumor is very difficult. Therefore the tumor activity are done with precious primary explants and need to be compared with its parent tumor enzyme activity so error is not necessary to show here.

Other meningiomas were shown in single figure with their graph for an enzyme activity in respective passages. Meningothelial meningioma G-I olfactory groove showed whorls, evenly distributed cells of monolayer that appears as an established cell lines (Fig 3:panel A) with very compact layer of cells that dispersed from tissue bit completely, which gave rise to longitudinal cells. cells in passage -3 and other transitional meningioma G-I (L) tempo parietal passage-3 showed longitudinal cells (Fig 3: panel D, G). Atypical meningioma G-II tempo parietal showed clear and evenly distributed spindle shaped cells with nucleoli (Fig 3: panel J). Again β-hexosaminidase has got highest activity in passage-1 (Table 3A, Fig 3: panel B, E, H, K) as compared to other two glycosidases, so the β-galactosidase and β-glucosidase activity was shown in the next graph (Table 3A, Fig 3: panel C, F, I and L).

Gliomas

Two gliomas with their respective monolayers having glycosidase activity were shown in Fig 4 and Table 3B. The monolayer of anaplastic astrocytoma G-III ® tempo parietal passage-1 showed cell sheath along with bunch of cells that are scattered with yellowish round bright spots (stem cell features) on the compact layer of cells with bundle of sheath at the centre (Fig 4: panel A). It showed higher activity in passages for all three glycosidases especially in passage-1. The monolayer of GBM WHO G-IV right frontal primary culture showed more compactly arranged cells, overlapping with bunch of cells from which bright cells (stem cell like features) were dispersing from the tissue with increased cellularity and sheet like growth (Fig 4:panel D). Both gliomas showed highest β-hexosaminidase activity in respective passage-1 and 2 (Fig 4: panel B, E), Anaplastic astrocytoma G-III ® tempo parietal showed higher β-galactosidase and β-glucosidase activity in passage-1 (Fig 4: panel C) however GBM IV R frontal showed significantly lower β-glucosidase activity in passage-2. (Fig 4: panel F). The increase of glycosidases in passage-1 may be due to the increased cellular density and mitotic potential of the cells in the culture.

These reports suggests the chance of solving the tumor problem related to cell surface, malignancy and catabolic activity in the future which is to be provided by the great theoretical advances in molecular biochemistry and the application of biotechnology.

The final outcome of the present study for glycosidases in brain tumors are interrelated to one another with respect to their specific activity and the type of the tumors. β-Hexosaminidase showed significantly very much higher activities in all menigiomas, majority of the gliomas and respective passages of meningiomas and all glioma cell lines (which proves membrane bound enzyme), when compared to β-galactosidase and β-glucosidase in tumors. However β-glucosidase and β-hexosaminidase has got higher activity only in anaplastic meningioma among meningiomas and in GBM among gliomas, but not in the case of cell culture. But β-galactosidase and β-hexosaminidase showed higher activity in many brain tumors than normal brain and in their respective passages of many meningiomas and glioma derived cell lines when compared to parent tumors. Such a findings has not been hitherto reported in human brain tumor tissue samples.

Therefore this is the first report of β-galactosidase and β-hexosaminidase, which exhibited similar in function related to the behavior of tumor cells, even though the activity of β-hexosaminidase was significantly higher than β-galactosidase in brain tumors and in their cell cultures of meningiomas and gliomas, especially in passage-1 for both enzymes, that confirms their identical enzymatic function in catabolism of tumors (degradation of glycoconjugates) related to stability in confluency of monolayer cells in primary explants.
If glycosidases are localized in the membranes' of the neuronal cell which helps in the degradation of complex lipids such as—sphingolipids, gangliosides etc., where there is defence activity against transformed cells and this is one of the factor in the case of neurodegenerative diseases if glycosidase are localized sufficiently, there is no chance of plaque formation which leads to neuronal loss.

In future glycosidase enzyme transplant with stem cells can be done for the treatment of brain tumors and with application of rDNA technology for lysosomal protein gene that may be modified and transfected to the cells to improve the treatment for brain tumor patients.

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SUPPLEMENTARY DATA

Table A: Brain tumor samples list- Meningiomas

Sl No	Brain tumors	Sl no/age /Neuronal Number , Neuro pathology number /year	
	Meningiomas G-I	**Male**	**Female**
1	Fibrous meningioma	1)49y,331304,243/02,2)45y,351935,14/033)55y, 362754, 1083/03, 4)47y, 373457,51/04, 5)65y, 368016, 1489/03	1) 42y, 348167,1598/02, 2) 36y, 345112,1284/02 3)73y, 776/02, 4)68y, 340374,1026/02 , 5)37y, 341753,1031/02, 6)55, 1379/02, 7)50y, 1813/02 , 8) 56y, 346052, 1396/02,9)45y, 369383383, 1579/03, 10)44y, 368208,1519/03, 11)45y, 367626, 1453/03, 12)30y, 371443, 1819/03, 13) 42y, 357929, 1294/03, 14) 45y, 360827, 993/03
2	Transitional meningioma	1)44y,347275,1453/02, 2)38y,340070, 873/02, 3)20y, 374821, 123/04	1)72y, 346444, 1645/02, 2)35y, 314589,315/03, 3)27y, 327893, 1565/01, 4)55y, 345120, 1460/02, 5)35y, 358303,914/03, 6)60y, 368806,1549/03, 7)60y, 369743, 1659/03, 8)36y, 212046, 226/04, 9)45y, 372184,341/04, 10)35y, 762/02
3	Microcystic meningioma	1)45y, 242855, 41/04	1) 40y,352686, 84/03 ,2) 55y, 358222, 600/03, 3) 52y, 365824, 1604/03
4	Meningothelial meningioma	1) 52y, 95/03, 2)35y, 349226, 771/03, 3) 61y, 361755, 941/03, 4)40y, 366437, 1363/03, 5) 43y, 371128, 1747/03, 6) 363917, 132/04	1)45y, 353318, 226/03, 2)45y, 928/03, 3)45y, 534/03, 4) 65y, 358286, 1213/03, 5)45y, 367764, 12/04
5	Angiomatous meningioma	1)47y, 347134, 1451/02, 2)42y, 355139, 507/03, 3)29y, 360445, 877/03	1) 58y, 361983, 992/03,
6	Secretory meningioma	1) 65y, 355877, 465/03,	1) 39y, 353334, 193/02, 2) 32y, 364106, 1184/03
	Meningiomas G-II		
7	Atypical meningioma	1)50y,339194, 842/02, 2)74y, 341303, 1015/02, 3)35y, 358719, 905/03, 4)62y, 368989, 1540/03, 5)50y, 374952, 189/04, 6)26y, 376814, 398/04, 7)46y, 361141, 973/03,	1) 40y, 344551, 1265/02, 2)48y, 358958, 714/03, 3)32y, 356906, 518/03, 4)53y, 361098, 985/03, 5)28y, 362158, 968/03, 6)65y, 368401, 1496/03, 7) 42y, 357929, 1294/03, 8)32y, 367110, 1438/03, 9)60y, 369203, 1605/03
	Meningiomas G-III		
8	Anaplastic meningioma	1) 46y, 298786, 475/03, 2)60y, 366881, 1424/03, 3)40y, 369788, 1632/03, 4)75y, 352027, 1697/03	1)55y, 347739, 1533/02,
	Total	**Meningiomas=30M**	**Meningiomas = 45F**

Table B: Brain tumor samples list- Gliomas and other tumors

Sl No	Brain tumors	Sl no/age /Neuronal No, Neuro pathology no/ year	
	Gliomas	**Male**	**Female**
	Gliomas G-I		
1	Low grade glioma	1) 26y, 319710, 962/01, 2) 17y, 305311, /02	
	Gliomas G-II		
2	Oligodendro glioma		1)60y, 377038, 345/04
	Gliomas G-III		
3	Anaplastic Astrocytoma	1)29y, 3447115, 1513/02, 2)32y, 347541, 1471/02, 3)23y, 349329, 1631/02, 4)32y, 354017, 212/02, 5)29y, 341142, 926/02, 6)35y, 349232, 161/02, 7)25y, 358626, 696/03, 8)26y, 358929, 689/03, 9)73y, 356652, 472/03, 10)50y, 361279, 894/03, 11)40y, 364059, 1234/03, 12)45y, 368873, 1564/03, 13)32y, 367863, 1529/03, 14)56y, 364669, 1167/03	1)30y, 345054, 1270/02, 2)23y, 345978, 1487/02, 3)53y, 367375, 1449/03, 4)27y, 377070, 352/04, 5)45y, 377286, 343/04, 6)27y, 358500, 660/03
4	Anaplastic Oligoastrocytoma	1) 29y, 328200, 1570/01,2)36y, 312571,/01	1) 25y, 321485, 1083/01, 2)24y, 336201, 20/03, 3)27y, 358500, 660/03, 4)30y, 323945, 541/03
5	Anaplastic Oligodendro glioma	1) 30y, 343283, 1171/02, 2)53y, 1444/01, 3)53y, 1444/01 , 4) 35y, 344363,1271/02, 5)32y, 1746/02, 6)32y, 363348, 1107/03, 7)40y, 363421, 1138/03, 8)369530, 1597 / 03, 9) 50y, 373974, 58/04, 10)40y, 371363, 1734/03, 11)45y, 349060, 1596/02	1) 50y, 340488,869/02, 2) 40y, 355633, 393/03, 3) 35y, 364859, 1255/03
6	Malignant Astrocytoma	1) 29y, 287075, 986/01, 2)24y, 337407, 656 /02, 3)30y, 314881, 1031/01, 4)50y, 328569, 1593/01, 5)32y, 307091, 1138/2000	1) 36y, 322864, 1187/01, 2)49y, 1000/01
7	Malignant Oligoastrocytoma	1) 30y, 328237, 1562/01, 2)55y, 317168, 736/01, 3)49y, 359456, 779/03	
	Gliomas G-IV		
8	Malignant Astrocytoma	1) 52y, 347888, 1548/02, 2)61y, 303923, 1008/01	1) 60y, 327375, 1487/01
9	Glioblastoma/ GBM	1) 50y, 354113, 236/03, 2)38y, 323743, 1463/01, 3) 45y, 342955, 1199/02, 4)80y, 349124, 1695/02, 5)35y, 328688,/01, 6)53y, 556200, 435/03, 7)50y, 358135, 602/03, 8) 48y, 355689, 395/03, 9)45y, 269891, 537/03, 10)34, 364709, 1174/03, 11)62y, 329859, 74/02, 12)33y, 364174, 1168/03, 13)35y, 360166, 802/03, 14)40y, 359474, 900/03,	1)46y, 339656, 837/02, 2)48y, 345629, 1535/02, 3)28y, 318224, 845/01, 4)55y, 348899, 1585/02, 5)65y, 324652,/ 01 , 6)30y, 343414, 1172/02, 7) 60y, 323707, 1270/01, 8)60y, 329794, 47/02, 9)52y, 327898,1537/01, 10)56y, 363545, 1071/03, 11)40y, 328421, 1575/01, 12)50y, 368661, 396/04
	Total	**Gliomas=53 M**	**Gliomas=29F**
	Other tumors		
1	Metastasis	1) 40y, 357338, 540/03, 2)305875/20003)20, 352194,55/03, 4)55y/M, 370302, 1728/03	1) 41y, 365320, 1254/03, 2)30y, 375219, 190/04, 3)50y, 340579, 935/02
2	Medulloblastoma	1)5y, 306473, 1089/2000 2)10y	

Abbreviations---Sl No→Serial Number; Note—Above table refer to the different types of Brain tumors that has taken from the respective patients - According to age, gender, neuronal number, neuropathology number and year is given to know if interesting results were obtained, further information can be collected by knowing the patients epidemiological data for the analysis of respective experimental study.

Materials: Human Brain tumors: Meningiomas (n=96): **Grade-I types (n=73)**: Meningiomas (n=10); Fibrous (n=17); Psammatous (n=2); Meningothelial (n=12); Transitional (n=15); Angiomatous (n=5); Secretory meningioma (n=3); Psammatous meningioma (n=2); Hemangiopericytic (variant 1 n=2); Hemangioblastoma (variant 3 of angioblastic n=1); Microcystic meningiomas (n=4); **Grade –II types:** Atypical meningioma (n=18); **Grade –III types:** Anaplastic meningioma (n=5);

Gliomas with different types (**n=101**): **Grade –I**: Low grade Astrocytoma (n=2); **Grade –II:**Fibrillary astrocytoma (n=2); Oligodendroglioma (n=1); **Grade-III:**Anaplastic astrocytoma (n=21); Malignant astrocytoma III (n=9); Protoplasmic Astrocytoma G-II progressing to G-III (n=1); **Mixed Glioma**: Anaplastic Oligodendroglioma (n=18); Anaplastic Oligoastrocytoma (n=4); Malignant Oligoastrocytoma (n=6); **Grade-IV:** Glioblastoma (n=7); Glioblastoma Multiforme (n=26); Malignant Astrocytoma IV (n=4). Other brain tumors including metastatic are 17 nos.