Mutated USP9X-Associated TRIM33 Inhibition in the Metastasis of Gingivobuccal Oral Squamous Cell Carcinoma

Suchitra Singh, Ajay Kumar Singh
Department of Bioinformatics, Central University of South Bihar, Gaya

Abstract

Objectives: Locoregional abortions of gingivobuccal oral squamous cell carcinoma (OSCC-GB) fabricate a global cancer burden. Ubiquitin Specific Peptidase 9 X-Linked (USP9X), a significantly mutated gene in OSCC-GB, usually aids SMAD family member4 (SMAD4) deubiquitination. The loss of USP9X prevents deubiquitination, which leads to SMAD4 inactivation. The TGF-β/SMAD signaling pathway is continuously regulated by a central transducer (SMAD4 protein). Inactivated SMAD4-deprived TGF-β/SMAD tumor suppressor response promotes the metastasis. USP9X inhibition promotes SMAD4 ubiquitination by E3 ligases TIF1-γ transcriptional intermediary factor1gamma (TRIM33). Overexpressed TRIM33 causes inactivation of SMAD4. The knockdown of TRIM33 inhibits tumor cell invasion. TRIM33 serves as a potential therapeutic target for OSCC-GB.

Methods: We conducted experiments to determine the possible interactions and inhibitions of target TRIM33 PHD-domain (PDB ID 3u5n) with natural as well as synthetic anticancerous drugs through molecular docking and virtual screening approach.

Results: Based on the lowest binding energy and rmsd values, our results suggest the probable synthetic inhibitor lapatanib having a binding energy of −9.22 and rmsd value of 0.00 and the natural inhibitor resveratrol having a binding energy of −8.22 and rmsd value of 0.00.

Conclusion: Our cumulative results formed a basis for investigating resveratrol and lapatanib as potent drugs for arresting active metastasis. Our findings can accentuate the importance of high-quality investigations in OSCC-GB.

Keywords: Gingivobuccal oral squamous cell carcinoma (OSCC-GB), TGF-β/SMAD signaling pathway, E3 ubiquitin-protein ligase (TRIM33/TIF1-γ), USP9X, SMAD4

Cite This Article: Singh S, Kumar Singh A. Mutated USP9X-Associated TRIM33 Inhibition in the Metastasis of Gingivobuccal Oral Squamous Cell Carcinoma. EJMO 2020;4(4):309–318.
vobuccal region that constitute the buccal mucosa, retro
molar trigone, and lower gum, together known as OSCC-
GB. The involvement of buccal mucosa is an aggressive
malignancy, with a significant propensity of invasion into
the surrounding tissues and metastasis into the cervical
lymph nodes.[5] Cervical lymph node metastasis is the most
critical prognostic factor of the head and neck cancer, as
evidenced by multiple studies.[6] We noted that OSCC-GB
substantially occurred at advanced stages (stages III and IV)
with high loco regional failure despite the best multimodal
treatment and therapy.[7]

Past studies on OSCC-GB identified some significantly
and commonly mutated gene specific to OSCC-GB (USP9X,
MLL4, UNC13C, ARID2, and TRPM3) as well as some shared
genes (with TP53, FAT1, CASP8, HRAS, and NOTCH1).[8]
Moreover, various pathways have been reported to be
altered in gingivobuccal cancer (including p53 signaling
pathways, apoptosis, viral carcinogenesis, neurotrophin
signaling pathway, Wnt signaling pathway, PI3K–Akt signal-
ing pathway, dorso-ventral axis formation, axon guidance,
MAPK signaling pathway, focal adhesion, cell adhesion
molecules, neuroactive ligand-receptor, Notch signaling
pathway, and serotonergic synapse).

Recent studies have shown that the alteration rate of
USP9X is the most frequent in OSCC-GB patients. USP9X
is a deubiquitinating enzyme for SMAD4,[8] which act as
an essential or crucial component of the TGF-β signaling
pathway.[9] The loss of USP9X prevents deubiquitination
of SMAD4, which in turn enhances the tumor progression.
The mutation of USP9X results in the overexpression of E3
ubiquitin-protein ligase TRIM33 protein that antagonized
the SMAD4 transcriptional and tumor suppressor activity.
The overexpression of ubiquitin ligases promotes the me-
tastasis of breast cancer.[10]

The TGF-β/SMAD signaling pathway helps in regulating cell
growth, differentiation, apoptosis, and homeostasis. The
alteration of the TGF-β/SMAD pathway have been shown
to be involved in a variety of human diseases, including
cancer, fibrosis diseases, atherosclerosis, cleidocranial dys-
plasia, and familial primary pulmonary hypertension.[11]
Different components of the TGF-β/SMAD pathway are
SMAD4, R-SMAD, 1-SMAD, ubiquitinating enzyme (TIF1g,
SMURF), deubiquitinating enzyme (USP9X, USP11, and
USP15), Transforming growth factor beta (TGF-β) is a family
of cytokines that help regulate a variety of biological pro-
cesses.[12] For instance, they bind to the serine/threonine ki-
nase domain of type I and type II receptor in the cytoplasm
and then transduce the signal to the nucleus by SMADs
proteins.[13] The receptor SMAD or R-SMAD (e.g., SMAD2
or SMAD3) gets directly phosphorylated by type 1 recep-
tor, hence forming an activated complex with co-SMAD
(SMAD4) protein.[14] These complexes then translocate to
the nucleus, where the regulation and transcription of the
target genes occur. The TGF-β/SMAD pathway is regulated
by ubiquitinating/deubiquitinating and the phosphoryla-
tion and dephosphorylation process. Ubiquitinating refers
to the post-translational modifications that modify or de-
grade a variety of SMAD components of the TGF-β/SMAD
signaling pathway. Ubiquitination antagonized the activity
of substrate protein (i.e., monoubiquitination). An ubiquiti-
ning enzyme (which ubiquitates substrate protein) acts
as a repressor and is a reversible process. Deubiquitinating
removes the covalently attached ubiquitin molecules from
the substrate protein and aids in the regulation of the sub-
strate protein.[15]

SMAD4 act as a central receptor of TGF-β signaling and
is crucial for most TGF-β biological effects, including em-
byronic development, tumor suppression, and metastasis,
and the reduction or absence of SMAD4 expression pro-
motes the carcinogenesis of OSCC.[16] Inactivated SMAD4
deplete the TGF-β/SMAD tumor suppressor response and
promotes the metastasis (Fig. 1). In the present study, we
performed the interactions and the possible inhibition of
the target (TRIM33/TIF1-γ) in the TGF-β/SMAD pathway
by using metastasis inhibitors via docking analysis.

Methods

Functional Information About Mutated Genes and
Selection of the Target

Uniprot database (http://www.uniprot.org) was opened,
and the name of the gene was typed, and the functional
information (i.e., about the molecular function and bio-
logical pathways) were retrieved and recorded. Among all
USP9X that encoded deubiquitinating enzyme for smad4
were significantly altered in OSCC-GB. The losses of USP9X
promotes the ubiquitination of smad4 and the expression
level of Trim33 protein. TIF1g/Trim33 inactivates Smad4
via ubiquitination, resulting in the loss of Smad4 in the
TGF-β/SMAD signaling pathway and thereby promoting
the metastasis. Ectodermín/Trim33a transcriptional cofac-
tor restricts the transcriptional activity of SMAD4 through
its PHD-domain overexpression of TIF1γ, which causes in-
activation of SMAD4. The knockdown of TIF1γ inhibits tu-
mor cell invasion. For the ubiquitination of Smad4, Trim33
requires its PHD-domain; therefore, for docking analysis,
only chain A and chain B were extracted from the complex
of Trim33 PHD-domain and Histone peptide. Therefore, in
silico interaction by docking analysis of TIF1g/Trim33 was
considered as the target molecule (Fig. 2).
Active Site Prediction of the Target Protein

The 3D coordinates of the active site of target protein is one of the important requirements for in silico docking analysis. The Ligsite software was used to predict the active sites of Trim33 protein. This software uses the Connolly surface and defines the surface-solvent-surface events instead of the protein-solvent-protein event for the prediction of active sites. Three active sites (x = −14.254, −25.255, 1.085, y = −2.469, −33.756, –13.597, z = −17.392, −53.977, 2.883) have been predicted by the Ligsite software (http://projects.biotec.tu-dresden.de/pocket/).

Protein Preparation

The structure of Tif1g/Trim33 proteins with pdb id 3u5n (Fig. 3) was opened in the Autodock window. Polar hydrogens were added, followed by the addition of Kollman charges and saved in the 3u5n.pdbqt format.

Ligand Selection and Preparation

Ligands selection is based on stage-specific metastasis of known inhibitors in various types of cancer that can be tested against the target of OSCC-GB. An interaction study was performed by selecting 10 natural inhibitors and 12 synthetic cancer inhibitors, as specified in Table 1 and Table 2. For molecular docking ligands, the files were opened in the Autodock window. The root and torsion numbers were detected and saved in the —.pdbqt format.

Grid Preparation

The grid box was prepared by giving X-Y-Z coordinates, and
the dimensions of the box were set to be 60-60-60 units for both the docking and virtual screening analysis. The grid file was saved as —sample_grid.gpf in the Autodock4.2 and conf.txt file in the Autodock Vina.

Docking Studies

Molecular docking software Autodock 4.2 (http://autodock.scripps.edu/) and Virtual screening software Autodock Vina (http://vina.scripps.edu) were used for the protein-ligand interaction studies.

| S.No | Inhibitors       | Pubchem ID | Cancer types | References                                                                 |
|------|------------------|------------|--------------|-----------------------------------------------------------------------------|
| 1    | Lapatinib (Tykerb/Tyverb, GW572016) | 208908     | HNSCC        | Agulnik, Mark, et al. “Phase II study of Lapatinib in recurrent or metastatic epidermal growth factor receptor and/or erbB2 expressing adenoid cystic carcinoma and non–adenoid cystic carcinoma malignant tumors of the salivary glands.” Journal of Clinical Oncology 25.25 (2007): 3978-3984. |
| 2    | Foretinib (GSK1363089) | 42642645   | HNSCC        | Seiwert, Tanguy, et al. “Phase II trial of single-agent foretinib (GSK1363089) in patients with recurrent or metastatic squamous cell carcinoma of the head and neck.” Investigational new drugs 31.2 (2013): 417-424. |
| 3    | Dasatinib (BMS-354825) | 3062316    | HNSCC        | Brooks, H. D., et al. “Phase II study of dasatinib in the treatment of head and neck squamous cell carcinoma (HNSCC).” Journal of Clinical Oncology 27.15S (2009): 6022-6022. |
| 4    | Erlotinib (Terceva, OSI-774) | 176870     | HNSCC        | Cohen, Ezra EW, et al. “Erlotinib and bevacizumab in patients with recurrent or metastatic squamous cell carcinoma of the head and neck: a phase I/II study.” The lancet oncology 10.3 (2009): 247-257. |
| 5    | Gemcitabine      | 60750      | Pancreatic cancer | Giroux, Valentin, et al. “p8 is a new target of gemcitabine in pancreatic cancer cells.” Clinical cancer research 12.1 (2006): 235-241. |
| 6    | Letrozole         | 3902       | Breast cancer | Geisler, Jürgen, et al. “Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, crossover study.” Journal of Clinical Oncology 20.3 (2002): 751-757. |
| 7    | Etoposide        | 36462      | Breast cancer | Bockbrader, Katrina M., Mingjia Tan, and Yi Sun. “A small molecule Smac-mimic compound induces apoptosis and sensitizes TRAIL-and etoposide-induced apoptosis in breast cancer cells.” Oncogene 24.49 (2005): 7381. |
| 8    | Palbociclib      | 5330286    | Breast cancer | Qin, Ge, et al. “Palbociclib inhibits epithelial-mesenchymal transition and metastasis in breast cancer via c-Jun/COX-2 signaling pathway.” Oncotarget 6.39 (2015): 41794. |
| 9    | Ifosfamide       | 3690       | Cervical cancer | Buda, A., et al. “Role of ifosfamide in cervical cancer: an overview.” Oncology 65.Suppl. 2 (2003): 63-66. |
| 10   | Aspirin          | 2244       | Colon Cancer | Ying, Jun, et al. “Aspirin inhibited the metastasis of colon cancer cells by inhibiting the expression of toll-like receptor 4.” Cell & bioscience 8.1 (2018): 1 |
| 11   | Carboplatin      | 10339178   | Lung cancer  | Cohen, Martin H., et al. "FDA drug approval summary: bevacizumab (Avastin®) plus carboplatin and paclitaxel as first-line treatment of advanced/metastatic recurrent nonsquamous non-small cell lung cancer." The oncologist 12.6 (2007): 713-718. |
| 12   | Topotecan        | 60700      | Lung cancer  | O’Brien, Mary Er, et al. “Phase III trial comparing supportive care alone with supportive care with oral topotecan in patients with relapsed small-cell lung cancer.” Journal of Clinical Oncology 24.34 (2006): 5441-5447 |
interaction study. All compounds were docked by keeping the protein rigid. The searching algorithm for docking was set as the Genetic Algorithm, and the input file was saved as — sample_dock.dpf.

**Virtual Screening**

Virtual screening is a high-throughput docking in which a large numbers of ligands are docked simultaneously at the target. In our study, we employed this method to discover new inhibitors for OSCC-GB cancer. Natural products were downloaded from the African database of Zinc Library (http://zinc15.docking.org/catalogs/afronp/). The drug likeness property or the ADMET properties of these compounds were checked using the DruLito tool (http://www.niper.gov.in/pi_dev_tools/DruLiToWeb). Compounds that did not follow the Lipinski rule of 5 were discarded. Out of 880 compounds, only 608 compounds followed the Lipinski rule. Hence, the virtual screening of 608 compounds was performed using the Autodock vina, and further top 10 compounds with the lowest binding energy and rmsd values were selected for the study.

**Analysis of the Interaction Studies**

Docking and virtual screening results were analyzed with the lowest binding energy, and the rmsd values of each were taken as the criteria of analysis. The best ligands with

| S.No | Natural compounds | Pubchem ID | Source | Cancer types | References |
|------|-------------------|------------|--------|--------------|------------|
| 1.   | Circumin          | 969516     | Turmeric | Cervical cancer | Zaman, Mohd S., et al. “Curcumin nano formulation for cervical cancer treatment.” Scientific reports 6 (2016): 20051. |
| 2.   | Genistein         | 5280961    | Soyabean, Lupin | Breast cancer | Chen, Jun, et al. “Genistein induces apoptosis by the inactivation of the IGF-1R/p-Akt signaling pathway in MCF-7 human breast cancer cells.” Food & function 6.3 (2015): 995-1000 |
| 3.   | Isoquercitin      | 5280804    | Apple, Onion | Colon cancer | Amado, Nathália G., et al. “Isoquercitrin suppresses colon cancer cell growth in vitro by targeting the Wnt/β-catenin signaling pathway.” Journal of Biological Chemistry 289.51 (2014): 35456-35467 |
| 4.   | Resveratrol       | 445154     | Peanuts, Grapes | Pancreatic cancer | Bonucci, Massimo, et al. “Integrated cancer treatment in the course of metastatic pancreatic cancer: complete resolution in 2 cases.” Integrative cancer therapies 17.3 (2018): 994-999 |
| 5.   | Sulforaphane      | 5350       | Cabbage, Broccoli, Sprouts | Oral Cancer | Liu, Chía-Ming, et al. “Sulforaphane targets cancer stemness and tumor initiating properties in oral squamous cell carcinomas via miR-200c induction.” Journal of the Formosan Medical Association116.1 (2017): 41-48 |
| 6.   | Gingerols         | 3473       | Ginger root | Breast cancer | Lee, Hyun Sook, et al. “[6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells.” The Journal of nutritional biochemistry19.5 (2008): 313-319 |
| 7.   | Aloe-emodin       | 10207      | Aloe Vera | Colon cancer | Suboj, Priya, et al. “Aloe emodin inhibits colon cancer cell migration/angiogenesis by downregulating MMP-2/9, RhoB and VEGF via reduced DNA binding activity of NF-κB.” European journal of pharmaceutical sciences 45.5 (2012): 581-591 |
| 8.   | Epigallocatechin gallocate | 65064 | Green Tea | Skin cancer | Siddiqui, Imtiaz Ahmad, Rohinton S. Tarapore, and HasanMukhtar. “Prevention of skin cancer by green tea: past, present and future.” Cancer biology & therapy 8.13 (2009): 1288 1291 |
| 9.   | Honokiol          | 72303      | Magnolia tree | Lung cancer | Singh, Tripti, and Santosh K. Katiyar. “Honokiol inhibits non-small cell lung cancer cell migration by targeting PGE2-mediated activation of β-catenin signaling.” PloS one 8.4 (2013): e60749. |
| 10.  | Daurinol          | 14704582   | Arilynaphthalene lignin plant | Lung and Breast cancer | Woo, Jong Kyu, et al. “Daurinol blocks breast and lung cancer metastasis and development by inhibition of focal adhesion kinase (FAK).” Oncotarget 8.34 (2017): 57058 |
the least energy of binding with the protein and the rmsd value were selected.

The LIG-PLOT (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) was used to study the H-Bond interaction interactions of the protein-ligand complex.

Results

Functional Analysis of Newly Identified or Significantly Mutated Genes

The functional and pathways' information of mutated genes were retrieved from uniprot (as mentioned in Table 3). Our focus was mainly on USP9X considering that its mutation rate is extremely common in gingivobuccal cancer. The loss of USP9X lead to the inactivation of SMAD4 protein, and both are crucial for the TGFβ/SMAD signaling. Based on the literature, this pathway is altered in several cancer types, such as HNSCC, OSCC, and breast cancer. The alteration in this pathway can be attributed to smad4 reduction and post-transcriptional changes.

Molecular Docking and Virtual Screening

The Autodock 4.2 predicted the best conformation of known ligand (synthetic and natural) with TRIM33. The lowest binding energy and the rmsd values were used as the criteria to analyze the docking result. The binding energy of Trim33 docked with known natural and synthetic inhibitors are listed in Table 4 and Table 5. Out of 3 active sites, the second active site coordinates were best in interaction with ligand based on binding affinity (Fig. 4).

Based on the abovementioned criteria, lapatanib and palbociclib (synthetic inhibitors) and aloe-emodin and reseveratrol (natural inhibitors) were found to be the best interacting molecules (Fig. 5) that are useful for further H-Bond interaction analysis through ligplot (https://www.ebi).

Table 3. List of newly identified genes in OSCC-GB and their functions

| Genes  | Function                                                                 | Involvement in Pathways                                                                 |
|--------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| USP9X  | Deubiquitination of protein (e.g., SMAD4), crucial for regulation of protein at the level of protein turnover by preventing degradation of protein through inhibition of ubiquitin molecules. Also involved in axonal growth, neuronal cell migration and tumor suppressor. | TGFβ/SMAD signaling, BMP signaling pathway mTor pathway, axon guidance pathway          |
| MLL4   | Histone methyl transferase, DNA binding, chromatin regulator and act as a co-activator of p53 (tumor suppressor gene). | P53 signaling pathway                                                                  |
| ARID2  | Chromatin regulator, DNA binding.                                         | Hippo signaling pathway                                                                 |
| UNC13C | Calcium ion binding, phospholipids binding, neurotransmitter.             |                                                                                         |
| TRPM3  | Calcium channel activity, cation channel activity, and neurotransmitter and also has a tumor suppressor activity. |                                                                                         |

Table 4. Molecular docking analysis of synthetic drugs against Trim33

| S.No | Compound Name | Pocket 1 | Pocket 2 | Pocket 3 |
|------|---------------|----------|----------|----------|
|      |               | Pose     | Binding Energy | RMSD | Pose     | Binding Energy | RMSD | Pose     | Binding Energy | RMSD |
| 1.   | Lapatinib*    | 2        | -7.43     | 0.00    | 10       | -9.22       | 0.00    | 5        | -5.42       | 0.00   |
| 2.   | Foretinib     | 6        | -6.73     | 0.00    | 1        | -6.34       | 0.00    | 3        | -3.92       | 0.00   |
| 3.   | Dasatinib     | 5        | -6.79     | 0.00    | 2        | -7.63       | 0.00    | 3        | -5.71       | 0.00   |
| 4.   | Erlotinib     | 8        | -6.10     | 0.00    | 4        | -7.19       | 0.00    | 1        | -5.40       | 0.00   |
| 5.   | Letrozole     | 6        | -6.12     | 0.00    | 1        | -8.12       | 0.00    | 10       | -6.14       | 0.00   |
| 6.   | Palbociclib*  | 4        | -8.55     | 0.00    | 2        | -8.33       | 0.00    | 3        | -5.71       | 0.00   |
| 7.   | Aspirin       | 4        | -4.73     | 0.00    | 1        | -6.29       | 0.00    | 7        | -5.44       | 0.00   |
| 8.   | Etoposide     | 1        | -7.05     | 0.00    | 3        | -8.62       | 0.00    | 4        | -6.54       | 0.00   |
| 9.   | Isoflamide    | 9        | -4.43     | 0.00    | 8        | -5.00       | 0.00    | 10       | -4.52       | 0.00   |
| 10.  | Carboplatin   | 9        | -4.46     | 0.00    | 9        | -4.62       | 0.00    | 2        | -4.40       | 0.00   |
| 11.  | Topotecan     | 4        | -7.43     | 0.00    | 8        | -8.77       | 0.00    | 1        | -6.96       | 0.00   |
| 12.  | Gemcitabine   | 1        | -4.68     | 34.99   | 6        | -5.67       | 0.00    | 4        | -5.40       | 0.00   |

*Best docked inhibitor
The criteria for evaluating virtual screening results were similar to that of molecular docking. Since the binding affinity of known drugs was better in the second active site, virtual screening was performed on the second active site. By using the above paradigm, top 10 compounds were listed in Table 6, and the interaction studies were performed for the compounds whose binding energy was greater than that of known drugs.

### Ligplot Interaction Analyses

TRIM33 and inhibitors interaction are displayed in the Table 7 and Table 8 in the form of H-Bond. E3 ubiquitin-protein ligase TRIM33 shows the best interaction with the natural

**Figure 4.** Binding energy of Trim33 with natural and synthetic compounds in all the three pockets Graph showing the binding energy of target and inhibitors interactions among all the pockets, pocket 2 have best binding energy in both natural and synthetic compounds docking. (a) Binding energy of Trim33 with natural inhibitors in all the three pockets. (b) Binding energy of Trim33 with synthetic inhibitors in all the three pockets.

**Figure 5.** Binding mode of natural and synthetic inhibitors with Trim33 protein (a) Structure and Binding mode of Resveratrol with different residues of Trim33. (b) Structure and Binding mode of Lapatanib with different residues of Trim33 protein. (c) Binding mode of natural lead compound (croman-4-one) with Trim33 protein.
drug resveratrol that forms 5 H-Bond with GLU 967 at a distance of 3.10 Å, Arg932 forms the H-Bond at a distance of 3.74 Å, Leu958 forms H-Bond at 3.75 Å distance, Tyr941 forms H-Bond at 3.75 Å, and His 1017 forms H-Bond at a distance of 3.33 Å (Fig. 6a).

Among the synthetic drugs, lapatinib showed the best interaction with Trim33. The residues involved in the formation of H-Bond include GLU981 and MET1001, which were at a distance of 3.02 Å and 3.00 Å (Fig. 6b). The interaction of E3 ubiquitin-protein ligase with the lead compound was not as good as that of known drugs, although the binding energy of lead compounds was higher than that of known inhibitors. The lead compounds screened by virtual screening had lesser H-Bond than the known drugs. The top two were lead residues.

**Discussion**

Our analysis revealed the involvement of the TGFβ/SMAD signaling pathway and its associated protein E3 ubiquitin ligase TRIM33 in cervical lymph node metastasis. The TGF-β signaling pathways promote cancer metastasis when both smad4 and USP9X functionality are lost. USP9X loss and E3 ubiquitin-protein ligase TRIM33 overexpression is an important factor involved in the metastasis of OSCC-GB.
fact, it may act as a new therapeutic target in OSCC-GB. A list of 12 well-known synthetic drugs along with 10 natural drugs are used for molecular docking. In silico docking studies on the current work provides evidence that, among all screened inhibitors, lapatanib (synthetic) and resveratrol (natural), have been proved to have a good potential in targeting metastasis in OSCC-GB. The cessation of metastasis may decrease the loco regional failure as well as mortality due to OSCC-GB, which is one of the crucial issues in India. Although the provided analysis and methodologies are adequate and constitute a set of powerful tools to guarantee the real-time requirements of the in silico approach, there is some scope for improvements. To determine the exact strength and duration of H-Bond, MD (Molecular Dynamics) Simulation can be performed with the docked molecules. Our results need to be validated through wet lab experiments in the future.

Disclosures

Peer-review: Externally peer-reviewed.
Conflict of Interest: None declared.

Authorship Contributions: Concept – S.S.; Design – S.S.; Supervision – A.K.S.; Materials – S.S.; Data collection &/or processing – S.S.; Analysis and/or interpretation – S.S., A.K.S.; Literature search – S.S.; Writing – S.S.; Critical review – A.K.S.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: a cancer journal for clinicians 2010;60:277–300.
2. Szafarowski T, Szczepanski MJ. Cancer stem cells in head and neck squamous cell carcinoma. Otolaryngologia Polska 2014;68:105–11.
3. Dikshit R, Gupta PC, Ramasundararhatte C, Gajalakshmi V, Aleksandrowicz L, Badwe R, et al; Million Death Study Collaborators. Cancer mortality in India: a nationally representative survey. Lancet 2012;379:1807–16.
4. Ghantous Y, Abu I.E. Global incidence and risk factors of oral cancer. Harefuah 2017;156:645–9.
5. Ahmed S.Q, Junaid M, Awari S, Choudhary M.M, Kazi M, Masoom, et.al., Relationship of tumor thickness with neck node metastasis in buccal squamous cell carcinoma: An experience at a tertiary care hospital. International archives of otolaryngology 2017;21:265–9.
6. Mamelle G, Pampurik J, Luboinski B, Lancar R, Lusinchi A, Bosq. Lymph node prognostic factors in head and neck squamous cell carcinomas. The American journal of surgery 1994;168:494–8.
7. Walvekar RR, Chaukar DA, Deshpande MS, Pai PS, Chaturvedi P, Kakade A, et al. Squamous cell carcinoma of the gingivo-buccal complex: predictors of locoregional failure in stage III-IV cancers. Oral Oncol 2009;45:135–40.
8. India Project Team of the International Cancer Genome Consortium. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. Nat Commun 2013;4:2873.
9. Dupont S, Mamidi A, Cordenonsi M, Montagner M, Zacchigna L, Adorno M, et al. FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. Cell 2009:136:123–35.
10. Gallo LH, Ko J, Donoghue DJ. The importance of regulatory ubiquitination in cancer and metastasis. Cell Cycle 2017;16:634–48.
11. Massagué J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. EMBO J 2000;19:1745–54.
12. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med 2000;342:1350–8.
13. Akhurst RJ, Derynck R. TGF-beta signaling in cancer--a double-edged sword. Trends Cell Biol 2001;11:544–51.
14. Massagué J. TGFBeta signal transduction. Annu Rev Biochem 1998;67:753–91.
15. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, et al. A genomic and functional inventory of deubiquitinating enzymes Cell 2005;123:773–86.
16. Iamaroon A, Pattamapun K, Pibooniyom S.O. Aberrant expression of Smad4, a TGF-β signaling molecule, in oral squa-
mous cell carcinoma Journal of oral science 2006;48:105-109.
17. Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, et al. Effect of high intensity intermittent training on heart rate variability in pubescent children. European journal of applied physiology 2009;105:731–8.
18. Pathak KA, Gupta S, Talole S, Khanna V, Chaturvedi P, Deshpande MS, et al. Advanced squamous cell carcinoma of lower gingivobuccal complex: patterns of spread and failure. Head & Neck: Journal for the Sciences and Specialties of the Head and Neck 2005;27:597–602.
19. Sadaie M, Salama R, Carroll T, Tomimatsu K, Chandra T, Young AR, et al. Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. Genes & development 2013;27:1800–8.
20. Hussain S, Zhang Y, Galardy P. DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. Cell cycle 2009;8:1688–97.
21. Hoge CW, McGurk D, Thomas JI, Cox AL, Engel CC, Castro CA. Mild traumatic brain injury in US soldiers returning from Iraq. New England journal of medicine 2008;358:453–63.
22. Liu T, Ghosal G, Yua J, Chen J, Huang J. FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. Science 2010;329:693–6.
23. Bornstein S, White R, Malkoski S, Oka M, Han G, Cleaver T, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. The Journal of clinical investigation 2009;119:3408–19.
24. Zhao M, Mishra L, Deng CX. The role of TGF-β/SMAD4 signaling in cancer. International journal of biological sciences 2018;14:111–23.
25. Fadlullah MZH, Chiang IKN, Dionne KR, San Yee P, Gan CP, Sam KK, et al. Genetically-defined novel oral squamous cell carcinoma cell lines for the development of molecular therapies. Oncotarget 2016;7:27802–18.
26. Ligr M, Wu X, Daniels G, Zhang D, Wang H, Hajdu C, et al. Imbalanced expression of Tif1γ inhibits pancreatic ductal epithelial cell growth. Am J Cancer Res 2014;4:196–210.
27. Chen XF, Zhang HJ, Wang HB, Zhu J, Zhou WY, Zhang H, et al. Transforming growth factor-β1 induces epithelial-to-mesenchymal transition in human lung cancer cells via PI3K/Akt and MEK/Erk1/2 signaling pathways. Mol Biol Rep 2012;39:3549–56.

Web servers
- http://www.rcsb.org/pdb/home/home.do
- http://projects.biotec.tu-dresden.de/pocket/
- http://autodock.scripps.edu/
- www.ebi.ac.uk/thornton-srv/software/LigPlus/
- https://www.pymol.org
- http://www.niper.gov.in/pi_dev_tools/DruLiToWeb