



Deciphering Role of EGFR and the Different Inhibitory Mechanisms in Glioblastoma

Aditi Dixit¹ Tarun Gupta*

¹Department of Biotechnology , BSITC , I. T. College , Uttar Pradesh , Lucknow -226007 , INDIA

*Assistant Professor, Bansal classes Pvt. Ltd., Rajasthan , Kota -324005 , INDIA

Abstract:-Glioblastoma is major Astrocytoma which has been found out to be formed from the positive regulation of the different factors such as EGFR, NF-1 and PDGFR, etc.. It leads to the brain more cellularly differentiated and the metastasis is found to lead the cellular proliferation. Several other factors such as GADD 14-3-3 Σ and RAD001 is responsible to activate the major signalling in the glioblastoma pathways . The p21 acts the major apoptotic gene to regulate the GS Cell-lines. The EGFR is found to be associated with the TERT-Dependent as well as AKT dependent manner of signalling for the glioma cells to acquire stem-like features . There has been developed a signalling cascade for the GS Cell-lines to function as the tumorous cells. These tumorous cells can be inhibited by the different types of antioxidating factors, histone deacetylase inhibitors, CDK Inhibitors and miRNAs .

Keywords: GS Cell-lines, EGFR, STAT, NF-1, p21, Inhibitors.

I. INTRODUCTION

The Glioblastoma is a type of Grade – IV Astrocytoma, found to be a malignant brain tumor . It has been described according to the major intra- and intertumoral heterogeneity at histological and molecular level and are characterized as the mesenchymal , neural, proneural, and classical subtypes, according to specific genetic aberrations or expression of marker genes (mesenchymal: NF1; neural: SYT1; proneuronal: PDGFR α /IDH1, classical: EGFR).[Kristyn Galbraith et al.]The CD 133 (Cluster of Differentiation 133 ;also termed as Prominin -1) is a probable marker to differentiate the GS (Glioblastoma stem) Cell – lines from the other type of brain tumor. The ability to form tumors in GS – Cells show the heterogeneous phenotype of their parent tumor when implanted into the brain of immunodeficient mice is considered the major differentiation role for GS-cells by nonstem-like tumor cells), GS-cells contain the ability to self-renew and to differentiate along neural lineages ,that is similar to adult cells , which means , astrocytes , neurons, and oligodendrocytes, when cultured in differentiation medium (fetal calf serum, retinoic acid, and cyclic adenosine monophosphate) are able to clone their own. GS-cells are also found to be highly resistant to radio- and chemotherapy in vitro and in vivo and to adapt rapidly to changes in the tumor microenvironment, that is, acidic stress or hypoxia. [Lee JS et al., Mitsudomi T et al.]

II. FACTORS RESPONSIBLE FOR GLIOMA DEVELOPMENT

The Nuclear factor I (NFI) family of transcription factors (site-specific DNA-binding proteins that bind to a specific motif (TGGC(N₅)GCCA)) functions as activators or repressors of gene expression. NFIA, a member of the NFI family, correlates an essential role in glial development in the central nervous system. It specifies glial identity, maintains glial progenitors, and regulates astrocyte differentiation in part through transcriptional regulation of glial fibrillary acidic protein (GFAP). More recently, there had been discovered an association between NFIA and human gliomas which showed that NFIA is abundantly expressed in astroglial tumors compared with non-neoplastic brains. It was unknown, however, whether NFIA is involved in regulation of glioma growth. [Lee JS *et al.* Liffers K *et al.*] NFIA increases cell proliferation and survival by repressing p53 and p21. Furthermore, NFIA promoted GBM cell migration by repression of PAI 1 (plasminogen activator inhibitor 1), an important regulator of cell migration. Importantly, these effects were mediated via specific NFIA-recognition sequences in the promoters of p53, p21, and plasminogen activator inhibitor 1 (PAI 1). [Lee JS *et al.*] Epidermal growth factor (EGF), isolated by Stanley Cohen in 1962, located on chromosome 7p12-13, codes for a 170kDa receptor tyrosine kinase. All EGFR proteins have four functional domains: an extracellular ligand-binding domain; a transmembrane domain; an intracellular tyrosine kinase domain; and a C-terminal regulatory domain. The extracellular domain is subdivided further into four domains. The tyrosine kinase domain consists of an N-lobe and a C-lobe, and ATP binds to the cleft formed between these two lobes. The C-terminal regulatory domain has several tyrosine residues that are phosphorylated specifically upon ligand binding. [Liffers K *et al.* - Mitsudomi T *et al.*]

There have been three different types of deletion mutations (categorized according to the extent of deletion, and termed EGFR vI, EGFR vII and EGFR vIII, which have been reported in the extracellular domain of the EGFR gene, out of which EGFR vIII (de2-7EGFR or pEGFR) is reported to occur in 30–50% of glioblastomas. [Kanu OO *et al.*] EGFRvIII results from a nonrandom 801-bp inframe deletion of exons 2–7 of the EGFR gene that occurs at the genomic level leading to expression of aberrant transcripts and proteins. This mutated protein lacks a portion of the extracellular ligand-binding domain as a result of genomic deletions, resulting in a constitutively autophosphorylated receptor, albeit, at a lower level than wild type. In addition to enhancing growth, proliferation, migration, and tumor neovascularization, this truncated receptor also confers resistance to chemotherapies such as cisplatin through modulation of Bcl-XL and caspases in cell death pathways. Multiple murine glioma models have also been confirmed of this aberrant growth factor signaling in gliomagenesis. Platelet derived growth factor receptor (PDGFR) is also a studied receptor expressed in most types of gliomas, while EGFR which is expressed mainly in GBM. PDGFR signals through phosphoinositide 3-kinase (PI3K) and phospholipase C gamma (PLC- γ) to express the mTOR pathway leading glioblastoma. [Mitsudomi T *et al.*]

III. STAGING OF GLIOBASTOMA

The staging of the tumor is based on hypercellularity, mitosis rates, presence of necrosis, and vascular proliferation. [Gabrielle L. Brown et al.]

Grading system for GBM	
Grade	Symtoms
Grade 1 tumor (Juvenile pilocytic astrocytoma)	It is slow growing tumor ; usually associated with long – term survival and low re- occurrence.
Grade 2 tumor (astrocytoma)	Increased hypercellularity with no mitosis ; no vascular proliferation ; no necrosis ; can re – occur as high – grade tumor
Grade 3 tumor (anaplastic astrocytoma)	High rate of hypercellularity with no mitosis ; no vascular proliferation ; no necrosis associated with high rate of tumor re - occurrence
Grade 4 tumor (Glioblastoma)	Very high rate of hypercellularity with very high rate of mitosis . There is also presence of vascular proliferation alongwith necrosis

Fig.1 Staging of glioblastoma

IV. GENE INTERACTIONS RESPONSIBLE FOR HIGH RATE OF HYPERCELLULARITY

➤ *EGFR Amplification:*

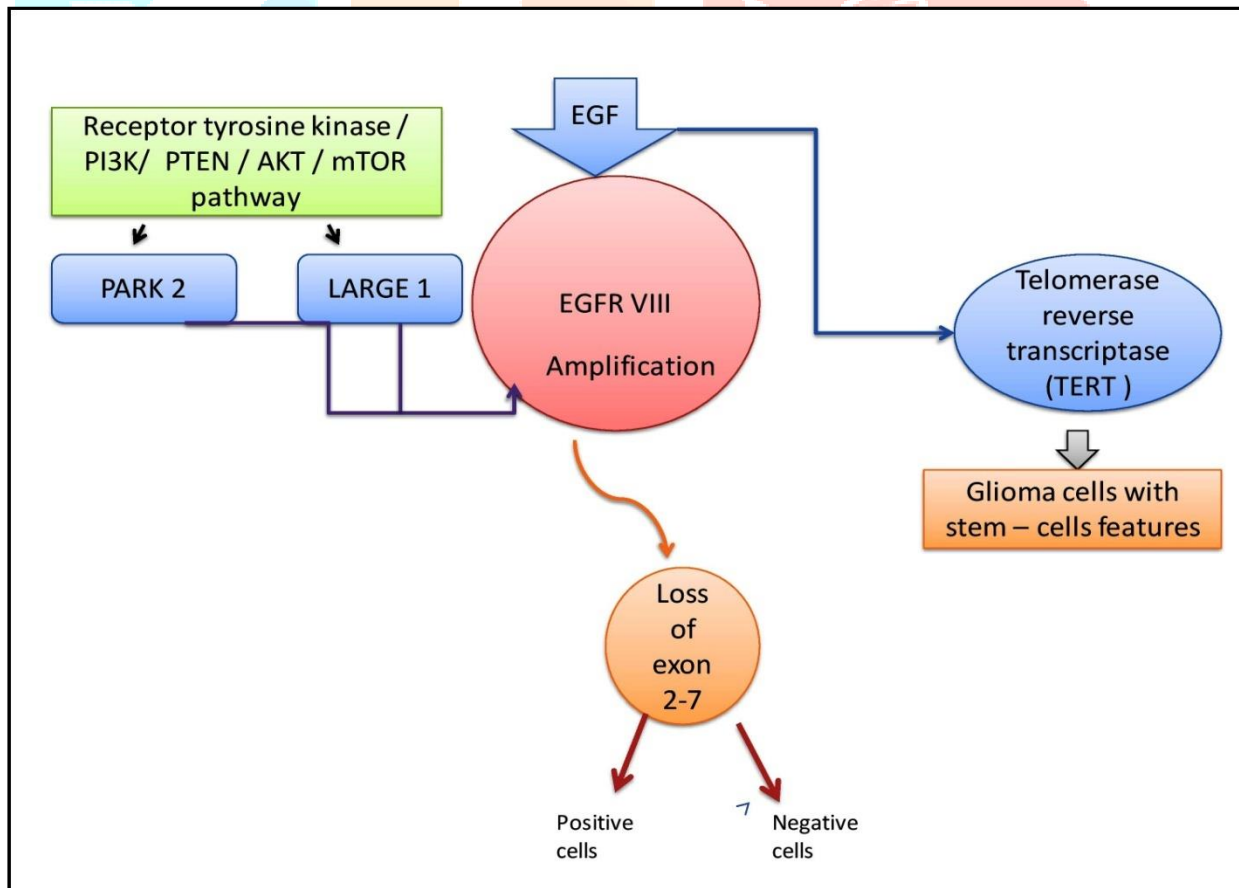


Fig.2 The EGFR Amplification is associated with the PARK2 and LARGE 1 factors of the mTOR pathway , which is also related with the TERT activation resulting to loss of exons 2-7

EGF induces the apoptosis by activating the EGFR in A431 cells, which can be revoked by tyrosine kinase inhibition. When there is much EGFR signaling due to increased receptor expression and subsequent ligand-induced overstimulation, the EGFR pathway seems to be a major negative selector for EGFR-amplified GBM cells in vitro. [Liffers K et al.] Experimental evidence from a genetically engineered mouse brain tumor model allows the lineage tracking of neural stem/progenitor cells, that indicates that NSCs are considered to be the prime suspects for the cell of origin in GBM. EGF-induced activation of EGFR increases proliferation, survival, and migration, while inhibiting differentiation, whereas withdrawal of EGF from NSC cultures leads to differentiation and cell death. [Liffers K et al., Mitsudomi T et al.] EGFR amplification and the EGFR gene rearrangement events leading to the loss of exons 2–7 resulting in EGFRvIII expression, which is considered as early events in GBM development. It has been also illustrated that EGFRvIII-positive cells can give rise to both EGFRvIII-positive and -negative cells. Furthermore upregulation of EGFR in a telomerase reverse transcriptase- (TERT-) dependent manner allows differentiated glioma cells to acquire stem-like features [Liffers K et al.]. There has been also revealed that PARK2 and LARGE1 CNAs are also associated to EGFR amplification. It is seen that PARK2 and LARGE1 are related to receptor tyrosine kinase/PI3K/PTEN/AKT/mTOR-signaling pathway [Shamloo, B et al.]

➤ *Role of p21 (WAF1 /CIP1) gene:*

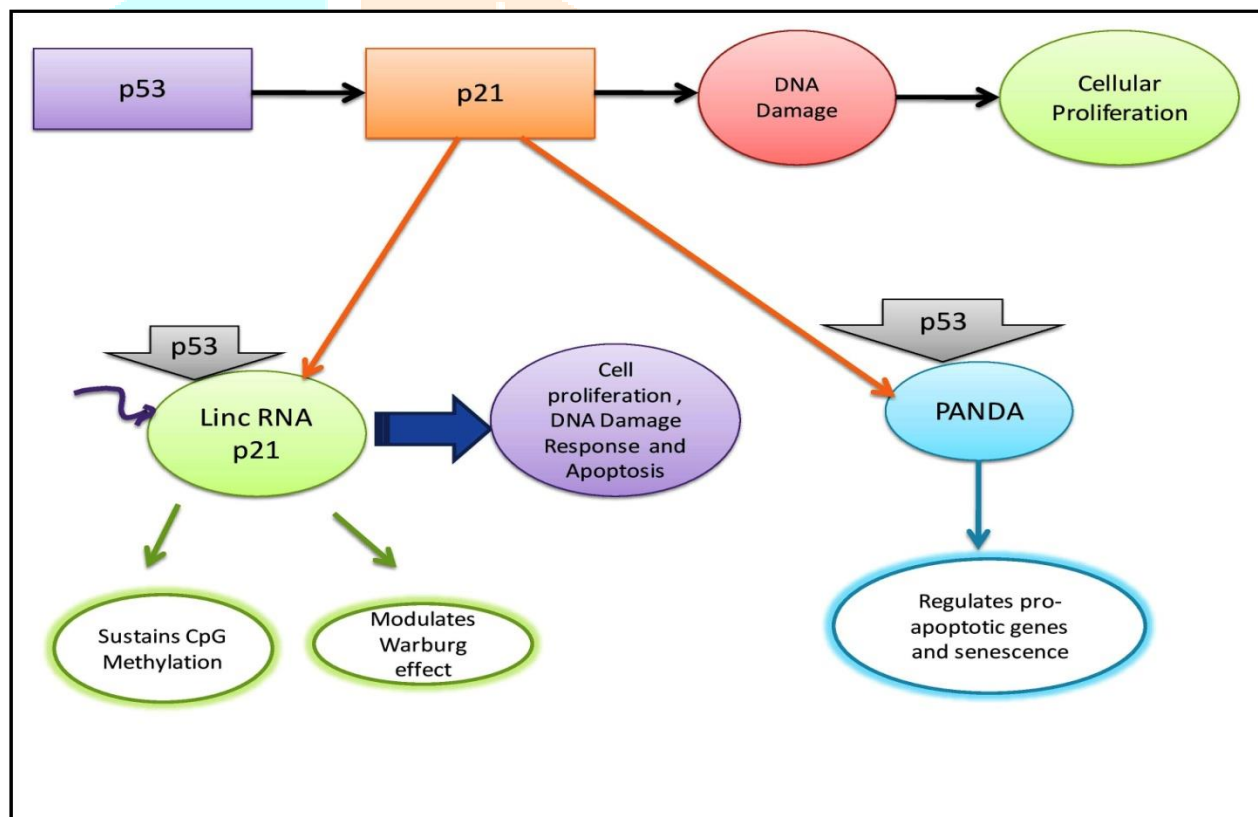


Fig.3 Induction of Warburg Effect and sustaining CpG Methylation by LincRNA p21 with the regulation of pro-apoptotic genes by PANDA - Two sites of p21 gene p21 has a critical function in the regulation of cell cycle initiation by exerting inhibitory effects on the activity of cyclin -dependent kinase (CDK), of which, the activation controls cell cycle alterations. The regulation of p21 activity initially occurs at the transcriptional level. p53 after phosphorylation corresponds with p21 activation. However, p53-independent pathways that control p21 expression, involving Stat3 and Sp1 have also been reported. The physiologic function of p21 involves proliferation, apoptosis, and DNA repair. It is also studied that the Up-regulation of p21 gene expression leads to arrest cell growth to allow for the damage to be repaired. [Dongsun Cao et al.]. LincRNA-p21 (long intergenic noncoding RNA-p21) which is located 15 kb upstream of p21 gene regulates gene expression both at the transcriptional level as well as post-transcriptional level. When regulated by p53, lincRNA-p21 physically interacts with heterogeneous nuclear ribonucleoprotein K (hnRNP K), functioning as a key repressor. LincRNA-p21 regulates cell proliferation, DNA

damage response, and apoptosis through its regulatory role in p53 target gene expression. LincRNA-21 also regulates reprogramming with the help of several mechanism, example, lincRNA-p21 sustains CpG methylation and/or H3K9me3 at the promoter region of pluripotency genes, causing somatic cell reprogramming prevention. LincRNA-p21 also modulates Warburg affect, hence playing an important role in cancer cell metabolism. PANDA (P21-associated noncoding RNA DNA damage-activated) is another linc (intergenic non-coding) RNA located 5 kb upstream of p21 gene that regulates proapoptotic genes and senescence through stabilizing p53 tumor suppressor gene. p 53 binds to transcription start site of p21 and activates PANDA and p21 transcription in response to DNA damage. [Shamloo, B et al.]

➤ Regulation of p21 and EGFR :-

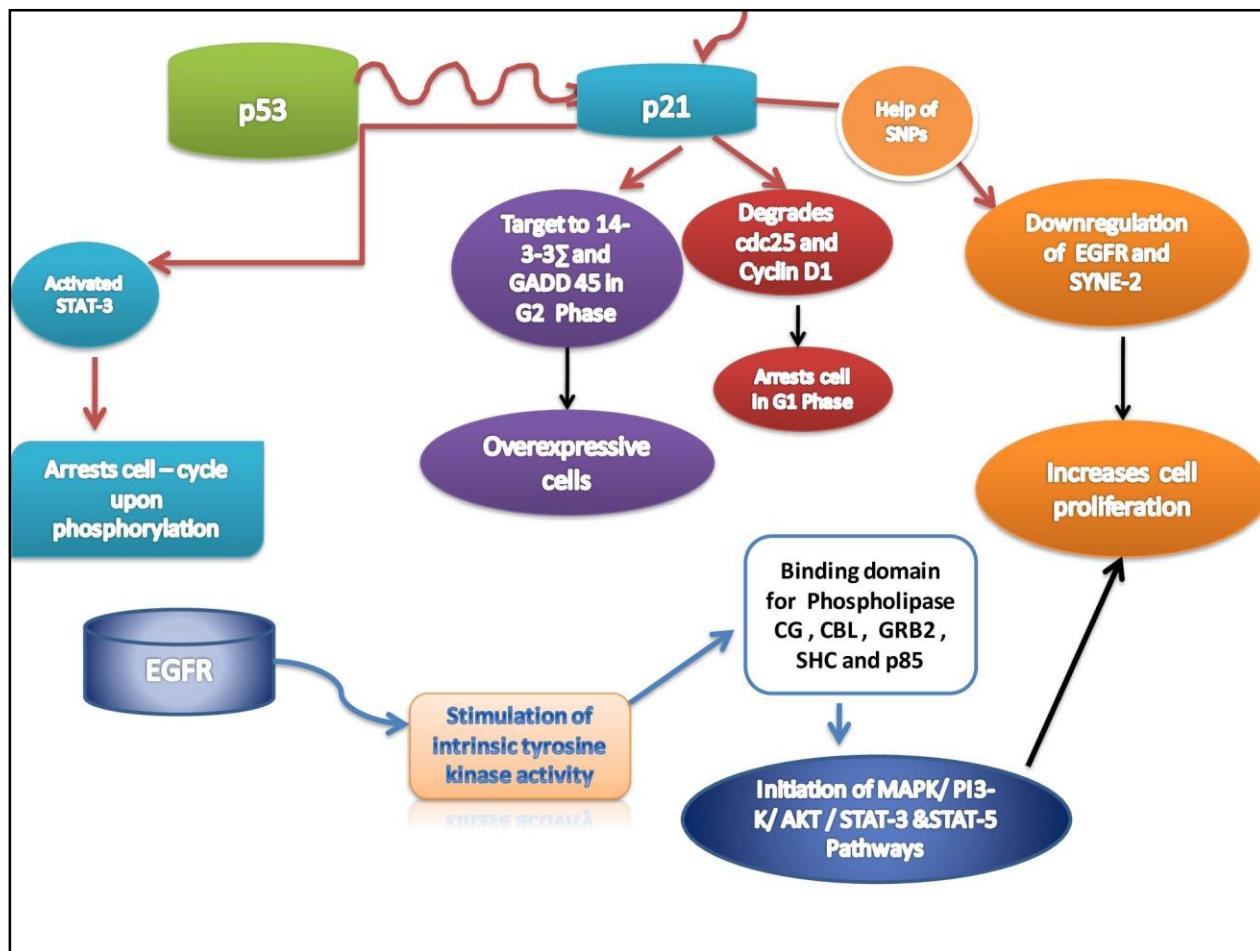


Fig .4 The intrinsic and extrinsic activation of pathways which are regulated by EGFR and p21

The p21 gene is transcriptionally initiated by p53 gene and arrests the cells in G1 phase. Although p21 is a tumour suppressor gene, it also promotes oncogenesis [Han C et al.] . Furthermore in some cases it has been shown that the p21 directly degrades the cdc25 and Cyclin D1 that leads to cell cycle arrest. There is some mechanism of action of p21 in G2 phase arrest also, it targets the 14-3-3 Σ (which sequesters cyclin B1–CDK1 complexes outside the nucleus) and GADD45 (Growth arrest and DNA-Damage inducible protein) and makes the cells overexpressive. [El-Deiry WS]. The p21 is also found to be associated with SYNE -2 (Spectrin repeat containing, nuclear envelope 2). The p21 with the help of different SNPs downregulates the EGFR and the SYNE- 2 proteins , which thereby increases the cell proliferation [Han C et al.]. When the p21 gets activated , it also activates the STAT 3 genes , which arrests the cell cycle upon phosphorylating. [Dongsun Cao et al.]. The ligands which are synthesized as transmembrane proteins, and soluble ligands (growth factors) are also released into the extracellular environment via proteolytic processing , mediated by ADAM (a disintegrin and metalloprotease) proteins that are membrane-anchored metalloproteases . [Liffers K et al.]The Dimerization of EGFR consequently stimulates intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues within the cytoplasmic regulatory domain. The tyrosine residues when phosphorylated serves as the binding domain for phospholipase Cg, CBL,

GRB2, SHC and p85. There have been several signal transducers, which then bind to these adaptors to initiate multiple signalling pathways, which includes mitogen-activated protein kinase, phosphatidylinositol 3-kinase/AKT and the signal transducer and activator of transcription (STAT)3 and STAT5 pathways. These pathways then resulting to the angiogenesis [Liffers K *et al.*].

➤ *Role of Calmodulin in GBM:-*

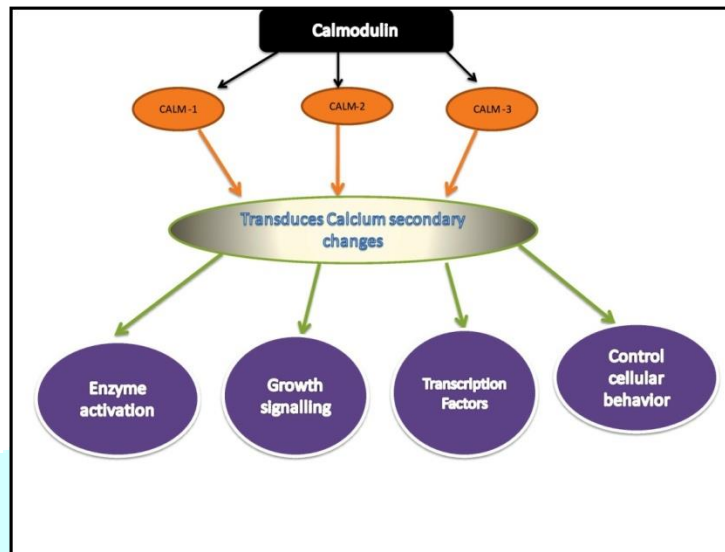


Fig .5 CALM-1, CALM -2, CALM -3 ; Three sub-units of Calmodulin along with their function

Calmodulin is higher in glioblastoma than low- grade glial tumors and has a correlation with prognosis. It transduces calcium secondary changes in the eukaryotic cell. Ca²⁺ calmodulin complex does a master role in subcellular variable effects as enzyme activation, growth signaling, transcription factors which consequently control the cellular behavior. Three genes were identified to encode Ca²⁺ calmodulin protein, CALM1, CALM2, and CALM3, and each has specificity for transcription activity in different tissues. . A study to analyze the expression levels of different types in GBM found that CALM1 and CALM2 are significantly expressed more than CALM3 in GBM [Mohammed A Azab *et al.*].

V.SIGNALLING PATHWAYS OF GLIOBLASTOMA WITH THEIR INHIBITORS

Pathways

➤ *AKT Pathway:-*

The AKT regulation is multifactorial, i.e., it directly phosphorylates the cell cycle machinery as well as the cell death machinery. It increases the cyclin D1 levels by inhibiting its degradation which is responsible for G1/S phase transition. Also it activates the pro-apoptotic process such as Caspase – 9 and BAD which provides the cell longevity. It was also seen that the activated AKT deactivates the Rb gene and downregulates the p21 gene leading to transcriptional upregulation of p21 expression [Vivanco I *et al.*], i.e., inhibition of CDK-2 [Bhunia AK *et al.*].

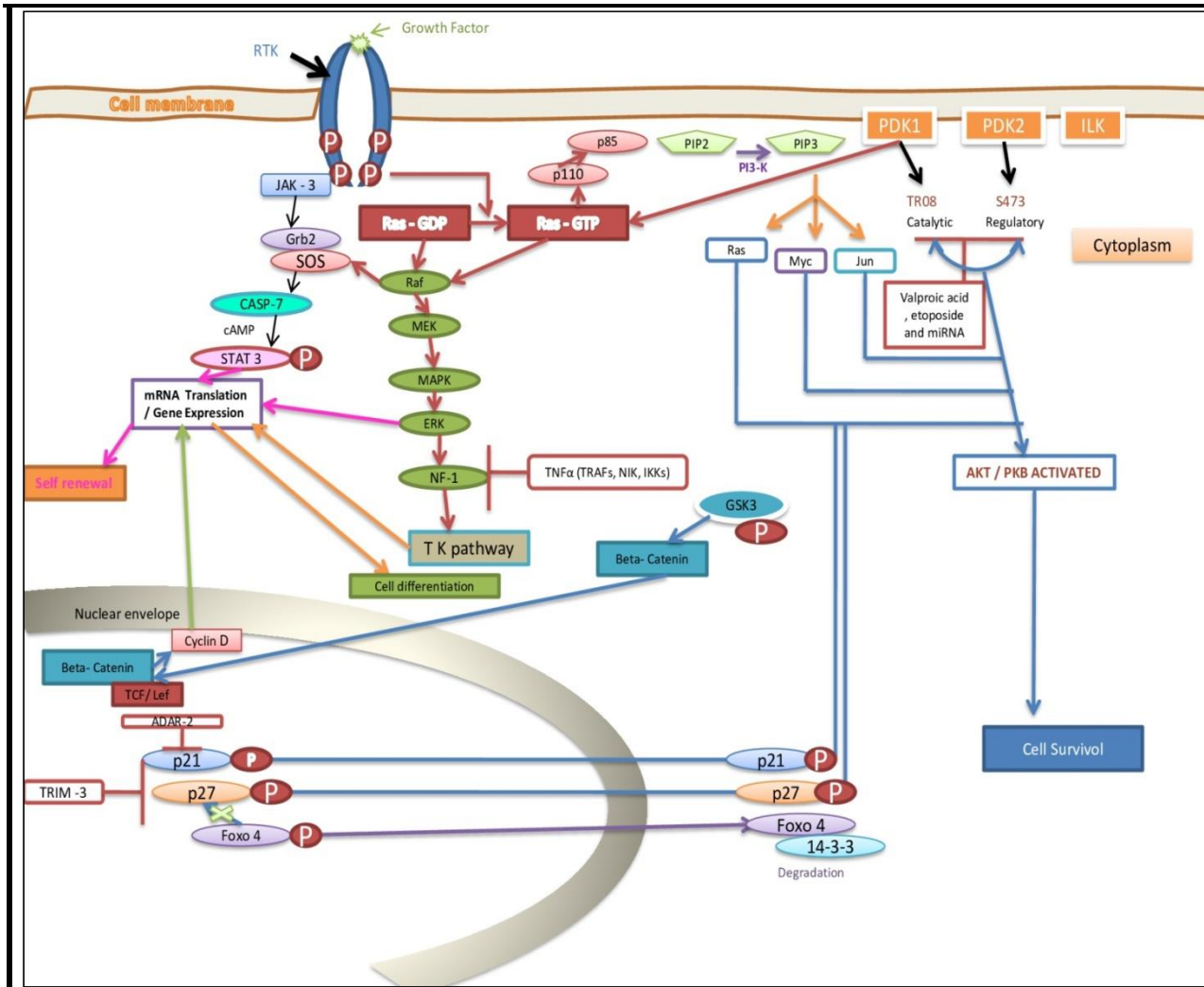


Fig.6 The AKT , JAK-STAT , mTOR and NF Pathways along with their regulation and the inhibitors associated

➤ *STAT Pathway:-*

The polycystin group of genes are responsible for direct activation of the STAT pathways of tyrosine kinase family. The polycystin -1 when phosphorylated directly activates the JAK -2, which is also supported by the activation of polycystin -2. Upon the activation by polycystin-1, it phosphorylates the PKD1 (Polycystin kinase domain -1) which induces the activation of STAT -3 ,leading to the cell differentiation , invasiveness , consequently the angiogenesis . [Bhunia AK et al.][Fig.6]

➤ *MAP Kinase Pathway:-*

The RAD001 upon activation by the IRK-1 leads to the EGFR and PGDFR downregulation which responds with the PI-3 K pathway[Wullschlegler S et al.] , thereby activating the ras , myc and jun genes , eventually increasing the cellular proliferation, thus leading metastasis . [Carracedo A et al.] The low concentration of EGF activate the ras genes , thereby activating PIP3 genes which upregulates the GAP/Shp2 genes.[Wullschlegler S et al. , Nichols R.J. et al]. Also, the mTORC1 inhibition increases RTK/ IRS-1/PI3K activity toward Ras/MAPK, therefore promoting both AKT activation and ERK phosphorylation in a dual feedback manner.[Carracedo A et al. - Nichols R.J. et al.] [Fig.6]

➤ *NF-1 Signalling:-*

NF -1 Signalling is a Ras independent pathway in which the Caspase 3 upon being phosphorylated corresponds with the Camp which facilitates the Caspase - 7 activation thereby increasing the NF-1 expression . This NF-1 expression then downregulates the EGFR, which upon phosphorylated, induces the Thymidine kinase pathway. [Guha A et al., Shapira S et al.] It has been also studied in the

canonical manner that when there is any mutation in the NF-1 receptor, it is activated upon binding with the p21 promoter (p21-Luc-mt), which then leads to de-phosphorylation of CDK-2 and Rb genes. [Liffers K et al.] [Fig.6]

Inhibitors:-

➤ *Inhibition through antioxidants:-*

The activation of NF-1 cascade can be triggered by TNF α (TRAFs, NIK, IKKs) that are anti-oxidative in nature and enable the identification of the redox-responsive target(s). It has also been reported that various agents like TNF α , IL-1, phorbol ester (e.g., PMA), lipopolysaccharide (LPS) or ultraviolet (UV) light can also induce oxidative stress as well as induce NF- κ B. Reducing agents like dithiothreitol and β -mercaptoethanol have also been studied to reverse the direct and specific interference of DNA binding by NF- κ B. [Epinat JC et al.][Fig.6]

➤ *Inhibition through ADAR-2 molecules:-*

ADAR3 is only expressed in the brain and catalytically inactive. ADARs bind to dsRNA through their N-terminal RNA-binding domains and convert adenosine into inosine via their C-terminal catalytic domain (deaminase domain). Inosine is recognized as guanosine by both splicing and translational machineries. Consequently, ADARs have the potential to 'fine-tune'/modulate genomic information with important consequences on editing RNA and brain proteins (CDC14B/Skp2/p21/p27) [Galeano F et al.]. [Fig.6]

➤ *Inhibition through TRIM :-*

TRIM3 binds with the CDK inhibitor p21^{WAF1/CIP1}. TRIM3 is a tumor suppressor mapping to chromosome 11p15.5 and it blocks the tumor growth by sequestering p21 and preventing it from facilitating the accumulation of cyclin D1-cdk4 [Liu Y et al.]. [Fig.6]

➤ *Valproic Acid and Etoposide inhibition:-*

These are the histone deacetylase inhibitors which modulates the gene expression and sensitize the GBM cell-lines. They act alone or in combination to different GBM cell-lines, i.e., U87, LN 18 and U251. It was seen that the Valproic acid enhanced the G1 accumulation of U87 and G2/M accumulation of U251 AND LN 18 Cells. It has been also studied that both lead to the increase in Caspase-3 activity leading to apoptosis. [Tseng JH et al.] [Fig.6]

➤ *Inhibition through MiRNA:-*

Several studies with miR-21 showed that when miR-specific antisense oligonucleotide when treated with GBM Cell-lines U251 (mutant PTEN) and LN 229 (wild-type PTEN) showed decreased expression of EGFR, activated AKT, Cyclin-D and Bcl-2. Similarly, miR-26a mimics decreased PTEN protein levels and increased AKT phosphorylation. miR-451 upon downregulation led to increase p27 levels, which thus shows the invasive ability, induced apoptosis in GBM cells in vitro, which shows that the miR-451 affects GBM cells via regulation of the PI3K/AKT signaling pathway. It is also seen that the miR-7 directly inhibits EGFR expression via its 3'-UTR and independently suppresses the AKT pathway via targeting upstream regulators (IRS-1 and IRS-2). miR-7 downregulates EGFR mRNA and protein expression in GBM cell-lines via two of the three predicted sites, which induces cell-cycle arrest leading to apoptosis. miR-128 downregulation and BMI-1 expression leads to decrease in H3K27 methylation and modulation of p21 and AKT and inhibits cell-cycle arrest. [Papagiannakopoulos T et al.] [Fig.6]

VI. CONCLUSION

- ❖ The EGFR and p21 plays a major role in the regulation of Glioblastoma.
- ❖ They supports the major AKT signalling with the NF-1 and TK signalling.
- ❖ It has also been found that the JAK-STAT and mTOR signalling is also found along with AKT signalling.

- ❖ The AKT signalling provides the huge vascular and cellular proliferation ,with the NF-1 and TK signalling providing cellular differentiation.
- ❖ The STAT signalling promotes the phosphorylation of the cellular receptors, helping in cellular arrest, invasiveness, thus the Metastasis.
- ❖ Role of Calmodulin is also been found with the GS-Cell lines.
- ❖ It has been reported that the Glioblastoma signalling can be inhibited with the help of antioxidants, ADAR molecules, TRIM molecules, Valproic acid , etoposide and the mi RNA .

VII. ACKNOWLEDGEMENT

I am sincerely thankful to Tarun Gupta and Ms. Madhurima Mishra for helping me a lot in completing this review. It is my honor to showcase every effort that has been done for completing this review on the topic” Deciphering role of EGFR and different inhibitory mechanisms in Glioblastoma” He has supported me a lot in finding the related researches of the following and also supported in creating the images , and checking the plagiarism .

VIII. REFERENCES

Andrei L. Gartel and Angela L. Tyner . The Role of the Cyclin-dependent Kinase Inhibitor p21 in Apoptosis 1 Supported in part by NIH Grant R01 DK56283 (to A. L. T.) for the p21 research and Campus Research Board and Illinois Department of Public Health Penny Severns Breast and Cervical Cancer grants (to A. L. G.).¹ *Mol Cancer Ther* 2002;1:639-649.

Antony M. Carr Piecing Together the p53 Puzzle *Science* 10 Mar 2000: Vol. 287, Issue 5459, pp. 1765-1766 DOI: 10.1126/science.287.5459.1765.

Bhunia AK, Piontek K, Boletta A, et al. PKD1 induces p21(waf1) and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2. *Cell*. 2002 Apr 19;109(2):157-68. doi: 10.1016/s0092-8674(02)00716-x.PMID:. 12007403

Blume-Jensen, P., Hunter, T. Oncogenic kinase signalling. *Nature* 411, 355–365(2001). <https://doi.org/10.1038/35077225>

Cantley LC. The phosphoinositide 3-kinase pathway. *Science*. 2002 May 31;296(5573):1655-7.doi: 10.1126/science.296.5573.1655.PMID:1204016.

Carracedo A, Ma L, Teruya-Feldstein J, et al . Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest*. 2008 Sep;118(9):3065-74. doi: 10.1172/JCI34739. PMID: 18725988; PMCID: PMC2518073.

Dongsun Cao ¹, Philip A. Bromberg ¹, and James M. Samet ² Diesel Particle–Induced Transcriptional Expression of p21 Involves Activation of EGFR, Src, and Stat3 <https://doi.org/10.1165/rcmb.2008-0455OC> PubMed: 19329556

El-Deiry WS. Insights into cancer therapeutic design based on p53 and TRAIL receptor signaling. *Cell Death Differ*. 2001 Nov;8(11):1066-75. doi: 10.1038/sj.cdd.4400943. PMID: 11687885.

Epinat JC, Gilmore TD. Diverse agents act at multiple levels to inhibit the Rel/NF-kappaB signal transduction pathway. *Oncogene*. 1999 Nov 22;18(49):6896-909. doi: 10.1038/sj.onc.1203218. PMID: 10602465.

Gabrielle L. Brown , Matthew Eckley, Kurt A. Wargo, A Review of Glioblastoma Multiforme *US Pharm*. 2010;35(5)(Oncology suppl):3-10

Galeano, F., Rossetti, C., Tomaselli, S. et al. ADAR2-editing activity inhibits glioblastoma growth through the modulation of the CDC14B/Skp2/p21/p27axis. *Oncogene* 32, 998–1009(2013). <https://doi.org/10.1038/onc.2012.125>

Gartel AL, Tyner AL. The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. *Mol Cancer Ther.* 2002 Jun;1(8):639-49. PMID: 12479224.

Guha A, Lau N, Huvar I, Gutmann D, Provias J, Pawson T, Boss G. Ras-GTP levels are elevated in human NF1 peripheral nerve tumors. *Oncogene.* 1996 Feb 1;12(3):507-13. PMID: 8637706.

Han C, Liao X, Qin W, et al. EGFR and SYNE2 are associated with p21 expression and SYNE2 variants predict post-operative clinical outcomes in HBV-related hepatocellular carcinoma. *Sci Rep.* 2016 Aug 9;6:31237. doi: 10.1038/srep31237. PMID: 27502069; PMCID: PMC4977508.

J. Wade Harper, Guy R. Adami, Nan Wei, et al. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases, *Cell*, Volume 75, Issue 4, 1993, Pages 805-816, ISSN 0092-8674, [https://doi.org/10.1016/0092-8674\(93\)90499-G](https://doi.org/10.1016/0092-8674(93)90499-G).

Kanu OO, Hughes B, Di C, et al Glioblastoma Multiforme Oncogenomics and Signaling Pathways. *Clin Med Oncol.* 2009 Apr 8;3:39-52. doi: 10.4137/cmo.s1008. PMID: 19777070; PMCID: PMC2748278.

Kristyn Galbraith, , Ashwani Kumar, MS, Kalil et al. , Molecular Correlates of Long Survival in IDH-Wildtype Glioblastoma Cohorts, *Journal of Neuropathology & Experimental Neurology*, Volume 79, Issue 8, August 2020, Pages 843–854, <https://doi.org/10.1093/jnen/nlaa059>

Lee JS, Xiao J, Patel P, Schade J, et al. A novel tumor-promoting role for nuclear factor IA in glioblastomas is mediated through negative regulation of p53, p21, and PAI1. *Neuro Oncol.* 2014 Jan;16(2):191-203. doi: 10.1093/neuonc/not167. Epub 2013 Dec 4. PMID: 24305710; PMCID: PMC3895381.

Liffers K, Lamszus K, Schulte A. EGFR Amplification and Glioblastoma Stem-Like Cells. *Stem Cells Int.* 2015;2015:427518. doi: 10.1155/2015/427518. Epub 2015 Jun 2. PMID: 26136784; PMCID: PMC4468289.

Liu Y., Raheja, R., Yeh, N. et al. TRIM3, a tumor suppressor linked to regulation of p21^{Waf1/Cip1}. *Oncogene* 33, 308–315(2014). <https://doi.org/10.1038/onc.2012.596>

Louis DN. A molecular genetic model of astrocytoma histopathology. *Brain Pathol.* 1997 Apr;7(2):755-64. doi: 10.1111/j.1750-3639.1997.tb01062.x. PMID: 9161727.

Matakidou A, Eisen T, Houlston RS. TP53 polymorphisms and lung cancer risk: a systematic review and meta-analysis. *Mutagenesis.* 2003 Jul;18(4):377-85. doi: 10.1093/mutage/geg008. PMID: 12840112.

Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *FEBS J.* 2010 Jan;277(2):301-8. doi: 10.1111/j.1742-4658.2009.07448.x. Epub 2009 Nov 18. PMID: 19922469.

Miyauchi H, Minamino T, Tateno K, et al. Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway. *EMBO J.* 2004 Jan 14;23(1):212-20. doi: 10.1038/sj.emboj.7600045. Epub 2004 Jan 8. PMID: 14713953; PMCID: PMC1271675.

Mohammed A Azab, Abdurraheem Alomari, Ahmed Y Azzam, Featuring how calcium channels and calmodulin affect glioblastoma behavior. A review article, *Cancer Treatment and Research Communications*, Volume 25,2020, 100255, ISSN 2468-2942, <https://doi.org/10.1016/j.ctarc.2020.100255>.

Muñoz-Hidalgo L, San-Miguel T, Megías J, et al. Somatic copy number alterations are associated with EGFR amplification and shortened survival in patients with primary glioblastoma. *Neoplasia.* 2020 Jan;22(1):10-21. doi: 10.1016/j.neo.2019.09.001. Epub 2019 Nov 18. PMID: 31751860; PMCID: PMC6864306.

Nichols, R.J., Haderk, F., Stahlhut, C. et al. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. *Nat Cell Biol* 20, 1064–1073(2018). <https://doi.org/10.1038/s41556-018-0169-1>

Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res.* 2004 Oct 1;64(19):6892-9. doi: 10.1158/0008-5472.CAN-04-1337. PMID: 15466178.

Papagiannakopoulos T, Shapiro A, Kosik KS. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. *Cancer Res.* 2008 Oct 1;68(19):8164-72. doi: 10.1158/0008-5472.CAN-08-1305. PMID: 18829576.

Pietsch T, Wiestler OD. Molecular neuropathology of astrocytic brain tumors. *J Neurooncol.* 1997 Dec;35(3):211-22. doi: 10.1023/a:1005843913095. PMID: 9440021.

Sandra H. Bigner, Peter A. Humphrey, Albert J. Wong et al Characterisation of Epidermal growth factor receptor in Human Glioma Cell lines and Xenografts *Cancer Res* 1990;50:8017-8022.

Shamloo, B.; Usluer, S. p21 in Cancer Research. *Cancers* 2019, *11*,1178. <https://doi.org/10.3390/cancers11081178>

Shapira, S., Barkan, B., Fridman, E. et al. The tumor suppressor neurofibromin confers sensitivity to apoptosis by Ras-dependent and Ras-independent pathways. *Cell Death Differ* 14, 895–906 (2007). <https://doi.org/10.1038/sj.cdd.4402057>

Tseng JH, Chen CY, Chen PC, et al. Valproic acid inhibits glioblastoma multiforme cell growth via paraoxonase 2 expression. *Oncotarget.* 2017;8(9):14666-14679. doi:10.18632/oncotarget.14716

Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer.* 2002 Jul;2(7):489-501. doi: 10.1038/nrc839. PMID: 12094235.

Vogelstein, B., Lane, D. & Levine, A. Surfing the p53 network. *Nature* 408, 307–310 (2000). <https://doi.org/10.1038/35042675>

Walid MS. Prognostic factors for long-term survival after glioblastoma. *Perm J.* 2008 Fall;12(4):45-8. doi: 10.7812/tpp/08-027. PMID: 21339920; PMCID: PMC3037140.

Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006 Feb 10;124(3):471-84. doi: 10.1016/j.cell.2006.01.016. PMID: 16469695.