

EXPERIMENTAL STUDY

Characterization of G protein β subunit expression of human brain Glioma tumor

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ABSTRACT

Proteome indicates the protein content of a genome. Proteome analysis is effective in a new system formulation and prediction, prevention, and treatment based on protein. One of the purposes of proteomics researches is to know and understand the cancer mechanism. In this study, we separated the proteins by the Two-Dimensional Electrophoresis method and analyzed and compared protein spots by statistical and software data. The spots were separated and identified by the proteins' Isoelectric PH differences, molecular weights, and data bank. In continuation, the protein profiles were clustered by MALDI-TOF-TOF and the main element was identified and confirmed. We have used site PhosphoSitePlus® to review post-translational modifications. The findings indicated that the G protein Beta subunit rate increased in the astrocytoma, oligodendroglia, and glioblastoma cerebral malignant tumors. The $\beta\gamma$ complex formation may prevent and activates many paths of cellular growth. The $\beta\gamma$ complex activity control of the changes after the conversion parallel to GTPase activity of this α subunit may be a formulation mechanism for the G signal path (Tab. 5, Fig. 4, Diagram 2, Ref. 29). Text in PDF www.elis.sk

KEY WORDS: glioma, G protein β subunit, proteomics.

Abbreviations: 2-DE, Two-Dimensional Gel Electrophoresis; 2D-PAGE, Two-dimensional polyacrylamide gel electrophoresis; MS, Mass Spectroscopy; EGFP, Epidermal Growth Factor Receptor; MW, Molecular Weight; PI, Isoelectric pH; BTK, Tyrosine protein kinase; NDPK, Nucleoside Diphosphate Kinase; LPA, Lysophosphatidic acid

Introduction

Glioma is a popular neoplasm created by the unusual growth of the glial cells (1). Such tumors are very offensive and grow into the cerebral parenchyma; they include three subunits: astrocytoma, oligodendroglia and oligoastrocytoma. Gliomas are primary cerebral tumors and always in malignant cases, it is possible that they can penetrate into the whole cerebral parenchyma (2–4).

The biological systems function depends on proteins. Moreover, the cellular proteome is influenced by the changes after the translation and transcription; that is why many biologic cellular and molecular phenomena may not be analyzed at RNA level

(5). Proteomics is the protein knowledge science, accordingly biomarker's knowledge distinguishing cancer is very developed by oncoproteomics; in such a study, it is possible to examine the protein profiles in the sound and sick cellular systems by Two-Dimensional Gel Electrophoresis or 2D-PAGE, Mass Spectroscopy (MS) and bioinformatics techniques (6, 7).

A considerable part of the proteins has changed in the malignant astrocytoma tumor after the translation (8); also the membrane proteins were increased and played an important role in concentrating glial cell processes such as G Protein (9) and Epidermal Growth Factor Receptor (EGFP) (10) Ous studies. In continuation, we present our previous studies in more details (1, 11). We examined the Folding changes, Molecular Weight (MW) and Isoelectric pH (PI) for a variety of malignant glioma tumors by two-dimensional electrophoresis and mass spectroscopy method; such tumors include astrocytoma and oligodendroglia with malignancy grade III and astrocytoma with malignancy grade IV whose changes and comparison was analyzed.

Tab. 1. Five voltage regulation steps in first electrophoresis dimension for separation based on Isoelectric PH.

Strip length	18 cm		
STEP 1	Step	150 v	3 h
STEP 2	Step	300 v	3 h
STEP 3	Gradient	1000 v	6 h
STEP 4	Gradient	10000 v	1 h
STEP 5	Step	10000 v	2/5 h
Total time/Duration v/h		15/5 h	32 kvh

Suitable for all IPG gradient, Temperature 20°C, Current/strip: up to 70 MA

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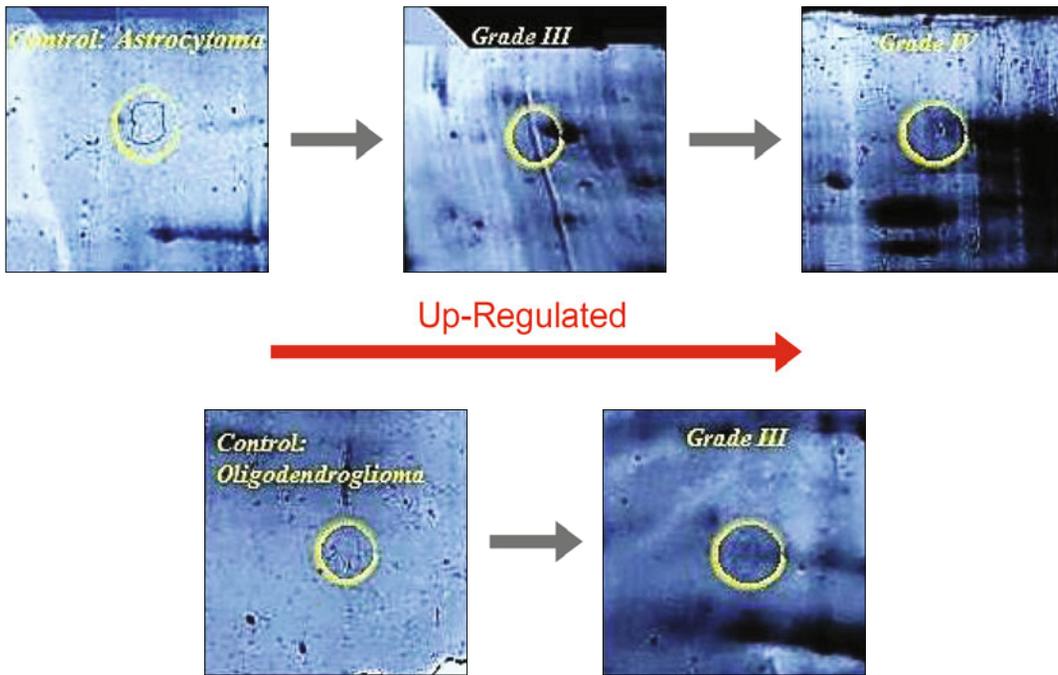


Fig. 1. Comparison of expression changes Spots (G Protein β subunit) in astrocytoma (III), astrocytoma (IV) and oligodendrogloma (III) with normal brain tissue.

Materials and methods

In the study 12 malignant glioma tumors including six astrocytoma cases with malignancy grade III, three astrocytoma cases with malignancy grade IV, and three oligodendroglia cases with malignancy grade III were removed (7 male and 5 female patients). Accordingly, the type and grade of the tumors were certified by the pathologists. The sound tissue was selected from the marginal tumor tissue for control. Both tumor tissues were kept at -80 °C.

Having washed and destructed cells and removed additional lipids the tumor proteins were extracted. The extraction method was described in previous articles completely (1, 10, 12). Finally, the final proteome concentration was examined by the Bradford method shown in Diagram 1.

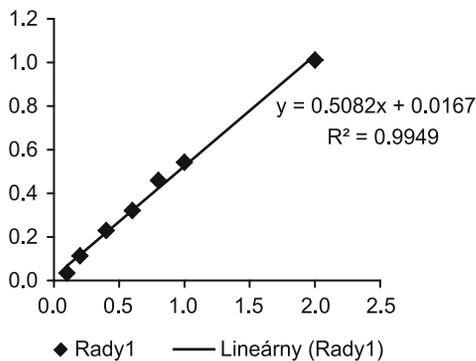


Diagram 1. Absorption based on concentration; if R2 is more and nearer to 1, the error rate is less.

The Two-Dimensional Electrophoresis method was used to separate the extracted proteomes. Accordingly, at first, the proteins were separated based on 3–10 isoelectric pH by 18 cm Strip. The solution samples were entered into the IPG gel by anode end and the primary separation was done in five steps (Tab. 1). In continuation, the second dimension was done based on 10–100 kDa

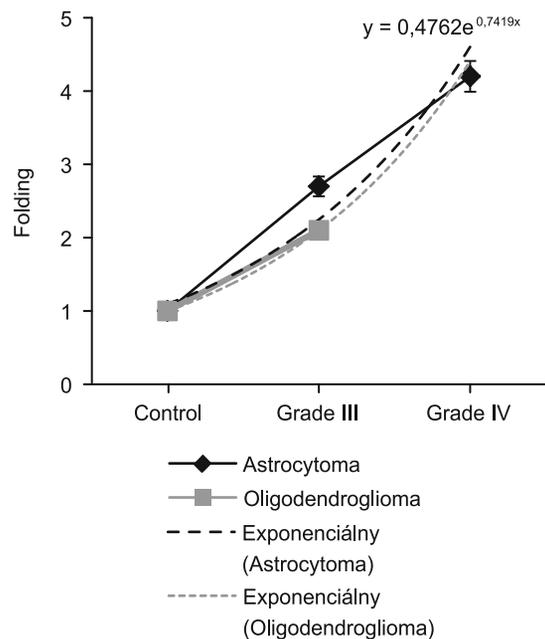


Diagram 2. Diagram Scatter, Trandline Drawings for the Molecular Weight and Isoelectric pH, Based on Data from Table 3.

Tab. 2. G protein β subunit Matching the Same set of Peptides by Databank.

	Expressed Proteins Change	Fold Change	Number of Peptides	Score	Matches	Sequences Coverage
Oligodendroglioma (III)	Up-Regulated	2.13	3	252	3(3)	3
Astrocytoma (III)	Up-Regulated	2.73	4	274	4(4)	4
Astrocytoma (IV)	Up-Regulated	4.23	3	260	3(3)	3

Tab. 3. Information on post-translational modifications of G protein β subunit adapted from the site PhosphoSitePlus.

Site	Site Information	Post-Translational Modifications	Ref
Ser2	MSELEQLRQ	Phosphorylation	13
Arg15	RQEAEQLRNQIRDAR	Mono-methylation	14
Lys23	NQIRDARKACGDSTL	Ubiquitylation	15, 16
Thr29	RKACGDSTLTQITAG	Phosphorylation	17
Ser72	TDSRLLVSASQDGKLL	Phosphorylation	18
Ser74	SRLLSASQDGKLLI	Phosphorylation	19
Lys78	VASQDGKLLIWDYSY	Acetylation	20
Tyr85	KLIIWDSYTTNKVHA	Phosphorylation	18
Lys89	WDSYTTNKVHAIPLR	Ubiquitylation	21
Ser136	REGNVRVSRELPGHT	Phosphorylation	13
Ser207	VSGACDASIKLWDVDR	Phosphorylation	13
Lys209	GACDASIKLWDVDRDS	Ubiquitylation	22
Ser216	KLWDVDRDSMCRQTFI	Phosphorylation	13
Tyr239	VAFFPNGYAFTTGSD	Phosphorylation	23
Lys301	CNIWDAMKGDRAVGL	Ubiquitylation	22

1 MSELEQLRQEAEQLRNQIRDAR **KACGDSTL** TQITAGLDPV GRIQMRTRRT
 51 LRGHLAKIYA **MHWGTDSRLL** VSASQDGKLLIWDYSYTTNKV HAIPLRSSWV
 101 MTCAYAXSGN FVACGGLDNI CSIYSLKTR EGNVRSRELP **GHTGYLSCCR**
 151 FLDDNQIITS SGTDTTCALWD IETGQQT VGF AGHSGDVMSL SLAPNGRTFV
 201 SGACDASIKL **WDVDRDSMCRQ** TFIGHESDIN AVAFFPNGYA FTGSDDATC
 251 RLFDLRADQE LLMYSHDNII CGITSVAFSR SGRLLLAGYD DFNCCIWDAM
 301 **KGDRAVGLAG HDNRV**SCLGV TDDGMAVATG SWDSFLKIWN

Tab. 4. The Molecular Weight and Isoelectric pH of Each of the Tumors (Oligodendroglioma III, and Astrocytoma III, IV) Compared to Control Independently for G protein β subunit.

	tumor	grade	sex	age	G protein β subunit		
					p < 0.05	PI	MW
Case 1	oligodendroglioma	III	Man	48	2.448e-008	5.93	36.82
Case 2	oligodendroglioma	III	Man	69	2.666e-008	5.88	39.12
Case 3	oligodendroglioma	III	Woman	63	5.736e-005	5.95	39.06
Case 4	astrocytoma	III	Man	39	3.206e-008	5.80	37.19
Case 5	astrocytoma	III	Man	60	3.604e-008	5.99	39.24
Case 6	astrocytoma	III	Man	39	5.691e-006	5.89	40.46
Case 7	astrocytoma	III	Man	60	4.330e-008	6.02	37.15
Case 8	astrocytoma	III	Woman	51	5.618e-008	6.04	37.61
Case 9	astrocytoma	III	Man	62	7.668e-006	5.79	39.09
Case 10	astrocytoma	IV	Woman	49	5.663e-007	5.92	38.53
Case 11	astrocytoma	IV	Man	55	7.691e-004	5.94	40.64
Case 12	astrocytoma	IV	Man	56	1.753e-007	6.01	39.77

molecular weight on an SDS-PAGE gel. At first, the 32 A, 400 V and 12 W conditions were regulated for 30 minutes; then we permitted the second dimension run with 48 A, 480 V and 10 W conditions for five hours.

Tab. 5. Statistical Analysis of MW and Isoelectric pH of G protein β subunit.

	Valid	Missing	Mean	Median	Error of Mean	Variance	Min	Max	Rande
PI	12	0	5.93	5.935	0.062	0.0065	5.79	6.04	0.25
MW	12	0	38.72	39.075	0.996	1.65	36.82	40.64	3.82

Staining, scanning and bioinformatics analyzing gel electrophoresis

Coomassie Blue dye was used to stain the gels. Then the gels were scanned and the images were processed and analyzed by Quantity One and Non-Linear Progenesis Same Spot programs. In continuation, the images of the protein spots separated from the tumor tissue were adapted with control tissue. The proteins were identified primarily by the data gained from the software based on the spots' dye rate and data bank. We have used site PhosphoSitePlus® to review post-translational modifications.

MALDI-TOF-TOF

The MS method (MALDI-TOF-TOF) was used to confirm primary identified spots. accordingly, the protein spots are separated based on their mass-load ratio in an electromagnetic field; then the proteins are identified by databank.

Statistical analysis, clustering and principal component analysis (PCA)

Finally, the gained data were analyzed statistically by t-Test and SPSS software. The significant rate was p < 0.05 in the study. The spots with p < 0.05 who were appropriate were divided into two groups (With an increased or decreased expression). Then the location of the protein spots was identified by clustering. Then the PCA was assessed to find if it was right or not.

Results

Mean folding change for G protein β subunit in proportion to control in astrocytoma with malignancy grade III is indicating +2.73 (for six tumors: 1.9, 2.6, 3, 2.6, 3.2, 3.1, respectively), also for astrocytoma with malignancy grade IV. Mean folding change for G protein β subunit in proportion

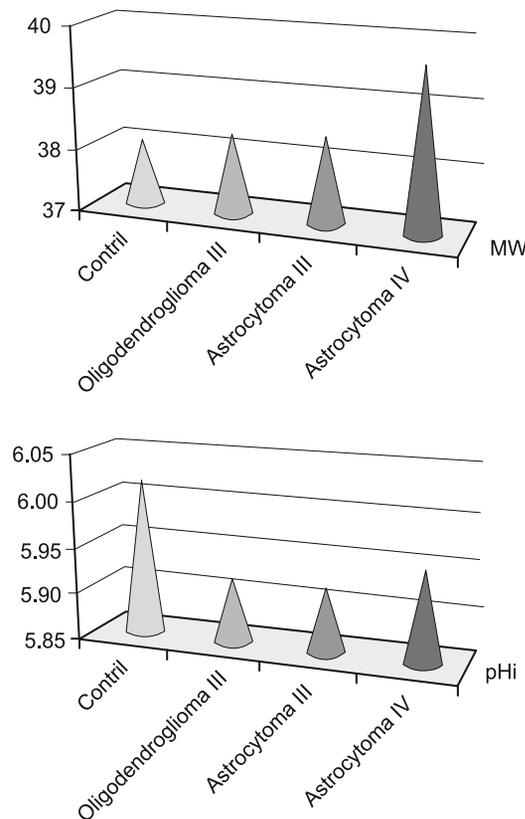


Fig. 2. Categorization of change in MW and pHi of Each of the Tumors (Oligodendroglioma III, and Astrocytoma III, IV) Compared to Control Independently for G protein β subunit.

$$\begin{aligned}
 & MW_{\text{Astrocytoma IV}} > MW_{\text{Astrocytoma III}} > MW_{\text{Oligodendroglioma III}} > MW_{\text{Control}} \\
 & pHi_{\text{Astrocytoma IV}} > pHi_{\text{Astrocytoma III}} = pHi_{\text{Oligodendroglioma III}} > pHi_{\text{Control}}
 \end{aligned}$$

to control in astrocytoma with malignancy grade III is indicating +4.83 (For three tumors: 3.9, 5.5 and 5.1, respectively) and for oligodendroglia with malignancy grade III. Mean folding change for G protein β subunit in proportion to control in astrocytoma with malignancy grade III is indicating +2.13 (For three tumors: 1.552.7 and 2.2, respectively). These findings indicate the folding change increased for the G protein β subunit in glioma malignant tumors (Fig. 1 and Diagram 2). The analysis and data related to the MS are presented in Table 2. Also, shown are the anticipated peptides, and matched peptides shown in blue (gi|306785). Below are the amino acids that have been changed to red. These sequences have undergone post-translational modifications that have been documented by other researchers, and the results from our study are shown in Table 3.

Our findings indicated the MW changes mean is 38.72 in proportion to the control for a variety of glioma malignant tumors indicating +0.659 for the molecular weight change and PI changes mean is 5.93 in proportion to the control for a variety of glioma malignant tumor indicating -0.09 for the isoelectric pH change. PI and MW are shown in Figure 2 and Table 4 for three types of glioma tumors in proportion to control. Data and statistical analysis are shown in Table 5.

Discussion

The Post-translation modifications of G protein β subunit are in relation to the varied structure of the protein. The β subunit is formed by seven butterfly shaped edges and each edge is made of a series of beta-sheets. One of these post-translation modifications of this protein includes the methionine removal from terminal N in situation 1 of this subunit. In continuation, these changes occur in the N2 acetylation situation for serine and it should be noted that the change mechanism is unknown yet (13–15). βγ complex formation may prevent and activate many cell growth paths (16, 17). βγ complex activity inhibition is by the changes after translation parallel to the GTPase activity of the α subunit which may be a regulation mechanism for the G protein signal path. This document contains 4 G-protein βγ complex signaling pathways (Fig. 3 from site PhosphoSitePlus) (18); G-protein βγ complex signaling:

- G-protein βγ complex signaling through PI3Kgamma
- G-protein βγ complex signaling through PLC beta
- G-protein βγ complex signaling through BTK
- G-protein βγ complex signaling through CDC42

It was shown before that histidine acid amino phosphorylation of the β subunit is a mechanism for G protein activity by Nucleoside Diphosphate Kinase (NDPK). The most relationships between the active and inactive situation of the α subunit are by β subunit (17, 19–21).

Smrcka and colleagues in 2008 reported that G protein βγ complex is a signal for primary receptors proliferation related to G protein complex (22). Previous studies indicated that some receptors such as Lysophosphatidic acid (LPA), Chemokines and Bradykinin increased in a big group of cancers (23). Also, LPA creates some cellular responses including proliferation, cellular differentiation, movement and survival in both normal and ill conditions. LPA shows the most distinguishable cancer diagnoses including angiogenesis and attack to the tissue. These biologic acts are with the abnormal state of the receptor and increased LPA in cancer. Also, these lipids do their biologic operations through the receptors attached to G proteins (24). In 2002, Kue and colleagues proved that G protein βγ complex may transfer freely the activity in the EGFR receptors (21, 25). Also, in our previous studies, we reported the EGFR increase for primary tumors of the brain, such as glioma. Our findings indicated EGFR increased +1.6 and +1.5 for astrocytoma and oligodendroglia tumors with malignancy grade III and +2.8 for glioblastoma, and it's MW mean changed from 14.2 to 13.3 and it's PI mean changed from 5.8 to 6.28.11 In continuation, we report the increase of G protein β subunit in this study which has a direct effect to form βγ complex leading to protein activity change in G protein. In Figure 4 we show the relationship between βγ complex and EGFR. This is an image adapted from Site PhosphoSitePlus (18). These protein changes are a part of the glial cell cancerating chain.

Kue and colleagues indicated that the G protein βγ complex control may be a preventive solution against the protein G complete receptors proliferation; this prevents the cell growth signal transfer. They prevent the primary prostate cancer cell growth in vitro (26); Also, it was shown that G protein βγ complex is an ele-

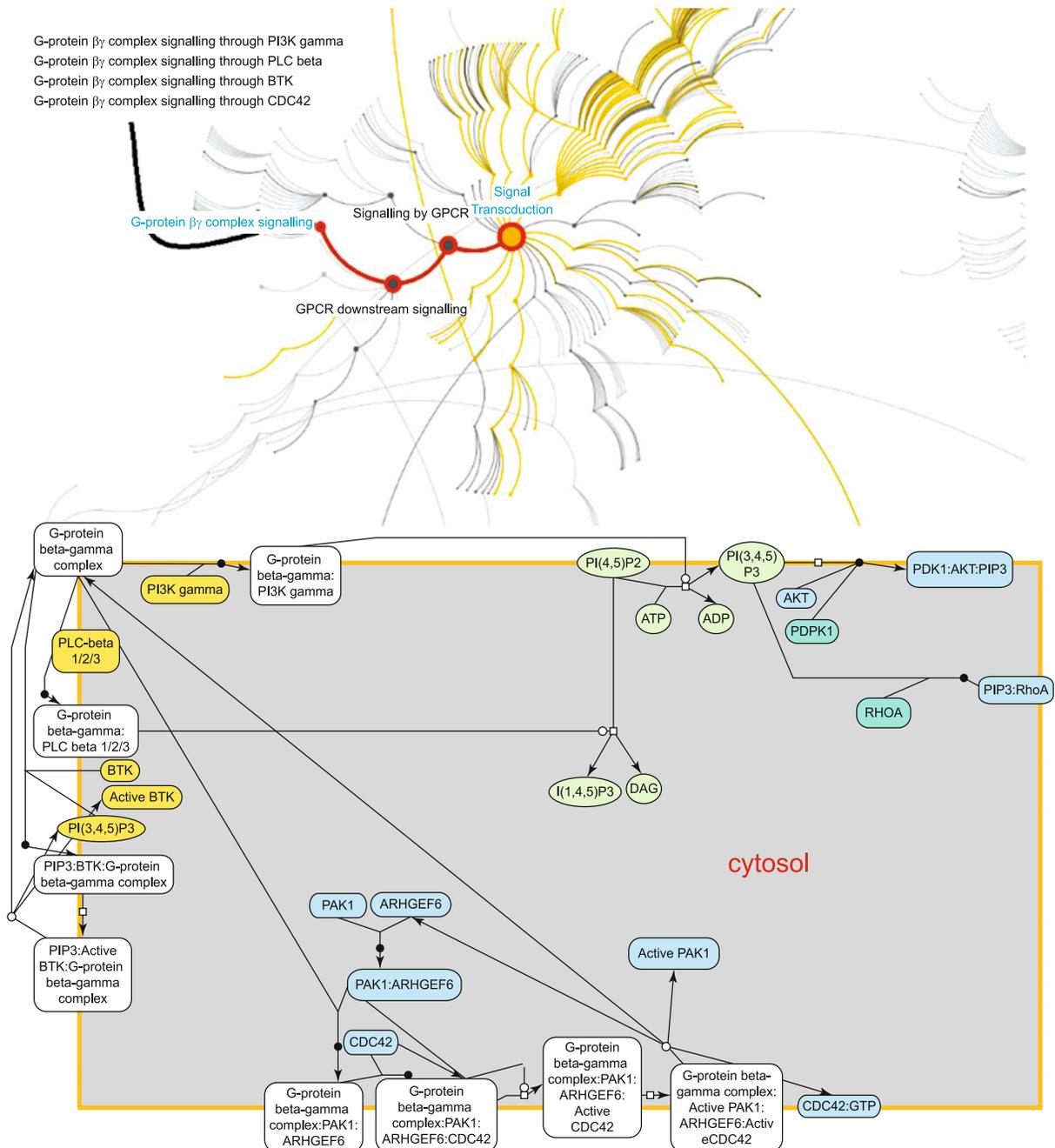


Fig. 3. G-protein $\beta\gamma$ complex signaling: The G protein beta subunit is a negative regulator of the G-protein signaling complex. G-protein $\beta\gamma$ complex signaling recruits PI3KG to the membrane, both activating PI3KG and providing access to its substrate PIP2, which is converted to PIP3. G protein β subunit is increasing the phospholipase activity and also causes increased hydrolysis of PIP2 to DAG and IP3. G-protein $\beta\gamma$ complex signaling, along with phosphatidylinositol 3,4,5-trisphosphate, recruits the nonreceptor Tyrosine-protein kinase (BTK) to the cell membrane. Here, the G-protein $\beta\gamma$ complex activates BTK. Subsequently, active BTK dissociates from the complex to phosphorylate downstream substrates. (Adapted from the site PhosphoSitePlus).

ment starting breast cancer metastasis and growth. In their report (27), Ye and colleagues showed the role played by G protein $\beta\gamma$ complex in metastasis (28), and then, Paudyal and colleagues in 2017 reported that G protein $\beta\gamma$ complex plays a protective role in metastasis formation (29).

Many studies were done on the proteome changes in a variety of cancers and each study had its special purpose and strategy. Some protein changes were reported for malignant glioma tumors including astrocytoma, oligodendroglia, and glioblastoma; such as an increase in G protein β subunit in relation to the ma-

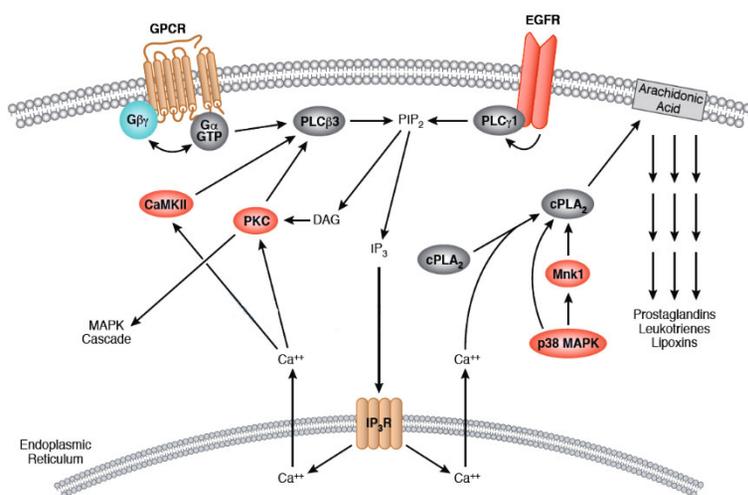


Fig. 4. Signaling relationship between G-protein $\beta\gamma$ subunit complex and EGFR. Both β subunit and EGFR proteins are overexpressed in glioma tumor. (This is an image adapted from Site PhosphoSitePlus).

lignancy rate in a glial cancerous cell. Our findings indicated a direct relation between the protein increase and the invasive rate of the glioma tumor.

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