IN SILICO ANALYSIS OF KEY miRNAs AND GENE- NETWORK ANALYSIS OF PTEN GENE INVOLVED IN HEAD AND NECK CANCER

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ABSTRACT:

Introduction: MicroRNAs (miRNA) are non coding RNAs that play an important role in regulating gene expression. It is used in gene profiling as a therapeutic target for complex diseases. miRNAs post transcriptionally control the gene expression via translational repression. In silico analysis led to discovery of miRNA that plays a vital role in cancer biology. PTEN acts as an tumor suppressor gene which regulates negatively on the survival and proliferation and down regulation and causes cancer.

Materials and methods: Prediction of microRNA target for PTEN gene was carried using Targetscan Human Software program. Predicted regulatory target of PTEN gene using program default settings.The targets of the conserved miRNA, has-miR-1297 miRNA with a miTG score of ≥ 0.99 were identified using miRdb online server program followed by the String database online server.

Results: The gene analysis showed varied genes like NEDD4,MAGI2,DLG1,PIK3CA that were correlated with the PTEN gene involved in head and neck cancer. From the prediction of miRNA the PTEN gene showed a target score more than 95 that was involved in the cancer.

Discussion: our study identified a total of 35 miRNAs associated with the PTEN gene above the target score of 95. From the previous study it shows PTEN has also been shown to be a direct target of miR-26a in high grade glioma. PTEN has shown multiple aspects of cancer development.

Conclusion: It can be concluded that miRNAs help in identifying at higher risk to cancer and this study helps to understand the role of PTEN gene in head and neck cancer and can be used as a biomarker for oncogenesis.

Keywords: cancer, genes ,miRNA, PTEN

INTRODUCTION:

MicroRNAs are an enormous class of newly discovered endogenous small RNAs with 20-24 nucleotides in length. miRNAs are produced by miRNA genes which are primarily found in intergenic regions but can also be found in exon and intron regions(1). RNA polymerase II primarily produces primary miRNAs from miRNA genes, which the microprocessor complex processes in the nucleus to create precursor miRNAs. At the post transcriptional levels, they negatively influence gene expression. miRNAs offer a special tactic and are essential in practically all biological and metabolic processes. By predominantly targeting their 3'untranslated regions (UTRs) in a sequence specific manner, miRNAs suppress cellular translation and stability of a variety of protein coding transcripts(2). Human health and disease are significantly impacted by miRNAs selective suppression of gene expression(3).

Phosphatase and tensin homolog (PTEN) is an essential tumor suppressor that reduces

cell proliferation and increases cellular susceptibility to apoptosis(4). PTEN's significance as a biomarker for cancer has grown over time(5). Changes in these genes or protein expressions are considered to be actionable molecular hallmarks which implicates clinical decision making(6). PTEN is also involved in modelling the adaptive component of the anti-tumor immune response and the overall DNA damage response. PTEN is also deeply involved in cell growth, proliferation and survival(7).

Squamous cell carcinomas of the head and neck cancer grow in the mucosal linings of the aerodigestive tract. It is a heterogeneous disease(8). The fact that head and neck cancer form within large preneoplastic fields of mucosal epithelium composed of genetically altered cells that are clonally related to the carcinoma, frequently extend into the surgical margins when tumors are excised, and can cause local recurrences and second primary tumors which is the key problem in head and neck cancer pathogenesis. Abrogation of the P53 and retinoblastoma (RB) pathways which disrupt cell cycle regulation , most likely in the setting of telomerase reverse transcriptase expression, gives head and neck cancer cells an infinite capacity for replication(9). The study's objective is to analyze the In silico analyses of the miRNA and gene network analyses of PTEN gene involved in head and neck cancer.

MATERIALS AND METHODS:

In Target scan prediction, Prediction of microRNA targets for PTEN gene in humans was carried out using Targetscan Human Software program. Targetscan is a database that predicts biological targets of miRNAs and we refined the results that were associated with GC. Broadly conserved, conserved and poorly conserved miRNA families were searched for the presence of 8mer, 7mer $\geq$ 0.99and 6mer sites matching each miRNA seed region. Predicted regulatory targets of the PTEN gene were identified using the program with default settings. The targets of the conserved miRNA, hsa-miR-122-5p miRNA with a miTG score of $\geq$ 0.99 were identified using the Dianalab software program. Gene network analysis of PTEN was carried out by STRING database online server program. Important gene interactions with a combined score of $\geq$ 0.99 were considered and listed.

miRDB prediction:

Target miRNAs were predicted using miRDB online server program. miRNA targets with a target score of more than 95 were considered for further analysis. The target details and the predicted genes for the miRNA hsa-miR-3922-5p were carried out and the details were collected.

Gene network analysis:

Gene network analysis of PTEN gene was carried out by STRING database online server program.The important genes that interact with the PTEN gene were identified.

RESULTS:

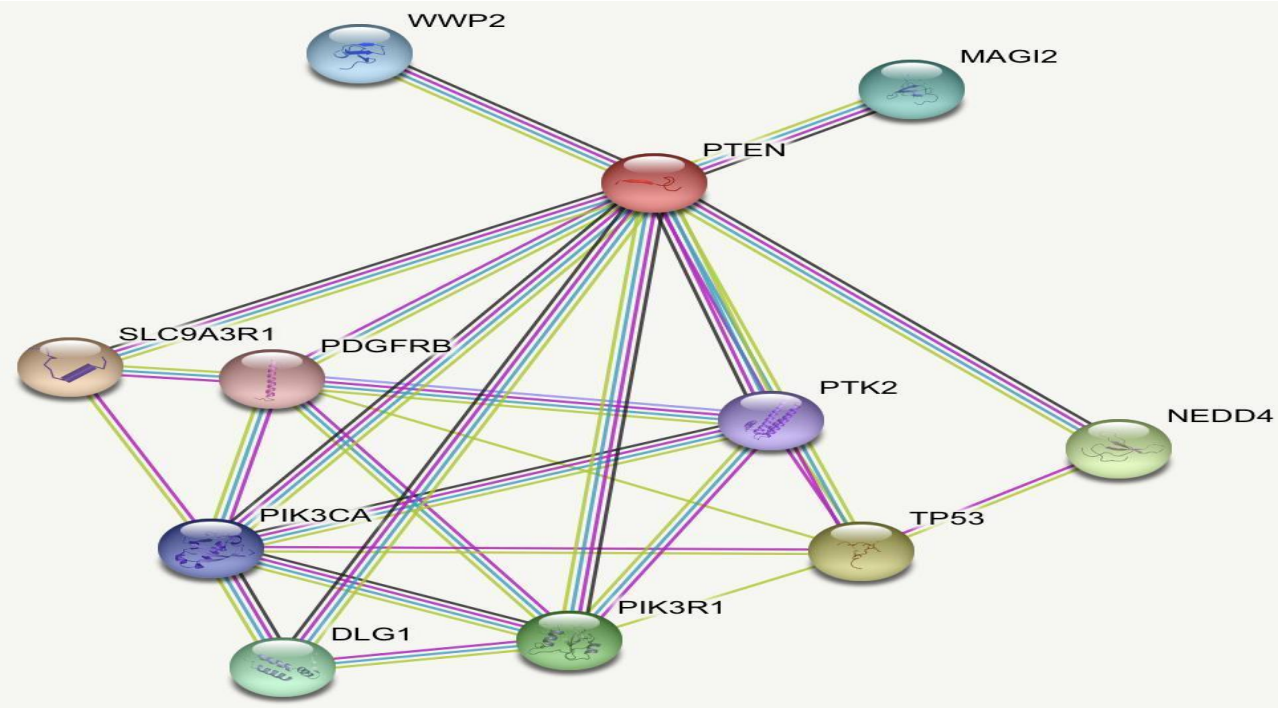

Figure 1: Gene analysis of PTEN gene . The above mentioned genes are correlated with the PTEN gene.

Target Rank	Target Score	miRNA Name	Gene Symbol
1	100	hsa-miR-1297	PTEN
2	100	hsa-miR-5011-5p	PTEN
3	99	hsa-miR-23a-3p	PTEN
4	99	hsa-miR-5692c	PTEN
5	99	hsa-miR-26a-5p	PTEN
6	99	hsa-miR-26b-5p	PTEN
7	99	hsa-miR-23c	PTEN
8	99	hsa-miR-23b-3p	PTEN
9	99	hsa-miR-4465	PTEN
10	99	hsa-miR-5692b	PTEN
11	98	hsa-miR-4775	PTEN
12	97	hsa-miR-1277-5p	PTEN
13	97	hsa-miR-513a-3p	PTEN
14	97	hsa-miR-29c-3p	PTEN
15	97	hsa-miR-29a-3p	PTEN
16	97	hsa-miR-486-5p	PTEN
17	97	hsa-miR-29b-3p	PTEN
18	97	hsa-miR-494-3p	PTEN
19	97	hsa-miR-190a-3p	PTEN
20	97	hsa-miR-513c-3p	PTEN
21	96	hsa-miR-32-5p	PTEN
22	96	hsa-miR-30d-3p	PTEN
23	96	hsa-miR-92b-3p	PTEN
24	96	hsa-miR-3944-5p	PTEN
25	96	hsa-miR-8485	PTEN
26	96	hsa-miR-30a-3p	PTEN
27	96	hsa-miR-3606-3p	PTEN
28	96	hsa-miR-373-5p	PTEN
29	96	hsa-miR-616-5p	PTEN
30	96	hsa-miR-30e-3p	PTEN
31	96	hsa-miR-92a-3p	PTEN
32	96	hsa-miR-371b-5p	PTEN
33	95	hsa-miR-367-3p	PTEN
34	95	hsa-miR-363-3p	PTEN
35	95	hsa-miR-188-5p	PTEN
36	95	hsa-miR-25-3p	PTEN

Figure 2 : Prediction of miRNAs for PTEN gene. These are few miRNAs with target scores more than 95.

DISCUSSION:

The PTEN gene is associated with genes like NEDD4, WWP2, PTK2, TP53, MAGI2, DLG1,PIK3CA, SLC9A3R1, PIK3CA and affects their activity. It was identified that 35 miRNAs associated with the PTEN gene had a target score of 95. PTEN gene showed higher range towards the proliferation and metastasis of cancer.Previous studies show that PTEN can control the growth and maintenance of cancer stem cells specifically impacting vital aspects of these cells via downstream signaling pathways such as P13K/Akt pathway. PTEN loss can influence tumor progression, metastasis, and radiation and chemotherapy(10).From another it shows that PTEN has been demonstrated to upregulate the putative tumor metastasis suppressor gene Drg1(differentiated related gene1) which in turn decreases tumor invasiveness in prostate and breast cancer cells via an Akt dependent mechanism, limiting metastasis(11). From another study it shows that the involvement of PTEN is more equivocal since in some tissue types , PTEN loss alone is sufficient to trigger carcinogenesis. It can be seen that when compared to the pre-treatment tumor, convergent reduction of PTEN expression was shown in the post-treatment metastatic samples, pointing to a potential function for PTEN in the emergence of resistance(12).

From another study it shows that PTEN which inhibits P13K activity is implicated in inhibition of cell cycle progression, causing cell death, modifying the arrest signal and also in promoting angiogenesis.The lipid phosphatase activity of PTEN is the best-characterized physiological function contributing to the tumor suppressor function of PTEN. PTEN is the only identified lipid phosphatase inhibiting the PI3K pathway, as no additional redundant or compensating family members have been identified. Thus the loss of PTEN function has an important impact on multiple aspects of cancer developments(13). Another study showed that PTEN appeared to have a stronger prognostic value than any of the other variables considered, including neck node involvement and EGFR expression(14). From another study it shows that PTEN genes are more frequent in endometrial,glial,prostate and melanoma cancer when compared with the present study PTEN is more prone to head and neck cancer with the development such as cell proliferation,apoptosis resistance, angiogenesis, metabolism regulation, genomic instability, stem cell self- renewal, cellular senescence, cell migration and metastasis(15) in accordance to previous studies (16-25).

CONCLUSION:

miRNAs contribute as a simple profiling strategy to identify individuals at higher risk of developing head and neck cancers. In silico studies reveal important miRNAs that could potentially be involved in the tumorigenesis process and help understand the role of PTEN gene. The study could help develop a predictive biomarker for oncologic patients therapy selection and also useful for designing miRNA based directed therapy.

Acknowledgement:

The authors would like to thank Saveetha Dental college for their support to conduct the study.

Conflict of interest : All the authors declare that there was no conflict of interest in the present study.

SOURCE OF FUNDING :

The present project was funded by

- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospital
- Saveetha University
- Pushpavin Hospital

REFERENCES:

1. Sempere LF. MicroRNA as Biomarkers in Cancer Diagnostics and Therapy. MDPI; 2019. 168 p.
2. Lambert NJ. Mechanism of Messenger RNA Target Recognition by MicroRNAs. 2010. 196 p.
3. Djuranovic S, Nahvi A, Green R. A parsimonious model for gene regulation by miRNAs. Science. 2011 Feb 4;331(6017):550–3.
4. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. *PTEN* , a Putative Protein Tyrosine Phosphatase Gene Mutated in Human Brain, Breast, and Prostate Cancer [Internet]. Vol. 275, Science. 1997. p. 1943–7. Available from: http://dx.doi.org/10.1126/science.275.5308.1943
5. Lozano R, Salles DC, Sandhu S, Aragón IM, Thorne H, López-Campos F, et al. Association between BRCA2 alterations and intraductal and cribriform histologies in prostate cancer. Eur J Cancer. 2021 Apr;147:74–83.
6. Vidwans SJ, Turski ML, Janku F, Garrido-Laguna I, Munoz J, Schwab R, et al. A framework for genomic biomarker actionability and its use in clinical decision making [Internet]. Vol. 1, Oncoscience. 2014. p. 614–23. Available from: http://dx.doi.org/10.18632/oncoscience.90
7. Hoxhaj G, Manning BD. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. Nat Rev Cancer. 2020 Feb;20(2):74–88.
8. Rajapurkar M. Molecular Biology of Head and Neck Cancer [Internet]. Basic Concepts in Head and Neck Surgery and Oncology. 2015. p. 227–227. Available from: http://dx.doi.org/10.5005/jp/books/12491_33
9. Burtness B, Golemis EA. Molecular Determinants of Head and Neck Cancer. Springer; 2018. 629 p.
10. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor [Internet]. Vol. 13, Nature Reviews Molecular Cell Biology. 2012. p. 283–96. Available from: http://dx.doi.org/10.1038/nrm3330
11. Uzoh CC, Perks CM, Bahl A, Holly JMP, Sugiono M, Persad RA. PTEN-mediated pathways and their association with treatment-resistant prostate cancer. BJU Int. 2009 Aug;104(4):556–61.
12. Park E, Dempsey DR, Cole P. the structural basis of PTEN regulation by multi-site phosphorylation [Internet]. 2021. Available from: http://dx.doi.org/10.2210/pdb7jtx/pdb
13. Snietura M, Jaworska M, Mlynarczyk-Liszka J, Goraj-Zajac A, Piglowski W, Lange D, et al. PTEN as a prognostic and predictive marker in postoperative radiotherapy for squamous cell cancer of the head and neck. PLoS One. 2012 Mar 7;7(3):e33396.
14. Portelli S, Barr L, de Sá AGC, Pires DEV, Ascher DB. Distinguishing between PTEN clinical phenotypes

through mutation analysis. Comput Struct Biotechnol J. 2021 May 21;19:3097–109.

15. Fusco N, Sajjadi E, Venetis K, Gaudioso G, Lopez G, Corti C, et al. Alterations and Their Role in Cancer Management: Are We Making Headway on Precision Medicine? Genes [Internet]. 2020 Jun 28;11(7). Available from: http://dx.doi.org/10.3390/genes11070719
16. Aldhuwayhi, Sami, Sreekanth Kumar Mallineni, Srinivasulu Sakhamuri, Amar Ashok Thakare, Sahana Mallineni, Rishitha Sajja, Mallika Sethi, Venkatesh Nettam, and Azher Mohiuddin Mohammad. 2021. "Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey." Risk Management and Healthcare Policy 14 (July): 2851–61.
17. Dua, Kamal, Ridhima Wadhwa, Gautam Singhvi, Vamshikrishna Rapalli, Shakti Dhar Shukla, Madhur D. Shastri, Gaurav Gupta, et al. 2019. "The Potential of siRNA Based Drug Delivery in Respiratory Disorders: Recent Advances and Progress." Drug Development Research 80 (6): 714–30.
18. Gan, Hongyun, Yaqing Zhang, Qingyun Zhou, Lierui Zheng, Xiaofeng Xie, Vishnu Priya Veeraraghavan, and Surapaneni Krishna Mohan. 2019. "Zingerone Induced Caspase-Dependent Apoptosis in MCF-7 Cells and Prevents 7,12-Dimethylbenz(a)anthracene-Induced Mammary Carcinogenesis in Experimental Rats." Journal of Biochemical and Molecular Toxicology 33 (10): e22387.
19. Jayaraj, Gifrina, Pratibha Ramani, Herald J. Sherlin, Priya Premkumar, and N. Anuja. 2015. "Inter-Observer Agreement in Grading Oral Epithelial Dysplasia – A Systematic Review." Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. https://doi.org/10.1016/j.ajoms.2014.01.006.
20. Li, Zhenjiang, Vishnu Priya Veeraraghavan, Surapaneni Krishna Mohan, Srinivasa Rao Bolla, Hariprasath Lakshmanan, Subramanian Kumaran, Wilson Aruni, et al. 2020. "Apoptotic Induction and Anti-Metastatic Activity of Eugenol Encapsulated Chitosan Nanopolymer on Rat Glioma C6 Cells via Alleviating the MMP Signaling Pathway." Journal of Photochemistry and Photobiology B: Biology. https://doi.org/10.1016/j.jphotobiol.2019.111773.
21. Markov, Alexander, Lakshmi Thangavelu, Surendar Aravindhan, Angelina Olegovna Zekiy, Mostafa Jarahian, Max Stanley Chartrand, Yashwant Pathak, Faroogh Marofi, Somayeh Shamlou, and Ali Hassanzadeh. 2021. "Mesenchymal Stem/stromal Cells as a Valuable Source for the Treatment of Immune-Mediated Disorders." Stem Cell Research & Therapy 12 (1): 192.
22. Mohan, Meenakshi, and Nithya Jagannathan. 2014. "Oral Field Cancerization: An Update on Current Concepts." Oncology Reviews 8 (1): 244.
23. Neelakantan, Prasanna, Deeksha Grotra, and Subash Sharma. 2013. "Retreatability of 2 Mineral Trioxide

Aggregate-Based Root Canal Sealers: A Cone-Beam Computed Tomography Analysis." Journal of Endodontia 39 (7): 893–96.

24. Paramasivam, Arumugam, Jayaseelan Vijayashree Priyadharsini, Subramanian Raghunandhakumar, and Perumal Elumalai. 2020. "A Novel COVID-19 and Its Effects on Cardiovascular Disease." Hypertension Research: Official Journal of the Japanese Society of Hypertension.
25. Sheriff, K. Ahmed Hilal, K. Ahmed Hilal Sheriff, and Archana Santhanam. 2018. "Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College." Research Journal of Pharmacy and Technology. https://doi.org/10.5958/0974-360x.2018.00101.4.