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PTEN – A MOLECULAR MARKER FOR THE DIAGNOSIS OF GLIOMA

BABUL REDDY TATIREDDY

Babul Reddy Tatireddy*

Research Scholar, Department Of Biotechnology, Rayalaseema University, Kurnool (Dist), Andhra Pradesh, India. *Corresponding Author

ABSTRACT Glioblastoma is an uncommon primary brain tumor accounts for upto 15.4% of all primary brain tumors. Although several modern therapies against glioma are discovered, management is still a critical concern. The existing therapies were relatively inconsistent; moreover, the procedures as well are difficult to treat. These ineffective therapeutic approaches led researchers to identify the novel biomarkers that can be implemented with the existing therapies for better management. In this view, along with biomarker research, a literature search for exosomal *PTEN* detection in glioblastoma was conducted and the recent studies observed that exosomes can transport tumor-suppressive proteins (*PTEN*) and oncogenic mRNAs, microRNAs to a recipient cell, which subsequently activates the downstream signaling pathways and influences the cellular phenotype. These exosomes facilitate the transfer of *PTEN* released from tumor cells to receipt cells that leads to tumor progression. Similarly, glioma was also associated with a reduction or loss of *PTEN* expression. Hence, our present review aimed to provide a holistic picture of glioblastoma, its pathogenesis and novel biomarkers with an emphasis on PTEN detection in exosomes for the early identification of glioblastoma.

KEYWORDS : Glioblastoma; Exosomes; PTEN; Biomarkers; Tumors

INTRODUCTION

Glioblastoma, a rare lethal primary brain tumor, accounts for 15.4% of all primary brain tumors and 60% of all brain tumor cases. Despite several modern therapies against glioma, it is still a devasting disease. Poor prognosis with a median survival rate of 14.6 months from the diagnosis makes it a crucial health issue.[1] It was reported that grade IV glioblastoma was the most prevalent form of glioma with an incidence rate of 3.2 per 100,000 population. [2]

The common symptoms among glioblastoma patients include vomiting, blood pressure, nausea, drowsiness and headaches caused by increased blood pressure in the brain. However, based on the site of tumor, symptoms may vary from patient to patient.[3] Several cellular/molecular pathways are involved in the pathogenesis of primary glioblastoma. The hallmark alterations of pathways that trigger primary glioblastoma include overexpression of mouse double minute 2 (MDM2), growth factor receptor (EGFR) gene mutation and amplification, deletion of p16 and loss of heterozygosity (LOH) of chromosome 10q holding phosphatase and tensin homolog (*PTEN*) and TERT promoter mutation. An integrated analysis reported that genetic alterations mainly affects mainly three signaling pathways such as receptor tyrosine kinase/ RAS/ PI3K pathway (altered in 88% of glioblastoma), P53 pathway (7% of glioblastoma) and RB signaling pathway (altered in 78% of glioblastoma).[1]

Although several modern therapies against glioma are discovered, management is still a critical concern. The existing therapies were relatively inconsistent; moreover, the procedures as well are difficult to treat. Therefore, the identification of novel biomarkers and strategies that can be implemented along with existing therapies to deal with glioblastoma is the need of an hour.[3]Hence, our present review was aimed to provide a holistic picture glioblastoma, its pathogenesis and novel biomarkers with an emphasis on several aspects of exosomesand *PTEN* detection in exosomes for the early identification of glioblastoma.

Role of exosomes in glioma

Extracellular vesicles mediate the intercellular communication for both normal as well as tumor cells. Exosomes are one such type of extracellular vesicles that mediate intercellular communication. Exosomes are nanosized spherical particles with a lipid bilayer that are mainly known as biological vehicles for nucleic acids, proteins, growth factors, ions, receptors, and lipids. Whereas exosomes released by the tumor cells facilitates the transport of signaling molecules, receptors, non-coding RNA (miRNA), oncogenic genes (EGFRvIII), and tumor suppressor protein (*PTEN*). All these components modify the surrounding microenvironment and supportthe tumor progression in the receipt cells and lead to the development of tumors in different stages of cancer/development of various types of cancer. Exosomes released from tumor cells that are rich in proteins and nucleic acids also serve as a source of tumor antigens and inhibit the immune system and promotes tumor cell growth, metastasis, and drug resistance.[3,4]

Studies reported that exosomes were one of the key components in the biogenesis of glioblastoma that serves as a tumor biomarker for tracking cancer progression. Recent studies also discovered exosomes can transport tumor-suppressive and oncogenic mRNAs, microRNAs, and proteins to a receipt cell, which subsequently activates the downstream signaling pathways and influences the cellular phenotype. It was proposed that tumor-derived exosomes containing miRNAs serve as an ideal prognostic marker for tumor. Studies also reported that tumor-derived exosomal miRNA was an ideal prognosticmarker for glioblastoma, esophageal cancer, lung cancer, leukemia, breast cancer, ovarian cancer etc [3]. Research has recently found these exosomes facilitate the transfer of *PTEN* released from tumor cells to receipt cells leads to tumor progression. Similarly, glioma was also associated with a reduction or loss of *PTEN* expression.[3,5,6]

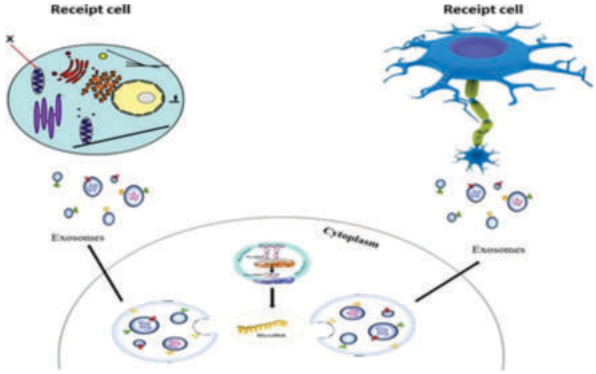

Figure 1: Schematic representation of exosomes releasing from tumor cell and reaching the receipt cells. [Source reference 3]

PTEN gene: An overview

PTEN (a phosphatase and tensin analog) is a tumor

suppressor protein encoded by the *PTEN* gene in human beings. This is the second most commonly mutated gene after p53 in most of the human cancers at high frequency. *PTEN* protein converts dephosphorylate phosphatidylinositol (3,4,5)-trisphosphate to biphosphate product (PIP_2) through phosphatase enzyme.[7] This phosphatase enzyme regulates the cell cycle and prevents the cells from rapidly growing or dividing.[8] This dephosphorylation, in turn, inhibits phosphatidylinositol 3-kinase signaling pathway and plays a vital role in cell growth, survival, and migration.[7]

The two major domains of *PTEN* include N-terminal tyrosine phosphatase domain and the C-terminal domain. Tumor suppressor action is mainly attributed to its N-terminal lipid phosphatase activity and >40% of *PTEN* mutations occur at its C-terminal domain. The tyrosine phosphatase domain has an active site and that perform the enzymatic action of the protein, whereas C2 binds to the phospholipid membrane. These two binds to the membrane and bring the active site to phosphatidylinositol (3,4,5)-trisphosphate to dephosphorylate.[7]

During tumor growth, mutations and deletions of *PTEN* gene inactivate the enzymatic activity leading to increased cell proliferation and reduced cell death. The defective *PTEN* protein encoded by defective *PTEN* gene neither unable to stop cell proliferation nor abnormal cells to die. In the mutated *PTEN* gene, phosphatase and tensin homolog was deleted on chromosome 10 (*PTEN*) and negatively control the phosphatidylinositol 3-kinasesignaling pathway. Research has found that *PTEN* mutations were mostly involved in two types of human cancers: glioblastomas and endometrial carcinomas.[9] And the mutated PTEN was transported to receipt cells through exosomes.[7]

Role of exosomal PTEN gene in glioblastoma

Studies conducted by Zhang et al.[6], and Lotan et al.[5], found *PTEN* deletion as the prognostic marker in breast cancer and prostate cancer. However, there is a lack of studies to report the role of *PTEN* mutation in glioblastoma. However, a recent study conducted by Manda et al.[10] reported the role of exosomal *PTEN* detection in glioblastoma patients. Serum exosomal RNA and biopsy tissue RNA isolated from the commercial kits were used for the study. *PTEN* expression was quantified using a semi-nested PCR with GAPDH as an internal control. The results reported that *PTEN* expression can be detected in exosomes of glioma. The study concluded that *PTEN* detection through a blood test could be a promising clinical tool for early identification of glioblastoma. As *PTEN* expression was found in glioblastoma patients, the research should be focused on developing therapeutic agents or therapies that target the exosomal *PTEN* expression in treating glioblastoma.

Glioma signaling pathways

EGFR/RAS/NF1/PTEN/PI3K Pathway

Growth receptors (e.g. EGFR, PDGFRA) become activated through the binding of their respective ligands (e.g. EGF, TGF-α, PDGF) to their extracellular domain, which results in recruitment of phosphatidylinositol 3-kinase (PI3K) to the cell membrane. The PI3K complex is composed of a catalytically active protein p110α and a regulatory protein p85α.

PI3K phosphorylates phosphatidynositol-4,5-bisphosphate (PIP2) to the respective 3-phosphate (PIP3) which activates downstream effector molecules such as AKT (protein kinase B) and mTOR, the mammalian target of rapamycin. This results in cell proliferation and increased cell survival by blocking apoptosis. PTEN inhibits the PIP3 signal, thereby inhibiting cell proliferation[11].

The NF1tumor suppressor gene encodes neurofibromin that functions primarily as a RAS negative regulator, and also plays a role in adenylate cyclase and AKT–mTOR mediated pathways. EGFR amplification occurs in approximately 40% of primary glioblastomas but is very rare in secondary glioblastomas[12,13]. The PTEN gene is mutated in 15–40% of primary glioblastomas, but rare in secondary glioblastomas[14].

PIK3CA mutations and amplification are rare (5% and 13%) in both primary and secondary glioblastomas. About two-thirds (63%) of primary glioblastomas and one-third of secondary glioblastomas showed alterations in at least one of EGFR, PTEN, or PIK3CA genes[15].

TP53/MDM2/MDM4/$p14^{ARF}$ Pathway

The TP53 gene encodes a protein that plays a role in several cellular processes, including the cell cycle, response of cells to DNA damage, cell death, and cell differentiation[16]. Following DNA damage, TP53 is activated and induces transcription of genes such as $p21^{Waf1/Cip1}$[17]. MDM2 is induced by wild-type TP53, which binds to mutant and wild-type TP53, thereby inhibiting the ability of wild-type TP53 to activate transcription[18]. The $p14^{ARF}$ binds to MDM2 and inhibits MDM2-mediated TP53 degradation and trans-activational silencing[19].

MDM4 (also called MDMX) also regulates TP53 activity [20]. $p14^{ARF}$ is negatively regulated by TP53[21]. Thus, loss of normal TP53 function may result from alterations in TP53, MDM2, MDM4, or $p14^{ARF}$. TP53mutations are significantly more frequent in secondary glioblastomas than in primary glioblastomas (65% vs 28%) [22]. At least one alteration in the TP53/MDM2/$p14^{ARF}$ pathway. (TP53 mutations, $p14^{ARF}$ homozygous deletion, $p14^{ARF}$ promoter methylation, MDM2 amplification) was observed in approximately 50% of primary glioblastomas, and in >70% of secondary glioblastomas[23].

$p16^{INK4a}$/CDK4/RB1 pathway

The RB1 protein controls the progression through G1 into the S-phase of the cell cycle. The CDK4□cyclin D1 complex phosphorylates the RB1 protein, thereby inducing the release of the E2F transcript factor that activates genes involved in the G1 to S transition. $p16^{INK4a}$ binds to CDK4, inhibits the CDK4/cyclin D1 complex, and thus inhibits the G1 S transition[24]. Therefore, loss of normal RB1 function may result from altered expression of any of the $p16^{INK4a}$, CDK4, or RB1 genes.

Homozygous deletion of the $p16^{INK4a}$ gene, CDK4 amplification, and loss of RB1 was largely mutually exclusive; the overall frequency of these alterations was 50% in primary glioblastomas and approximately 40% in secondary glioblastomas[25].

PI3K–PTEN-Akt-mTOR Signaling Pathway in glioma

The PI3K-PTEN-Akt-mTOR pathway regulates normal cellular functions that can also be critical in tumorigenesis, including cellular proliferation, apoptosis, cell invasion, and mobility. Activation of phosphatidylinositol 3-kinase (PI3K) complex, the first intracellular member of this signaling pathway, is regulated by many growth factors in conjunction with their receptors, such as epidermal growth factor (EGF) and its receptor (EGFR). Activated PI3K generates phosphatidy linositol 3, 4,5-triphosphate, which in turn activates Akt. Akt then activates mTOR, which integrates several upstream signals into effector actions on multiple downstream targets involved in cell growth and division. Tumor suppressors such as PTEN inhibit this pathway[26].

PTEN (phosphatase and tensin homologue, located on chromosome TEN) acts as a tumor suppressor gene, and mutations of PTEN lead to the development of many cancers including gliomas[27].

Diagnosis of Glioma

Clinical signs and symptoms

1. The symptoms and signs produced by gliomas vary according to the location of the tumor. In case of tumors located in or subjacent to cortical regions, the symptoms and signs relate to the functions of the brain regions affected. Patients present with progressive motor or sensory disturbances, language dysfunction, visual field abnormalities, or focal seizures. As the magnitude of tumor increases, the edema adjoining the tumor also increases, resulting in increased intracranial pressure and subsequent headaches. The headaches associated with increased intracranial pressure are typically worse when the patient is recumbent. When intracranial pressure rises to a critical threshold, changes in blood pressure due to dysfunctional autonomic reflexes may produce a syndrome of position-evoked crescendo headache, visual obscurations, lightheadedness, and exacerbation of focal symptoms. This cluster of symptoms is associated with intracranial pressure waves and is usually associated with papilledema[28].

2. A neurological examination: This examination tests vision, hearing, speech, strength, sensation, balance, coordination, reflexes and the ability to think and remember[29].

3. Scans of the brain: Magnetic resonance imaging (MRI) and computed tomography (CT or CAT scan) are the most common scans used to diagnose brain tumors. Gadolinium-enhanced magnetic resonance imaging (MRI) is the preferred modality because of its resolution and enhancement with contrast agents. If MRI cannot be performed (eg; in patients with metallic implants, embedded devices, or claustrophobia), head and neck spine tomography (CT) is acceptable, although the resolution is not as high as MRI and it cannot adequately assess lesions in the posterior fossa and spine [30].

4. Biopsy: This procedure involves examination of a small section of the tumor under a microscope. Depending on the location of the tumor, the biopsy and removal of the tumor may be performed at the same time[31].

Limitation in the diagnosis of glioma

Conventional imaging techniques like MRI and CT reveal morphological information but are of limited value for the assessment of more specific and reproducible information about biology and activity of the tumor[32]. Biopsies, on the other hand, are often performed for diagnostic purposes before treating patients whose imaging studies highly suggest glioma. It is frequently inaccurate in providing a correct diagnosis and is associated with additional risk and cost[33].

Tumor Marker Test for gliomas - (PTEN)

This is a test in which a sample of blood, urine or tissue is taken to measure the presence of certain substances that organs, tissues or tumor cells may produce when there are tumors present. There are presently no tumor marker tests in use for diagnosis of gliomas[34].

Cancer cells release vesicles into their surroundings. Microvesicles are one variety of shed vesicles, generated through the direct budding of the cell membrane. Exosomes are another, relatively smaller type of vesicle which is stored in multivesicular bodies and released when the multivesicular body fuses with the cell membrane[35,36].

Exosome content reflects its cellular source. Interestingly, these contents might include oncogenic proteins or tumor suppressor proteins. Consequently, one could predict that molecules transferred by exosomes bestow an acquired phenotype to acceptor cells, leading to positive or negative effects in relation to tumor progression dependent on the nature of the molecules transferred. The freight of exosomes might thus alter the balance between oncogenic and tumor suppressor characteristics. The analysis of such cargo could indicate the expression status of tumor suppressor proteins like PTEN in malignant cells without having to directly sample the malignancy[37,38].

In gliomas, PTEN is deleted due to LOH of chromosome 10q in 50-70% of primary cases and 54–63% of secondary glioma. It is also mutated in 14–47% of primary glioma[39]. This altered PTEN expression can be used as a diagnostic marker for gliomas.

CONCLUSION

It is well-known that exosomes mediate the intercellular communication for both normal as well as tumor cells. There is also evidence that exosomes released by the tumor cells facilitates the transport of signaling molecules, receptors, non-coding RNA (miRNA), oncogenic genes (EGFRvIII), and tumor suppressor protein (*PTEN*) and modify the surrounding microenvironment and supports the tumor progression in the receipt cells. However, the research regarding the expression of *PTEN* in exosomes is still in its infancy stage. Hence, further research is required to establish the prognostic role of *PTEN* expression in glioblastoma to develop targeted therapies based on exosomal delivery to reduce side-effects and improve outcome in glioblastoma patients.

This review highlights how in modern neuro-oncology, molecular markers like PTEN, a tumor suppressor protein can aid in tumor evaluation guiding the clinical decision. In contrast to the histology, to define tumor entities we formulated a concept of how diagnoses of tumors of the central nervous system (CNS) should be structured in the molecular era. PTEN detection can serve as a promising clinical tool for early tumor diagnosis.

Conflicts of Interest

Nothing to declare.

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REFERENCES

1. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: A review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pacific journal of cancer prevention: APJCP*. 2017;18:3.
2. Nørøxe DS, Poulsen HS, Lassen U. Hallmarks of glioblastoma: a systematic review. *ESMO open*. 2016;1:e000144.
3. Saadatpour L, Fadaee E, Fadaei S, Mansour RN, Mohammadi M, Mousavi S, et al. Glioblastoma: exosome and microRNA as novel diagnosis biomarkers. *Cancer Gene Therapy*. 2016;23:415.
4. Gourlay J, Morokoff A, Luwor R, Zhu H-J, Kaye A, Stylli S. The emergent role of exosomes in glioma. *Journal of Clinical Neuroscience*. 2017;35:13-23.
5. Lotan TL, Heumann A, Rico SD, Hicks J, Lecksell K, Koop C, et al. PTEN loss detection in prostate cancer: comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. *Oncotarget*. 2017;8:65566.
6. Zhang HY, Liang F, Jia ZL, Song ST, Jiang ZF. PTEN mutation, methylation and expression in breast cancer patients. *Oncology letters*. 2013;6:161-8.
7. Yin Y, Shen W. PTEN: a new guardian of the genome. *Oncogene*. 2008;27:5443.
8. Putz U, Howitt J, Doan A, Goh C-P, Low L-H, Silke J, et al. The tumor suppressor PTEN is exported in exosomes and has phosphatase activity in recipient cells. *Sci Signal*. 2012;5:ra70-ra.
9. Dominique B, Michel L. Mutations of the Human PTEN Gene. *Human mutation*. 2000;16:109122.
10. Sasidhar VM, Yogesh K, Babul R, Tati R, Alok R, Amitava R. Exosomal pten detection in glioma patients. *Neuro Oncol*. 2015;17.
11. Mellinghoff IK, Wang MY, Vivanco Iet al.Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med2005;353: 2012–24
12. Ekstrand AJ, Sugawa N, James CD, Collins VP. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and or C-terminal tails.Proc Natl Acad Sci USA1992;89: 4309–13
13. Watanabe K, Tachibana O, Sato K, Yonekawa Y, Kleihues P, Ohgaki H. Overexpression of the EGF receptor andp53mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol1996;6: 217–24.
14. Knobbe CB, Merlo A, Reifenberger G. Pten signaling in gliomas. Neuro Oncol2002;4: 196–211.
15. Kita D, Yonekawa Y, Weller M, Ohgaki H. PI3KCA alterations in primary(de novo)and secondary glioblastomas.Acta Neuropathol2007;113:295–302
16. Bogler O, Huang HJ, Kleihues P, Cavenee WK. The p53 gene and its role in human brain tumors.Glia1995;15: 308–27.
17. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression.Genes Dev1999;13: 1501–12.

18. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation.Cell1992;69: 1237–45.
19. Pomerantz J, Schreiber-Agus N, Liegeois NJ et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53.Cell1998;92: 713–23.
20. Toledo F, Wahl GM. MDM2 and MDM4: p53 regulators as targets in anticancer therapy.Int J Biochem Cell Biol2007;39: 1476–82
21. Stott FJ, Bates S, James MCet al.The alternative product from the human CDKN2Alocus, p14ARF, participates in a regulatory feedback loop with p53 and MDM2.EMBO J1998;17: 5001–14.
22. Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol2005;64: 479–89.
23. Zawlik I, Kita D, Vaccarella S, Mittelbronn M, Franceschi S, Ohgaki H. Common polymorphisms in the MDM2 and TP53 genes and the relationship between TP53 mutations and patient outcomes in glioblastomas.Brain Pathol 2009;19: 188–94
24. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression.Genes Dev1999;13: 1501–12.
25. Biernat W, Tohma Y, Yonekawa Y, Kleihues P, Ohgaki H. Alterations of cell cycle regulatory genes in primary (de novo) and secondary glioblastomas. Acta Neuropathol1997;94: 303–9.
26. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell. 1998;95:29–39.
27. Malati T. Tumour markers: An overview. Indian Journal of Clinical Biochemistry. 2007 Sep 1;22(2):17-31.
28. Ahmed, R., Oborski, M. J., Hwang, M., Lieberman, F. S., & Mountz, J. M. (2014). Malignant gliomas: current perspectives in diagnosis, treatment, and early response assessment using advanced quantitative imaging methods. Cancer Management and Research, 6, 149–170.
29. Lee J, Park SH, Kim YZ. Prognostic Evaluation of Neurological Assessment of the Neuro-Oncology Scale in Glioblastoma Patients. Brain tumor research and treatment. 2018 Apr 1;6(1):22-30.
30. Gao H, Jiang X. Progress on the diagnosis and evaluation of brain tumors. Cancer Imaging. 2013;13(4):466.
31. Meshkini A, Shahzadi S, Alikhah H, Naghavi-Behzad M. Role of stereotactic biopsy in histological diagnosis of multiple brain lesions. Asian journal of neurosurgery. 2013 Apr;8(2):69.
32. la Fougere C, Suchorska B, Bartenstein P, Kreth FW, Tonn JC. Molecular imaging of gliomas with PET: opportunities and limitations. Neuro-oncology. 2011 Jul 13;13(8):806-19.
33. Jackson RJ, Fuller GN, Abi-Said D, Lang FF, Gokaslan ZL, Shi WM, Wildrick DM, Sawaya R. Limitations of stereotactic biopsy in the initial management of gliomas. Neuro-oncology. 2001 Jul 1;3(3):193-200.
34. Malati T. Tumour markers: An overview. Indian Journal of Clinical Biochemistry. 2007 Sep 1;22(2):17-31.
35. Mathivanan S, Ji H, Simpson RJ (2010) Exosomes: extracellular organelles important in intercellular communication. Journal of proteomics 73: 1907–1920.
36. Nieuwland R, Sturk A (2010) Why do cells release vesicles? Thrombosis research 125 Suppl 1S49–51.
37. Al-Nedawi K, Meehan B, Kerbel RS, Allison AC, Rak J (2009) Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proceedings of the National Academy of Sciences of the United States of America 106: 3794–3799.
38. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, et al. (2008) Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nature cell biology 10: 619–624.
39. Simpson L., Parsons R. PTEN: Life as a tumor suppressor. Exp. Cell Res. 2001;264:29–41. doi: 10.1006/excr.2000.5130.