

## In Silico Prediction Of Mirna And Gene-Network Analysis Of Ttn Gene, A Key Regulator Of Squamous Cell Neoplasms

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### ABSTRACT

Squamous cell 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 encoded by the TTN gene. Titin is a giant protein, greater than 1 $\mu$ M 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. 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.

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. Its overexpression had a positive correlation of growing tumors and neoplasms

TTN mutation may be associated with squamous all carcinoma and positively correlated and can serve as a potential indicator of squamous cell cancer.

**Keywords:** In silico, miRNA, gene network, squamous cell, neoplasms

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### INTRODUCTION

Squamous cell carcinoma (SCC) is the second most common cutaneous malignancy after basal cell carcinoma, with an increasing incidence worldwide (Combalia & Carrera, 2020). 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 (Algahtani et al., 2019). Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80-90% of all malignant neoplasms of the oral cavity (Johnson et al., 2011). Local recurrence of OSCC most frequently occurs in regions such as the tongue, buccal mucosa, and gingivobuccal (*MOST COMMON SITE OF SQUAMOUS CELL CARCINOMA IN PATIENTS VISITING SAVEETHA DENTAL COLLEGE*, n.d.). 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 (Choi & Myers, 2008). Human papillomavirus (HPV) and Candida infections, nutritional deficiencies and genetic predisposition have been also associated with the cancer (Marur et al.,

2010). OSCC is typically treated using therapeutic methods such as surgery, chemotherapy, and radiation therapy (R et al., 2023). Thereby, understanding the underlying mechanisms of OSCC initiation and progression could contribute to the discovery of novel diagnostic biomarkers and therapeutic agents (Neralla, M H, et al., 2024).

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 (J. O'Brien et al., 2018). MicroRNAs are increasingly being explored for their potential role in the diagnosis and treatment of oral squamous cell carcinoma (OSCC), particularly as non-invasive biomarkers and therapeutic targets (Neralla, S H, et al., 2024). As key regulatory molecules, microRNAs (miRNAs) influence cellular activity by interacting with various genes and signaling pathways that contribute to either the promotion or inhibition of disease progression (Varshan et al., 2024). Elevated expression of microRNAs (miRs) has been observed in various cancers, including oral squamous cell carcinoma (OSCC) (*Website*, n.d.). The function of miRNAs regulated, is associated with RNA-induced silencing complex (RISC) (MacFarlane & Murphy,

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2010). The miRNAs are transcribed from DNA sequences turning into primary miRNAs and processed into precursor miRNAs, and finally into mature miRNAs (J. A. O'Brien et al., 2022). 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 (Chauveau et al., 2014). 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 (Miao et al., 2020). The chromosome in which TTN gene is located is 2q31 containing 364 exons (Hackman et al., 2002). Bioinformatics will allow us to analyze the correlation of TTN with progressing cancers (Zou et al., 2022). The aim of this study is to concentrate on the miRNAs associated with the TTN gene and the miRNAs regulation in squamous cell neoplasms.

## MATERIALS AND METHODS

### MATERIALS AND METHODS

This study was designed as an in silico investigation to explore the regulatory relationship between microRNAs (miRNAs) and the **TTN gene**, along with its associated gene interaction network in the context of squamous cell neoplasms. A multi-database bioinformatics approach was adopted to ensure reliability and high-confidence predictions.

Initially, detailed information regarding the TTN gene, including its sequence, chromosomal location, and functional relevance, was retrieved from established genomic repositories. This provided the foundational framework for subsequent analyses focusing on miRNA interactions and gene network associations.

To identify potential miRNAs targeting TTN, the TargetScan Human database was employed. This platform predicts miRNA binding sites based on conserved seed region complementarity. Both conserved and poorly conserved miRNA families were included in the analysis to ensure comprehensive coverage. The prediction algorithm identifies various binding site types, including 8mer, 7mer, and 6mer matches, allowing for a broad yet structured assessment of possible regulatory interactions.

To enhance the specificity of the predictions, results obtained from TargetScan were further validated using the DIANA microT-CDS tool. Only miRNAs with a miTG score of 0.99 or higher were considered, thereby ensuring that only high-confidence interactions were retained for further analysis. This step significantly reduced the likelihood of false-positive predictions and strengthened the robustness of the dataset.

In parallel, an independent miRNA target prediction was conducted using the miRDB database, which utilizes a machine learning-based algorithm trained on experimentally validated data. A stringent cutoff score

of 91 was applied to filter the results, as scores above this threshold are indicative of highly reliable target predictions. The top-ranking miRNAs were shortlisted based on their target scores, and their associated gene interactions were examined in detail.

To understand the broader biological context of TTN, gene network analysis was performed using the STRING database. This platform integrates known and predicted protein-protein interactions derived from multiple sources, including experimental data, computational prediction methods, and existing literature. A high confidence interaction score of 0.99 was set as the threshold to ensure that only the most significant and biologically relevant interactions were included. The resulting network highlighted several key genes that are functionally associated with TTN, providing insight into its potential role in cellular processes and cancer progression.

Following data acquisition, results from all three platforms were systematically compared and integrated. Only those miRNAs that consistently demonstrated high scores and biological relevance were selected for further interpretation. This integrative approach allowed for the identification of a refined set of miRNAs that may play a critical role in regulating TTN expression.

Finally, the predicted miRNAs and associated gene networks were interpreted in the context of cancer biology, with particular emphasis on their potential involvement in tumor proliferation, progression, and metastasis. Supporting evidence from existing literature was also considered to validate the biological significance of the findings.

## RESULTS

The in silico analysis performed in this study revealed significant insights into the regulatory landscape of the TTN gene and its potential involvement in squamous cell neoplasms. Using a multi-database approach, a set of high-confidence microRNAs (miRNAs) and interacting genes were identified, highlighting the complex regulatory network associated with TTN.

Gene interaction analysis using the STRING database demonstrated that TTN is functionally associated with several structurally and regulatory important genes. Notable interacting partners included NEB, TRIM63, CAPN3, TCAP, ACTN2, FHL2, OBSCN, and CMYA5, among others. These genes are primarily involved in muscle organization, cytoskeletal integrity, and signal transduction pathways. The high confidence interaction score ( $\geq 0.99$ ) suggests a strong functional linkage, indicating that alterations in TTN expression may influence multiple downstream pathways. This network supports the hypothesis that TTN may not act in isolation but rather as part of a broader regulatory system that could contribute to tumor progression and metastasis.

miRNA prediction analysis identified several candidate miRNAs with high target scores, suggesting a strong regulatory potential over TTN expression. Among these, hsa-miR-4738-3p and hsa-miR-4670-3p

exhibited the highest target scores (95), indicating a strong likelihood of interaction. Other notable miRNAs included hsa-miR-4538, hsa-miR-4453, hsa-miR-4531, and hsa-miR-3919 (target score 94), followed by hsa-miR-3913-3p (93), and hsa-miR-590-3p, hsa-miR-4666a-5p, and hsa-miR-3680-3p (92). The miRNA hsa-miR-4477a, with a score of 91, also met the inclusion criteria and was retained for further consideration.

Further examination of these miRNAs revealed their association with genes involved in key cellular processes such as proliferation, apoptosis, and transcriptional regulation. For instance, genes such as PPM1E, TNRC6B, BMP2, FZD3, HIPK1, and PGR were found to be linked with the predicted miRNAs, suggesting a broader regulatory network extending beyond TTN alone.

Importantly, the integration of results from TargetScan, DIANA, and miRDB ensured that only high-confidence miRNA–gene interactions were considered. The consistency observed across these platforms strengthens the validity of the findings and reduces the likelihood of computational bias.

Overall, the results indicate that TTN is embedded within a complex gene–miRNA interaction network, and its dysregulation may contribute to the molecular mechanisms underlying squamous cell neoplasms. These findings provide a foundation for further experimental validation and highlight potential biomarkers for disease prognosis and therapeutic targeting.

## DISCUSSION

By Ambros and colleagues the first miRNA, lin-4, was discovered in *Caenorhabditis elegans* (Lee et al., 1993). 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 (Calin et al., 2002). Dysregulated miRNAs affect the tumor initiation and progression in several of the cancer hallmarks, abnormal miRNAs are expressed in tumors (Weinberg & A., 2013). miRNA can function either as an oncogene or tumor suppressor under few conditions depending on their target genes in case of cancer (Wilczyński et al., 2015). miRNAs are involved in different kinds of cancers like, breast (Iorio et al., 2005), colon (Michael et al., 2003), prostate (Takamizawa et al., 2004), thyroid (He et al., 2005), and gastric (Cummins & Velculescu, 2006).

The TTN gene is highly oncogenic. It is associated with many other genes - few important ones are NEB, AKORDI, TRIM63, CAPN3, TCAP, ACTN2, 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 (Chauveau et al., 2014).

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 (Miao et al., 2020). 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 (Hackman et al., 2002).

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 (Zou et al., 2022).

The present study provides a comprehensive in silico perspective on the regulatory role of the TTN gene in squamous cell neoplasms, with a particular focus on its interaction with microRNAs (miRNAs) and associated gene networks. The findings suggest that TTN may play a more dynamic role in cancer biology than traditionally appreciated, extending beyond its well-established structural function in muscle physiology.

The gene network analysis revealed strong associations between TTN and several key genes, including NEB, TRIM63, CAPN3, and ACTN2, which are primarily involved in cytoskeletal organization and cellular integrity. While these genes are classically linked to muscle function, emerging evidence indicates that cytoskeletal remodeling is a critical component of tumor progression, invasion, and metastasis. The high-confidence interactions observed in this study support the idea that TTN may contribute to these processes through coordinated regulation with its interacting partners.

In parallel, the identification of multiple high-scoring miRNAs targeting TTN highlights an additional layer of post-transcriptional regulation. Notably, miRNAs such as hsa-miR-4738-3p and hsa-miR-4670-3p demonstrated strong predictive scores, suggesting their potential involvement in modulating TTN expression. Given that miRNAs can function either as oncogenes or tumor suppressors depending on the cellular context, their interaction with TTN may have significant implications for tumor behavior. The association of these miRNAs with genes involved in signaling pathways, apoptosis, and transcriptional control further reinforces their potential relevance in cancer progression.

Importantly, the integration of multiple bioinformatics tools enhanced the reliability of the findings by minimizing false-positive predictions. However, it is essential to acknowledge that in silico analyses are inherently predictive and require experimental

validation to confirm biological significance. Functional studies, including gene expression profiling and in vitro assays, would be necessary to establish the precise role of TTN and its associated miRNAs in squamous cell carcinoma.

Overall, this study highlights TTN as a potentially important molecular player in squamous cell neoplasms and underscores the value of integrative bioinformatics approaches in uncovering novel regulatory mechanisms. These findings may contribute to the identification of new biomarkers and therapeutic targets in cancer research.

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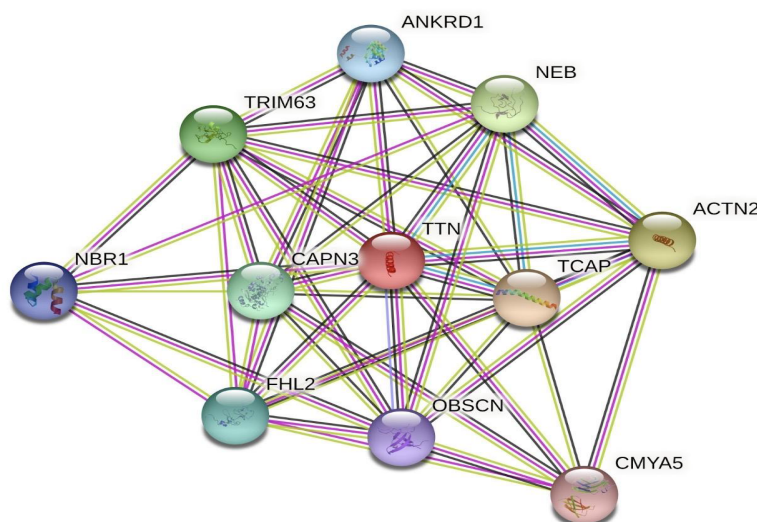
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**Figure 1: Gene analysis of TTN. The genes mentioned above are few genes which are co related to TTN. The upregulation of TTN effects in the regulation of the other stated genes.**

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Target Rank	Target Score	miRNA Name	Gene Sequence	Important Genes
1	95	hsa-miR-4738-3p	5'- ugaaacuggagcgcuggagga -3'	PPM1E, LRRTM3
2	95	hsa-miR-4670-3p	5'- ugaaguuacaucauggugcguu -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'- agacaucaagaucaguccaaa -3'	SHISA7, MEGF10
8	92	hsa-miR-590-3p	5'- uaauuuuauguauaagcuagu -3'	BPNT1, LTN1
9	92	hsa-miR-4666a-5p	5'- auacaugucagauuguauggcc -3'	COL4A3BP, PGR
10	92	hsa-miR-3680-3p	5'- uuuugcaugaccugggaguagg -3'	HIPK1, PGR
11	91	hsa-miR-4477a	5'- cuauuaaggacauuugauuc -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.