DRUG-GENE EXPRESSION PROFILES AND SYSTEMS BIOLOGY APPROACH TO IDENTIFY REPURPOSED DRUG CANDIDATES FOR TARGETING SCLEROSTIN IN PERI-IMPLANTITIS DISEASE

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Abstract

Successful identification of a therapeutic strategy to treat patients with periimplantitis remains extremely important as post-implant bone degradation leads to implant failure and extreme bone loss. Given that the establishment of a new drug is quite expensive and time-consuming, the drug repurposing approach has come in handy. It helps to identify the experimental drugs that are beyond the purview of the initial clinical indication. In our current study, we propose a three-step drug repurposing approach in treating peri-implant bone defects and investigating the action of the FDA approved drugs to inhibit the key protein Sclerostin, involved in bone degradation. As the preliminary step, we differentiated the gene expression pattern in periimplantitis and dentate patients with their drug-induced profiles to identify the primary lead candidates. As the second step, we employed the computational biology approach to evaluate the protein-drug interaction and segregate the best hits among the identified lead compounds. Finally, the mode of action network for each candidate is established with the help of literature support, and the drug enrichment and pathway analysis are performed on the target genes in the network to evaluate the drug efficacy. This approach provided us with a drug interaction profile and specific genes and biomarkers to target bone mineralization in peri-implantitis. Thus, our three-step drug repurposing method is consistent with identifying the drug molecules with high efficacy and developing an efficient therapeutic strategy to treat peri-implantitis.

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Introduction

- A successful dental implantation highly depends on the Osseo-integration of the dental implant and intra-oral tissue. Most of the time when the implants are introduced to the oral tissue, the implant-tissue interface breaks down at the crestal and further into the endosteal regions.
- **Peri-implantitis** is **caused** by the gum surrounding a tooth implant becoming inflected. **Peri-implantitis causes** and risk factors include: Poor oral hygiene. Tobacco use.
- This BMP (Bone morphogenic protein) antagonist mimic has its main function in promoting apoptosis of bone cells and inhibiting osteo-regeneration through catalyzing bone resorption.
- In order to minimize the bone loss, the mainstay treatment is to promote bone remineralization by blocking the sclerostin expression and activate Wnt signaling. It is a great deal to achieve such stability naturally and thus, it should be synthetically induced. It can be done using bone-analogies like Dicckopf-1 or sclerostin antibody (Romosozumab) treatment.
- Drug repurposing is an absolute strategy for discovering a novel application of the already existing drugs that are approved in the market. It helps to identify the experimental drugs that are beyond the purview of the initial medical indication, which is an added advantage over finding an entirely new drug for a given clinical indication.
- Therefore, we felt that there is a need discover and establish the new purpose of already approved drugs from the FDA drug database, as dual function drugs for sclerostin inhibition.

Materials and Methods

Data sources: 1) Gene expression data comparing peri implantitis and healthy controls were downloaded from Affymetrics Microarray data in Gene Expression Omnibus (GEO) with an accession code GSE57631. 2) Drug-induced gene expression data are retrieved from LINCS1000 FWD dataset that provides differentially expressed genes (DEGs) with z score. The FDA approved drug targets are retrieved from DrugBank database with gene signatures and known targets to perform repurposing analysis. 3) Gene ontology annotation and pathway enrichment analysis were performed using DAVID software. 4) The protein-protein (PPI) interaction were analyzed and visualized in cytoscape software. In the network, the nodes are genes, and the edges are PPI between nodes.

Data Analysis: 1) The top 250 up/down regulated DEGs with fold change were detected using GEO2r tool. 2) The drug repositioning is calculated using weighed/normalized/terminal connectivity scores. 3) The final drug targets are identified, and Drug Set Enrichment Analysis (DSEA) is done on the drug candidates. 4) Mode of Action (MOA) is developed for each drug candidate and the potential MOAs are identified using KEGG pathway analysis. 5) The Module is analysed using DAVID tool.

Results

- Our analysis identified drugs used in clinical practice including spiperone and hydrocortisone, out of which a few were already reported for peri-implantitis.
- The drug spiperone is known for successful nuclear translocation of SMAD, which is required for BMP and further Wnt pathway activation. Since sclerostin is a Wnt antagonist, the action of spiperone and similar drugs can be a possible therapy to prevent permanent bone loss.
- The pathway enrichment has identified the proteasome pathway being involved in peri-implantitis.
- The enrichment analysis and MOA network established the gene-drug relationship.
- The GSK3b, which is inactivated for Wnt activation, along with 150 other genes were overexpressed, while bone-forming BMPIR and related genes were downregulated in peri-implantitis samples.
- Additionally on inspecting the positive gene expression during peri-implantitis, we identified that HIST1H2AC, VCP, HSPA5, PPP1R7 and MSN present in the data set were key proteins interacting with Sclerostin SOST. (Further studies are being performed)

Sno.	ID	Gene name	LogFC	p-value	Regulation
1.	8339_at	HIST1H2BG	-1.498	0.000241	Up
2.	10480_at	EIF3M	-1.865	0.000305	Up
3.	857_at	CAV1	-1.808	0.000343	Up
4.	829_at	CAPZA1	-1.382	0.000385	Up
5.	10269_at	ZMPSTE24	-1.442	0.000425	Up
6.	84516_at	DCTN5	-1.963	0.000526	Up
7.	55845_at	BRK1	-1.16	0.000527	Up
8.	5691_at	PSMB3	-1.474	0.000684	Up
9.	5537_at	PPP6C	-1.644	0.00071	Up
10.	10383_at	TUBB2C	-1.733	0.000776	Up
11.	6637_at	SNRPG	1.522	0.000283	Down
12.	284805_at	C20orf203	1.275	0.000552	Down
13.	406949_at	MIR15B	1.21	0.000692	Down
14.	406996_at	MIR214	1.835	0.000828	Down
15.	407055_at	MIR99A	1.452	0.001108	Down
16.	28517_at	TRDV2	1.196	0.001622	Down
17.	65122_at	PRAMEF2	1.234	0.001687	Down
18.	653492_at	PSG10P	1.145	0.001845	Down
19.	2115_at	ETV1	1.832	0.002088	Down
20.	54967_at	CXorf48	1.219	0.002231	Down

Table 1: Top 20 differentially expressed genes (DEGs) in peri-implantitis.

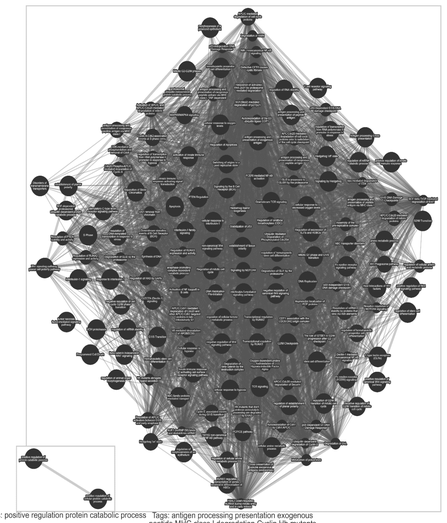

Fig 1: PPI network

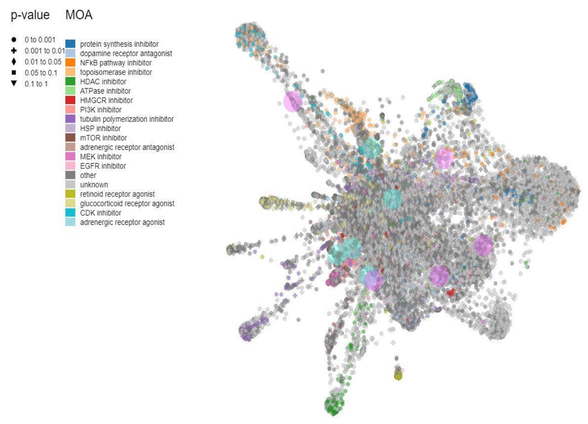

Fig 2: MOA analysis

Sno.	Final candidates for Peri-implantitis	Initial clinical indication	MOA
1.	Spiperone	Schizophrenia	Dopamine receptor Antagonist
2.	Dinoprostone	Vaginal suppository for labor	Protein synthesis inhibitor
3.	Vincristine	Chemotherapeutic drug	Tubulin polymerization inhibitor
4.	Hydrocortisone	adrenocortical insufficiency and inflammation	Glucocorticoid receptor antagonist
5.	Belinostat	Anticancer drug	HDAC inhibitor
6.	Lonidamine	Anticancer drug	Cyclooxygenase inhibitor
7.	Thiostrepton	Antibiotic	Tubulin polymerization inhibitor

Table 2: Final drug candidates.

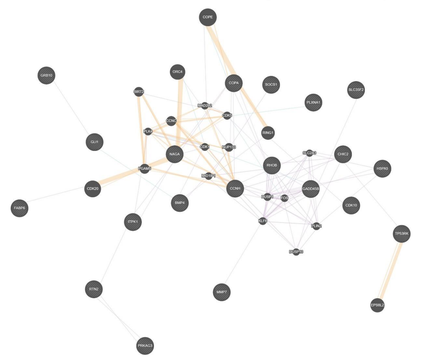

Fig 3a: Spiperone Gene Interaction network

score	type	name
98.69		HIV protease inhibitor
97.68		Tachykinin antagonist
97.45		Serotonin receptor antagonist
96.13		Estrogen receptor antagonist
95.67		Sigma receptor antagonist
3.95		Nucleophosmin inhibitor
3.65		Rho associated kinase inhibitor
3.05		NKL subclass homeoboxes and pseudogenes LOF
2.97		Zinc fingers RanBP2 type LOF
0.65		Cell Cycle Inhibition GOF

Fig 3b: Spiperone perturbagens

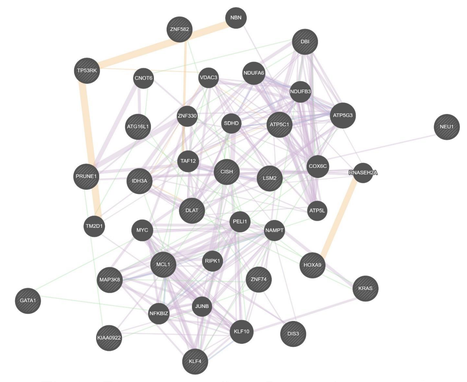

Fig 4a: Dinoprostone Gene Interaction network

score	type	name
96.83		DLAT
96.41		KIAA0922
96.26		TP53RK
95.91		DIS3
95.88		ZNF582
95.54		NEU1
95.13		ATP5C1
94.96		GATA1
94.60		KRAS
94.57		CISH
-15.38		KLF4
-15.73		DBI
-23.43		HOXA9
-27.85		ATG16L1
-30.54		IDH3A
-35.97		MCL1
-39.45		PRUNE
-44.02		ZNF74
-47.72		MAP3K8
-72.98		LSM2

Fig 4b: Dinoprostone perturbagens

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Proteasome	1.690e-10	5.206e-8	17.78	400.02
2	Protein export	0.002840	0.2187	10.43	61.19
3	Spliceosome	0.001499	0.1539	4.18	27.18
4	MicroRNAs in cancer	0.0004046	0.06232	3.21	25.08
5	Vasopressin-regulated water reabsorption	0.01753	0.6000	5.45	22.06
6	Protein processing in endoplasmic reticulum	0.004795	0.2953	3.39	18.12
7	Pentose phosphate pathway	0.05383	1.000	5.33	15.58
8	Alcoholism	0.007624	0.3914	3.11	15.17
9	Legionellosis	0.03144	0.9685	4.36	15.10
10	Mucin type O-glycan biosynthesis	0.05708	1.000	5.16	14.78

Table 2: KEGG pathway enrichment analysis

Annotation Cluster 1	Enrichment Score: 9.17			Count	P_Value	Benjamini
GOTERM_BP_DIRECT	regulation of cellular amino acid metabolic process	RT		11	5.3E-12	4.6E-9
GOTERM_BP_DIRECT	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	RT		11	4.9E-11	1.4E-8
GOTERM_BP_DIRECT	NIK/NF-kappaB signaling	RT		11	8.0E-11	1.7E-8
GOTERM_BP_DIRECT	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	RT		11	3.4E-10	4.9E-8
GOTERM_BP_DIRECT	anaphase-promoting complex-dependent catabolic process	RT		11	5.0E-10	5.6E-8
GOTERM_BP_DIRECT	Wnt signaling pathway, planar cell polarity pathway	RT		11	2.3E-9	2.0E-7
GOTERM_BP_DIRECT	tumor necrosis factor-mediated signaling pathway	RT		11	2.6E-8	1.6E-6
GOTERM_BP_DIRECT	T cell receptor signaling pathway	RT		11	2.3E-7	1.2E-5

Table 3: Functional Annotation Clustering

Conclusion

Thus, we established a framework for drug repurposing and presented a list of repositioning candidates for Peri-implantitis. we believe that this systematic drug discovery could be of particular use in the discovery of novel effective pharmacological therapies for peri-implant diseases.

References

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