In Silico Prediction of Mirna and Gene-Network Analysis of TTN Gene, A Key Regulator of Squamous Cell Neoplasms

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ABSTRACT

Introduction: Squamous all carcinoma (SCC) is the second most common form of skin cancer. It's usually found in areas of the body damaged by UV rays from the sun or tanning beds. Titin is a protein that is found in humans and is enclosed by the TTN gene. Titin is a giant protein, greater than 1μM in length, that functions as a molecular spring which is responsible for the passive elasticity of muscle. Predicting the targeted miRNA related to TTN in regulation of squamous cell neoplasm.

Materials and Methods: Gene network analysis of TTN was carried out by . STRING database where score of <0.99 considered. Target miRNAs predicted by miRDB prediction and target score 91 and alone considered.

Results and Discussion: TTN is highly oncogenic and is associated with many other genes and affects their activity when upregulated and downregulated. The TTN gene is highly expressed in the squamous cell neoplasms and helps in the proliferation and metastasis of the cancer. It's overexpression had a positive correlation of growing tumors and neoplasms

Conclusion: TTN mutation may be associated with squamous all carcinoma and positively correlated and serve as potential indicator of squamous all cancer.

Keywords: In silico, miRNA, gene network, TTN gene, squamous cell, neoplasms.

INTRODUCTION

Squamous cell carcinoma (SCC) is the second most common cutaneous malignancy after basal cell carcinoma, with an increasing incidence worldwide (1). SCC accounts for most nonmelanoma skin cancer–related metastatic disease; therefore, recognition and treatment of early SCC is important for the prevention of neoplastic progression (2). Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80-90% of all malignant neoplasms of the oral cavity (3). The development of oral squamous cell carcinoma (OSCC) is a multistep process requiring the accumulation of multiple genetic alterations, influenced by a patient's genetic predisposition as well as by environmental influences, including tobacco, alcohol, chronic inflammation, and viral infection (4). Human papillomavirus (HPV) and Candida infections, nutritional deficiencies and genetic predisposition have been also associated with the cancer (5).

MicroRNAs (miRNAs) were discovered in 1993 followed by developments in small RNA by Lee and colleagues. microRNAs (miRNAs) are a class of naturally occurring, small non-coding RNA molecules regulating gene expression (6). The function of miRNAs regulated, is

associated with RNA-induced silencing complex (RISC) (7). The miRNAs are transcribed from DNA sequences turning into primary miRNAs and processed into precursor miRNAs, and finally into mature miRNAs (8). MiRNAs guide gene expression by base-pairing with target mRNA to negatively.

TTN gene codes for titin. It is the largest known protein and it plays a key role in developmental, structural, mechanical and regulatory roles in skeletal and cardiac muscles (9). TTN protein has an important role in sarcomere organization, assembly of muscles, transmission of the force at the Z-line, passive myocyte stiffness, and resting tension maintenance in the I-band region (10). The chromosome in which TTN gene is located is 2q31 containing 364 exons (11). Bioinformatics will allow us to analyze the correlation of TTN with progressing cancers (12). Aim of this study's to concentrate on the miRNAs associated with the TTN gene and the miRNAS regulation in squamous cell neoplasms.

MATERIALS AND METHODS

Target scan prediction

Prediction of microRNA targets for the TTN gene in humans was carried out using Targetscan Human software program (https://www.targetscan.org/vert_80/).

Broadly conserved, and poorly conserved miRNA families were searched for the presence of 8mer, 7mer, and 6mer sites matching each miRNA seed region. Predicted regulatory targets of the TTN 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 ≥0.99 were identified using the Dianalab software program.

Gene network analysis of TTN was carried out by STRING database online server program. Important gene interactions with a combined score of ≥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 91 were considered for further analysis.

The target details and the predicted genes for the miRNA hsa-miR-3922-5p were carried out.

RESULTS

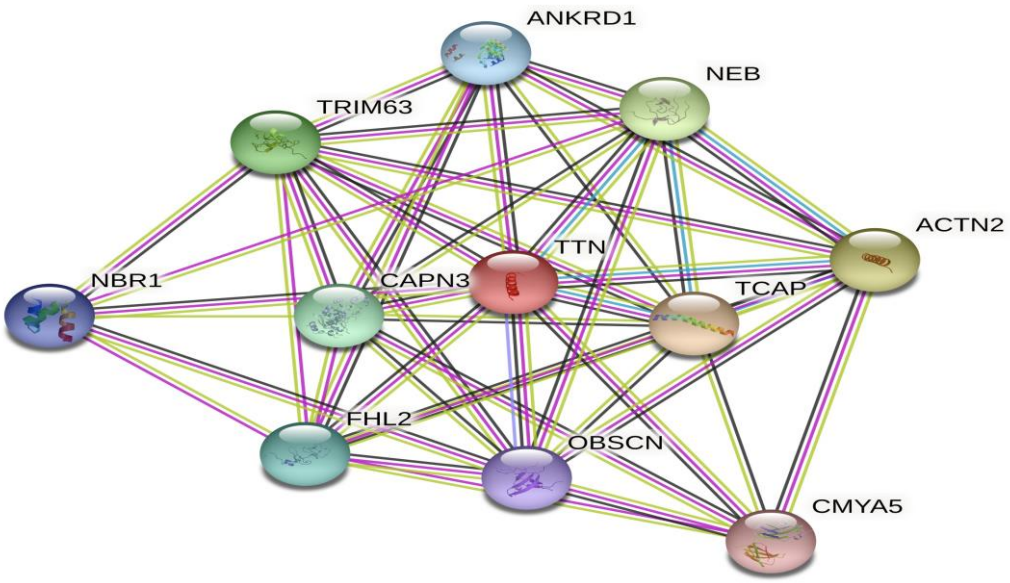

Figure 1: Gene analysis of TTN. The genes mentioned above are few genes which are co related to TTN. The upregulation of TTN effects in the regulation of the other stated genes.

Target rank	Target score	miRNA Name	Gene sequence	Important genes
1	95	hsa-miR-4738-3p	5' - **ugaaacuggagcgccuggagga** - 3'	PPM1E, LRRTM3
2	95	hsa-miR-4670-3p	5' - **ugaaguuacaucauggucgcuu** - 3'	TNRC6B, SDHAF4
3	94	hsa-miR-4538	5' - **gagcuuggaugagcugggcuga** - 3'	CCDC179,TTN
4	94	hsa-miR-4453	5' - **gagcuuggucuguagcgguu** - 3'	CCDC179,TTN
5	94	hsa-miR-4531	5' - **auggagaaggcuucuga** - 3'	CD276,BMP2
6	94	hsa-miR-3919	5' - **gcagagaacaaaggacucagu** - 3'	FZD3, SERTM1
7	93	hsa-miR-3913-3p	5' - **agacaucaagaucagucccaaa** - 3'	SHISA7, MEGF10
8	92	hsa-miR-590-3p	5' - **uaauuuuauguauaagcuagu** - 3'	BPNT1, LTN1
9	92	hsa-miR-4666a-5p	5' - **auacaugucagauuguaugcc** - 3'	COL4A3BP, PGR
10	92	hsa-miR-3680-3p	5' - **uuuugcaugacccugggaguagg** - 3'	HIPK1, PGR
11	91	hsa-miR-4477a	5' - **cuauuaaggacauuugugauuc** - 3'	EIF3J, NUFIP2

Figure 2: Prediction of miRNAs for TTN. These are few miRNAs related to Titin in which target scores of more than 91 were considered. Each miRNAs gene sequence and a few important genes related are listed.

TTN is the largest protein in humans which encodes titin. It is associated with a few other genes such as NEB, AKORDI, TRIM63, CAPN3, TCAP, ACTN 2, NBRI, FHL2, OBSCN, CMYA5. These other genes upregulation or downregulation are correlated to TTN in its function. The miRNAS whose target scores highest to TTN i.e., from 91 to 95 and its correlated genes are listed. hsa-miR-4738-3p and hsa-miR-4670-3p are miRNAS of target score 95; hsa-miR-4538, hsa-miR-4453, hsa-miR-4531 and hsa-miR-3919 are miRNAs of target score 94; hsa-miR-3913-3p is miRNA of target score 93; hsa-miR-590-3p, hsa-miR-4666a-5p and hsa-miR-3680-3p are miRNAs of target score 92 and hsa-miR-4477a is miRNA of target score 91.

DISCUSSION

By Ambros and colleagues the first miRNA, lin-4, was discovered in Caenorhabditis elegans (13). The earliest evidence of miRNA involvement in human cancer was identified from studies on tumor suppressors at chromosome 13q14 region in B-cell chronic lymphocytic leukemia cells by Dr Croce's group (14). Dysregulated miRNAs affect the tumor initiation and progression in several of the cancer hallmarks, abnormal miRNAs are expressed in tumors (15). miRNA can function either as an oncogene or tumor suppressor under few conditions depending on their target genes in case of cancer (16). miRNAs are involved in different kinds of cancers like, breast (17), colon (18), prostate (19), thyroid (20), and gastric (21).

The TTN gene is highly oncogenic. It is associated with many other genes - few important ones are NEB, AKORDI,TRIM63,CAPN3,TCAP,ACTN 2,NBRI,FHL2,OBSCN,CMYA5, and effects their activity when up-regulated or down-regulated. The TTN gene is highly exposed in the squamous cell neoplasms and tissues and helps in proliferation and metastasis of cancer, serving as a potential indicator.

Previous study proved that there are possible signal pathways in the tumorigenesis and development of LUSC patients with TTN mutation (9).

In another study cox regression analysis revealed that TTN mutation was an independent risk factor for LUSC development, suggesting that it can be used as a prognostic indicator for this disease (10).

In other studies overexpression of TTN-ASI correlated with poor prognosis in

breast cancer, lung cancer, digestive system cancer, reproductive system cancers, and other cancers. Furthermore, increased TTN-ASI expression correlates with more advanced pathology and tumor malignancy (11).

In another study, TTN-ASI/miR-15b-15p/FBXW7 axis identified in the work could help to identify treatment biomarkers for ovarian cancer. In the above stated studies it has been shown that there is a relation between TTN and cancers, in which its upregulation or downregulation causes cancers. It can be used as an indicator for suppression of oncogenic genes (12). This has be shown in previous studies (13-22)

CONCLUSION

TTN mutation may be positively correlated with prognosis of cancer and associated with squamous cell carcinoma. Therefore, this mutation may serve as a potential prognostic indicator of squamous cell cancer.

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CONFLICT OF INTEREST:

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

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