Efficacy of Quaratusugene Ozeplasmid TUSC2 Gene Therapy in Glioblastoma

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Abstract # 130

Abstract

Background: Tumor Suppressor Candidate 2 (TUSC2) is an established tumor suppressor in multiple cancer types. TUSC2 overexpression in lung cancer cells significantly decreases lung cancer growth and induces anti-tumor immunity, indicating the utility of TUSC2 gene therapy for lung cancer. Quaratusugene ozeplasmid (Quar Oze), TUSC2 plasmid DNA complexed with DOTAP-Cl: Cholesterol liposome solution, has been tested in lung cancer clinical trials including ONC-001, ONC-002, and active Acclaim-1 Phase II trial with osimertinib, Acclaim-2 Phase 1/2 trial with pembrolizumab, and Acclaim-3 Phase 1/2 study in combination with atezolizumab. While the role of TUSC2 in lung cancer is well established and TUSC2 restoration is being evaluated clinically, its importance in glioblastoma (GBM) was not elucidated until recently. In our 2022 study (Cancer Letters, 531:124-135, 2022), we reported TUSC2 as a novel tumor suppressor for GBM, the most common and deadliest primary brain tumor in adults associated with dismal prognosis. We reported that TUSC2 protein is preferentially degraded in GBM compared to normal brain cells, through NEDD4 E3 ligase polyubiquitination and subsequent proteasomal degradation. CRISPR/Cas9-mediated TUSC2-knockout promoted *in vivo* progression of GBM intracranial xenografts. TUSC2 expression restoration induced apoptosis and inhibited the development of GBM intracranial xenografts *in vivo*, suggesting that Quar Oze may suppress GBM growth and progression.

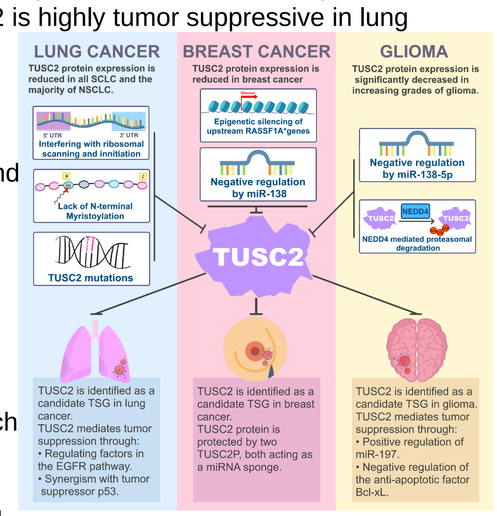
Material and Methods: Patient-derived GBM cell lines and patient-derived glioma stem cell (PD-GSC) lines were used. Quar Oze was used to restore TUSC2 expression. CellTiter Blue Cell Survival assay, neurosphere-forming assay, Annexin V staining apoptosis assay, and scratch-wound migration assay were used. Student's t-test was used to calculate p-values.

Results: We observed that Quar Oze significantly reduced GBM cell viability. In our 2022 study, we reported that TUSC2 inhibits PD-GSCs. Here, we found that Quar Oze strongly suppressed the glioma stem cell population that is highly resistant to therapy. Furthermore, since our 2022 study showed that ectopic TUSC2 expression promotes apoptosis whereas TUSC2 knockdown decreases apoptosis, we validated that Quar Oze indeed induced significant apoptosis in GBM and PD-GSC cells. Since GBM cells are highly infiltrative, we next determined if Quar Oze had the ability to inhibit GBM cell migration. The results of our migration assay demonstrated that Quar Oze suppressed GBM cell migration independent of its ability to suppress cell viability.

Conclusions: We report, for the first time, that Quar Oze demonstrates promising *in vitro* efficacy in GBM and PD-GSCs. These results serve as supporting evidence for further evaluation of its *in vivo* anti-tumor efficacy in gliomas using mouse models.

Background

Tumor Suppressor Candidate 2 (TUSC2) was first discovered as a potential tumor suppressor gene (TSG) residing in the frequently deleted 3p21.3 chromosomal region in non-small cell lung cancer (NSCLC) [1]. Multiple studies have demonstrated that TUSC2 is highly tumor suppressive in lung cancer [2-7]. Injection of a REQORSA®, TUSC2 plasmid complexed inN-[1-(2,3-dioleoyloxy)propyl]-N,N,N- (DOTAP): Cholesterol trimethylammoniumchloride liposomes resulted in decreasedprimary tumor growth, decreased metastatic nodules, increased tumor apoptosis, and prolonged survival in a lung cancer xenograft mouse model model [3, 8]. Restoration of tumor suppressor TUSC2 expression through targeted nanoparticle delivery systems has shown promise as a potential therapeutic strategy, as seen with TUSC2 and lung cancer [9].



In 2012, a Phase I clinical trial (NCT00059605) was conducted on 31 recurrent or metastatic lung cancer patients previously treated with platinum based chemotherapy, in which they were treated with nanoparticles containing a TUSC2 expression plasmid [9]. Patients were treated intravenously every three weeks with one of 6 doses (ranging from 0.01 to 0.09 mg/kg) of the TUSC2 carrying nanoparticle, showing a maximum tolerated dose of 0.06 mg/kg. The trial resulted in 5 patients achieving stable disease, as well as a large portion of patients successfully having increased TUSC2 mRNA and protein expression in post-treatment biopsy samples. Additionally, multiple apoptotic proteins involved in the intrinsic apoptosis pathway were significantly upregulated in patients post-treatment. The results from this trial demonstrated that treatment with nanoparticles carrying a TUSC2 expression plasmid are able to be safely administered through IV treatment and result in increased expression of the tumor suppressor TUSC2, as well as upregulation of pro-apoptotic genes in recurrent and metastatic lung cancer patients, with the potential of stabilizing disease progression [9]. A more recent Phase I/II clinical trial is currently in progress using the REQORSA® in combination with FDA-approved EGFR inhibitor Osimertinib (NCT04486833) in advanced lung cancer patients who experienced disease progression on Osimertinib alone [10].

While the role of TUSC2 in lung cancer is well established and TUSC2 restoration is being evaluated clinically, its importance in glioblastoma (GBM) was not elucidated until recently in our 2022 study [11]. We identified TUSC2 as a novel tumor suppressor for GBM [11], the most common and deadliest primary brain tumor in adults associated with dismal prognosis. In this 2022 study [11], we reported that TUSC2 protein is preferentially degraded in GBM compared to normal brain cells, through NEDD4 E3 ligase-mediated K71 polyubiquitination and subsequent proteasomal degradation. CRISPR/Cas9-mediated TUSC2-knockout promoted *in vivo* progression of GBM intracranial xenografts. Conversely, TUSC2 expression restoration induced apoptosis and inhibited the development of GBM intracranial xenografts *in vivo* [11], suggesting that TUSC2 restoration using REQORSA® may suppress GBM growth and progression.

References

[1] M.I. Lerman, and Minna, J.D., The 630-kb Lung Cancer Homozygous Deletion Region on Human Chromosome 3p21.3: Identification and Evaluation of the Resident Candidate Tumor Suppressor Gene, Cancer Research, 60 (2000) 6116-6133.
[2] M. Kondo, Ji, L., Kamibayashi, C., Tomizawa, Y., Randle, D., Sekido, Y., Yokota, J., Kashuda V., Zabarovsky, E., Kuzmin, I., Lerman, M., Roth, J., and Minna, J.D., Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells, Nature, 20 (2001) 5.
[3] I. Ito, Tanaka, F., Saito, Y., Gopalan, B., Branch, C.D., Xu, K., Atkinson, E.N., Bekele, B.N., Stephens, L.C., Minna, J.D., Roth, J.A., and Ramesh, R., Liposomal vector mediated delivery of the 3p *FUS1* gene demonstrates potent antitumor activity against human lung cancer *in vivo*, Cancer Gene Therapy, 11 (2004) 7.
[4] J. Meng, Majidi, M., Fang, B., Ji, L., Bekele, B.N., Minna, J.D., and Roth, J.A., The Tumor Suppressor Gene TUSC2 (FUS1) Sensitizes NSCLC to the AKT Inhibitor MK2206 in LKB1-dependent manor, PLOS One, 8 (2013).
[5] C. Xiaobo, M. Majidi, M. Feng, R. Shao, J. Wang, Y. Zhao, V. Baladandayuthapani, J. Song, B. Fang, L. Ji, TUSC2 (FUS1)-erlotinib induced vulnerabilities in epidermal growth factor receptor (EGFR) wildtype non-small cell lung cancer (NSCLC) targeted by the repurposed drug auranofin, Scientific reports, 6 (2016) 1-10.
[6] F. Uno, Sasaki, J., Nishizaki, M., Carboni, G., Xu, K., Atkinson, E.N., Kondo, M., Minna, J.D., Roth J.A., and Ji, L., Myristoylation of the fus1 protein is required for tumor suppression in human lung cancer cells, Cancer Research, 64 (2004) 2969-2976.
[7] A. Arrigo, A.T. Regua, M.K. Najjar, H.W. Lo, Tumor Suppressor Candidate 2 (TUSC2): Discovery, Functions, and Cancer Therapy, Cancers (Basel), 15 (2023).
[8] L. Ji, M. Nishizaki, B. Gao, D. Burbee, M. Kondo, C. Kamibayashi, K. Xu, N. Yen, E.N. Atkinson, B. Fang, M.I. Lerman, J.A. Roth, J.D. Minna, Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities in vitro and in vivo, Cancer Res, 62 (2002) 2715-2720.
[9] C. Lu, D.J. Stewart, J.J. Lee, L. Ji, R. Ramesh, G. Jayachandran, M.I. Nunez, I.I. Wistuba, J.J. Erasmus, M.E. Hicks, Phase I clinical trial of systemically administered TUSC2 (FUS1)-nanoparticles mediating functional gene transfer in humans, PloS one, 7 (2012) e34833.
[10] R. Uzhachenko, Shimamoto, A., Chirwa, S.S., Ivanov, S.V., Ivanova, A.V., and Shanker, A., Mitochondiral Fus1/Tusc2 and cellualr Ca2+ homeostasis: tumor suppressor, anti-inflammatory and aging implications, Cancer Gene Therapy, (2022).
[11] T.K. Rimkus, A.B. Arrigo, D. Zhu, R.L. Carpenter, S. Sirkisoon, D. Doheny, A.T. Regua, G.L. Wong, S. Manore, C. Wagner, H.K. Lin, G. Jin, J. Ruiz, M. Chan, W. Debinski, H.W. Lo, NEDD4 degrades TUSC2 to promote glioblastoma progression, Cancer Lett, 531 (2022) 124-135.
[12] A.F. Cardona, D. Jaramillo-Velásquez, A. Ruiz-Patiño, C. Polo, E. Jiménez, F. Hakim, D. Gómez, J.F. Ramón, H. Cifuentes, J.A. Mejía, F. Salguero, C. Ordoñez, Á. Muñoz, S. Bermúdez, N. Useche, D. Pineda, L. Ricaurte, Z.L. Zatarain-Barrón, J. Rodríguez, J. Avila, L. Rojas, E. Jaller, C. Sotelo, J.E. Garcia-Robledo, N. Santoyo, C. Rolfo, R. Rosell, O. Arrieta, Efficacy of osimertinib plus bevacizumab in glioblastoma patients with simultaneous EGFR amplification and EGFRvIII mutation, J Neurooncol, 154 (2021) 353-364.
[13] G. Chagoya, S.G. Kwatra, C.W. Nanni, C.M. Roberts, S.M. Phillips, S. Nullmeyergh, S.P. Gilmore, I. Spasojevic, D.L. Corcoran, C.C. Young, K.V. Ballman, R. Ramakrishna, D.A. Cross, J.M. Markert, M. Lim, M.R. Gilbert, G.J. Lesser, M.M. Kwatra, Efficacy of osimertinib against EGFRvIII+ glioblastoma, Oncotarget, 11 (2020) 2074-2082.
[14] D. Doheny, S. Sirkisoon, R.L. Carpenter, N.R. Aguayo, A.T. Regua, M. Anguelov, S.G. Manore, A. Arrigo, S.A. Jalboush, G.L. Wong, Y. Yu, C.J. Wagner, M. Chan, J. Ruiz, A. Thomas, R. Strowd, J. Lin, H.W. Lo, Combined inhibition of JAK2-STAT3 and SMO-GLI1/tGLI1 pathways suppresses breast cancer stem cells, tumor growth, and metastasis, Oncogene, 39 (2020) 6589-6605.
[15] R.L. Carpenter, S. Sirkisoon, D. Zhu, T. Rimkus, A. Harrison, A. Anderson, I. Paw, S. Qasem, F. Xing, Y. Liu, M. Chan, L. Metheny-Barlow, B.C. Pasche, W. Debinski, K. Watabe, H.W. Lo, Combined inhibition of AKT and HSF1 suppresses breast cancer stem cells and tumor growth, Oncotarget, 8 (2017) 73947-73963.
[16] T.K. Rimkus, R.L. Carpenter, S. Sirkisoon, D. Zhu, B.C. Pasche, M.D. Chan, G.J. Lesser, S.B. Tatter, K. Watabe, W. Debinski, H.W. Lo, Truncated Glioma-Associated Oncogene Homolog 1 (tGLI1) Mediates Mesenchymal Glioblastoma via Transcriptional Activation of CD44, Cancer Res, 78 (2018) 2589-2600.
[17] R.L. Carpenter, I. Paw, H. Zhu, S. Sirkisoon, F. Xing, K. Watabe, W. Debinski, H.W. Lo, The gain-of-function GLI1 transcription factor TGLI1 enhances expression of VEGF-C and TEM7 to promote glioblastoma angiogenesis, Oncotarget, 6 (2015) 22653-22665.
[18] H. Zhu, R.L. Carpenter, W. Han, H.W. Lo, The GLI1 splice variant TGLI1 promotes glioblastoma angiogenesis and growth, Cancer Lett, 343 (2014) 51-61.
[19] H.W. Lo, H. Zhu, X. Cao, A. Aldrich, F. Ali-Osman, A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion, Cancer Res, 69 (2009) 6790-6798.

Results

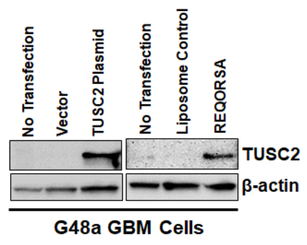


Fig. 1: Validation of Genprex's TUSC2 plasmid and Quar Oze/REQORSA®. G48a GBM cells transfected with control plasmid, TUSC2 plasmid, liposome control, or REQORSA® were analyzed for TUSC2 protein expression using Western Blotting.

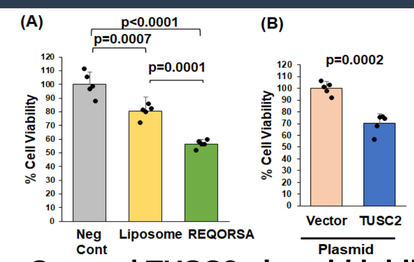


Fig. 2: Quar Oze and TUSC2 plasmid inhibit GBM cell viability. G48a GBM cells transfected with liposome control, REQORSA®, control plasmid, or TUSC2 plasmid for 48 hrs were analyzed for cell viability using Celltiter Blue Assay.

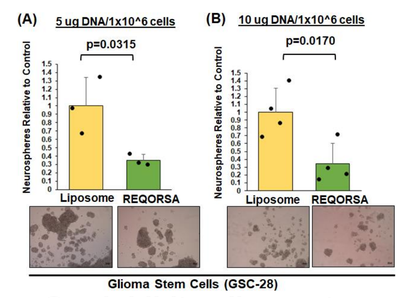
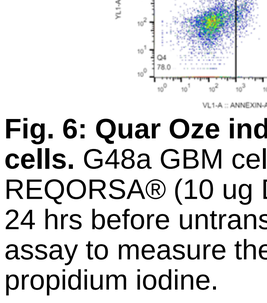


Fig. 3: Quar Oze inhibits glioma stem cells (GSCs). GSC-28 cultured as neurospheres were treated with liposome control or Quar Oze/ for 5-7 days, the number of neurospheres were counted.



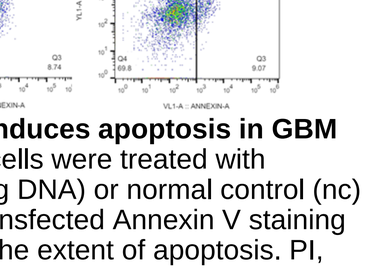


Fig. 4: Quar Oze inhibits GSCs as shown by the ALDEFLUOR stem cell Assay. G48a GBM cells were treated with or without REQORSA® for 48 hrs.

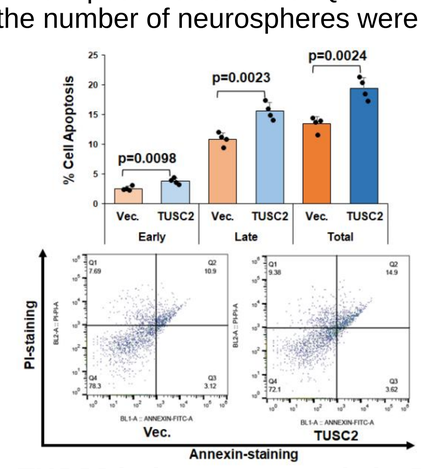


Fig. 5: TUSC2 induces apoptosis in GBM cells. G48a GBM cells were treated with TUSC2 plasmid or control plasmid for 24 hrs before Annexin V staining assay to measure the extent of apoptosis. PI, propidium iodine.

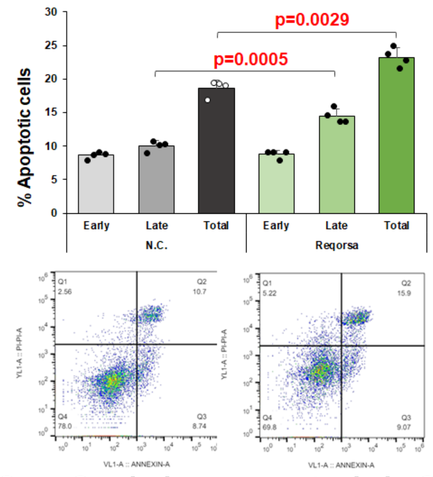


Fig. 6: Quar Oze induces apoptosis in GBM cells. G48a GBM cells were treated with REQORSA® (10 ug DNA) or normal control (nc) for 24 hrs before untransfected Annexin V staining assay to measure the extent of apoptosis. PI, propidium iodine.

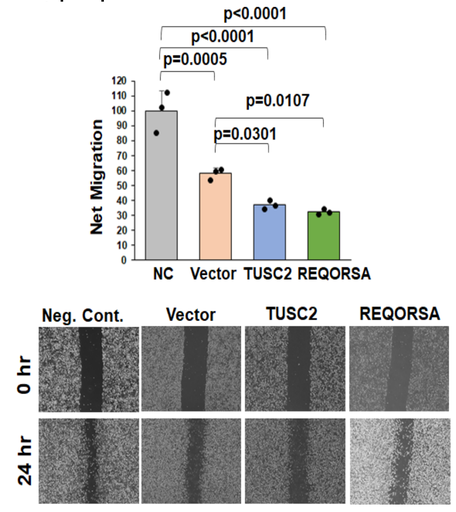


Fig. 7: Quar Oze can suppress GBM cell migration independent of its ability to suppress cell viability. G48a GBM cells were treated with liposome control or REQORSA®, and then subjected to migration scratch-wound assay. Migration rate was normalized against cell viability to derive net migration. NC, negative control without transfection.

Conclusion

We observed that Quar Oze/REQORSA® significantly reduced GBM cell viability. In our 2022 study, we reported that TUSC2 inhibits PD-GSCs. Here, we found that Quar Oze/REQORSA® strongly suppressed the PD-GSC population that is highly resistant to therapy. Furthermore, since our 2022 study showed that ectopic TUSC2 expression promotes apoptosis whereas TUSC2 knockdown decreases apoptosis, we validated that REQORSA® indeed induced significant apoptosis in GBM and PD-GSC cells. Since GBM cells are highly infiltrative, we next determined if Quar Oze/REQORSA® had the ability to inhibit GBM cell migration. The results of our migration assay demonstrated that Quar Oze/REQORSA® suppressed GBM cell migration independent of its ability to suppress cell viability. In summary, we report, for the first time, that Quar Oze/REQORSA® demonstrates promising *in vitro* efficacy in GBM and PD-GSCs. These results serve as supporting evidence for further evaluation of its *in vivo* anti-tumor efficacy in GBM using mouse models.

Table 1: Quar Oze Effects on GBM Cells

	Quar Oze Effects Over Controls	P-Values
Proliferation	Decreased 45%	<0.0001
Glioma Stem Cells	Decreased 63%	0.017
Apoptosis	Increased 32%	0.0029
Cell Migration	Decreased 70%	<0.0001

Future Directions

The *in vivo* effect of Quar Oze*/*REQORSA® in GBM will be determined in mouse models. Quar Oze*/*REQORSA® has been shown to sensitize lung cancer patients to EGFR inhibitor Osimertinib [10] and Osimertinib in combination with bevacizumab has showed efficacy in a subset of GBM patients with EGFR and EGFRvIII amplification [12, 13]. Therefore, we further hypothesize that REQORSA® sensitizes GBM cells and GSCs with EGFR/EGFRvIII overexpression to Osimertinib. Therefore, additional future studies include: (1) To determine whether Quar Oze*/*REQORSA® effectively targets GBM cells and GSCs *in vivo*, and (2) To examine whether Quar Oze*/* REQORSA® in combination with the EGFR inhibitor Osimertinib effectively suppresses EGFR/EGFRvIII-expressing GBM cells and GSCs *in vitro*.

Acknowledgement

Genprex provides funding, Quar Oze*/*REQORSA®. and TUSC2 plasmid. We thank Ms. Munazza S. Khan for assistance in preparing the poster.

Conflict of Interest

Dr. Mark Berger is an employee (CMO) of Genprex, the company that developed the new drug Quaratusugene ozeplasmid.