Systems Biology Unraveled the Relationship of lncRNA OIP5-AS1 with CD25 and its Co-Expression Analysis in Cancers

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Research

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Abstract

Background: Treg cells function in the immune homeostasis, these cells express high level of CD25. Even though the molecular mechanisms of CD25-mediated signaling pathways has been reported, some questions are still unclear, e.g. the relationship and function of the relative lncRNA. It is known that the CD25 expression levels are various among different cancers. Thus, we intended to dissect systems biology of a lncRNA pertained to CD25 and CD25 protein interactors-targeting miRNAs.

Methods: Apart from using the available RNA-seq data, the co-expression analysis of the lncRNA pertained to some cancers was performed. Our analysis was done for protein interactors of CD25 by STRING 11.0, ShinyGO v0.60 and KEGG web servers were used for enrichment and network analysis of CD25. TargetScan 7.2, miRTargetLink Human and mirDIP were applied for determining the CD25 and CD25 interactors-targeting miRNAs. To find the lncRNA-miRNA and lncRNA-protein interactions, starBase v3.0, LncBase Predicted v.2 and SFPEL-LPI were recruited, respectively. Also, using Co-LncRNA, the co-expressed lncRNA analysis and the relative signaling pathways in some cancers including bladder, breast, head and neck, kidney, liver, lung, prostate and thyroid cancers using RNA-seq data were achieved.

Results: OIP5-AS1 was shown to have the interaction with CD25 and CD25 protein interactors-targeting miRNAs. In addition, the co-expression of OIP5-AS1 in cancers and their signaling pathways was identified.

Conclusions: Possibly, OIP5-AS1 can effect on CD25 expression in all relative signaling pathways of these cancers.

Background

Treg cells play an important role in immune homeostasis. These CD4⁺ Foxp3⁺ Tregs express high levels of CD25 (known as IL-2RA). Tregs are the solitary immune cell type identified to express the full heterotrimeric receptor including CD25, CD122 (IL-2RB), and CD132 (IL-2RG), constitutively [1]. The IL-2 functions via high and low affinity in these cell surface receptors. The high affinity receptor complex is begun by binding the IL-2 to CD25 and next, CD122 and CD132 are engaged in the process. The CD122 and CD132 form a receptor with a 10-100 fold lower affinity for IL-2, in the absence of CD25 [2]. Notably, the IL-2 signaling is essential for the generation, survival and function of Treg cells. The heterotrimeric receptor is able to turn on MAPK/ERK, PI(3)K and STAT5 pathways [3]. Even though much is recognized about the molecular mechanisms of CD25 signaling, some questions are remained. Nowadays, some bioinformatic tools have been extended to carry out the functional annotations. The most well-known bioinformatic tool called ORA is recruited to gain the significant functional data (enrichment) from sets of related genes/proteins. This tool is used to detect the related and over-represented biological and functional annotations that are significantly enriched in a list of genes/proteins [4]. Also, another important bioinformatic tool consists of the network analysis describing and visualizing the protein-protein interactions of signaling pathways related to the reference genes/proteins list [5]. In these cases,

we aimed to describe the enrichment and network analyses of CD25. Also, using the prediction of miRNA targets we intended to describe the most significant relative miRNAs for CD25 and its protein interactors. Through the most significant and annotated data, we dissected the relative and regulator lncRNA pertained to CD25 and CD25 protein interactors-targeting miRNAs. It was reported that the CD25 expression level was changed in some cancers [6, 7]. With this aim, exploiting the available RNA-seq data, the co-expression analysis of the lncRNA pertained to some cancers was performed and the relative systems biology was dissected. Remarkably, these analyses were done for the first time to explain the predicted and annotated data of enrichment and network signaling pathways, non-coding RNAs and co-expression analysis in some cancers related to CD25 expression.

Methods

Protein-protein interaction (PPI) analysis

The PPI of human CD25 was evaluated using STRING web server version 11.0 (<https://string-db.org>). In this web server, the medium confidence and the max number of interactors in first shell were optioned as 0.4 and no more than 20 interactors, respectively.

Enrichment and network analysis

Using ShinyGO v0.60 web server (<http://bioinformatics.sdstate.edu/go>) and KEGG (<https://www.genome.jp/kegg>), the enrichment and network analysis for the significant pathways related to CD25 was gained. The P-value cutoff (FDR)= 0.05 was considered.

MiRNA prediction

For predicting the engagement of miRNA and CD25, TargetScan 7.2 (<http://www.targetscan.org>), miRTargetLink Human (<https://ccb-web.cs.uni-saarland.de/mirtargetlink>) and mirDIP (<http://ophid.utoronto.ca/mirDIP/index.jsp>) were utilized. The miRNA targets of CD25 were gained by the options including weaker evidence and predicted interactions in miRTargetLink Human. Also, the targets of CD25 were gained based on the score class (very high, high and medium) in mirDIP. The mirDIP web server was used for the CD25 protein interactors-targeting miRNAs.

LncRNA-miRNA interaction

In order to find the lncRNA-miRNA interaction, the open-source platform of starBase v3.0 (<http://starbase.sysu.edu.cn>) [8] was applied based on the CLIP-seq data. Also, for further confirming these data, LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2%2Findex-predicted) [9] from DIANA tools was utilized.

LncRNA-protein interaction

SFPEL-LPI web server (<http://www.bioinfotech.cn/SFPEL-LPI>) was applied to find the lncRNA-protein interactions [10].

Co-expression analysis of lncRNA

Using Co-LncRNA web server (<http://bio-bigdata.hrbmu.edu.cn/Co-LncRNA>) the co-expressed lncRNA analysis and the relative signaling pathways in some cancers including bladder, breast, head and neck, kidney, liver, lung, prostate and thyroid cancers using RNA-seq data were achieved. All these data are available at The Cancer Genome Atlas Program (TCGA) (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>).

Results

The PPI outcome of CD25

The PPI result of CD25 was depicted by the number of nodes= 21, number of edges= 182 and PPI enrichment p-value< 1E-16 (Fig. 1a and 1b).

The enriched signaling pathways and network of CD25

This analysis showed JAK-STAT (Fig. 1c) and Influenza A signaling pathways with the lowest Enrichment FDR= 6E-30 and the highest Enrichment FDR= 0.000025 utilizing KEGG option were placed in the resulted Table 1, respectively. Also, the network signaling pathways pertained to CD25 was depicted (Fig. 1d).

MiRNA prediction of CD25 and its interactors

The TargetScan 7.2 showed the conserved sites in miRNA-CD25 interaction (Table 2). The miRTargetLink Human displayed 50 interactions with weak support (Fig. 2a). Also, mirDIP demonstrated that 2049 miRNAs were engaged in CD25 (Table S1). Also, 33051 miRNAs were predicted by mirDIP web server for the CD25 protein interactors-targeting miRNAs (Table S2).

lncRNA-miRNA interaction outcomes

The resulted miRNAs from TargetScan 7.2, miRTargetLink Human and mirDIP were evaluated by starBase v3.0 to find miRNA-lncRNA interaction. Based on CLIP-seq data, the starBase v3.0 showed that TargetScan conserved miRNAs including hsa-miR-30c-5p, hsa-miR-30b-5p, hsa-miR-30a-5p, hsa-miR-30e-5p and hsa-miR-30d-5p possessed the interactions with lncRNA of OIP5-AS1 (For instance, hsa-miR-30b-5p was illustrated in Fig. 2b and other mentioned miRNAs were not shown). Also, miRTargetLink Human miRNAs had no interaction with any lncRNA in starBase v3.0 web server. Furthermore, among mirDIP miRNAs some of them with score class of “very high” including hsa-miR-30d-5p, hsa-miR-30a-5p, hsa-miR-30e-5p, hsa-miR-140-5p, hsa-miR-143-3p, hsa-miR-30c-5p, hsa-miR-211-5p and hsa-miR-324-3p had the interactions with lncRNA of OIP5-AS1 (For example, hsa-miR-324-3p was shown in Fig. 2c and other mentioned miRNAs were not shown). For further proving these results, LncBase Predicted v.2 showed that

OIP5-AS1 interacted with human CD25 and its protein interactors-targeting miRNAs from mirDIP (Table S3).

LncRNA-protein interaction outcomes

Based on the results of SFPEL-LPI web server, it was found that OIP5-AS1 possessed the interaction with some proteins. The most score was 0.999 pertained to ELAV-like protein 1 (Table 3).

Co-expressed lncRNA outcomes

According to Co-LncRNA web server results and its RNA-seq data, the co-expression of OIP5-AS1 in bladder, breast, head and neck, kidney, liver, lung, prostate and thyroid cancers in normal vs. tumor samples were proved (Table 4, Table S4, S5, S6, S7, S8, S9 and S10).

Discussion

The purpose of systems biology is to combine comprehensive biological data from varied experimental approaches to realize complex interactions at the molecular stage [11]. One of the important protein components of the human immune system is CD25 expressed on cell surface of Treg cells [1]. CD4⁺CD25⁺ Tregs cells inside TME known as TI-Tregs possess the essential function in cancer immune escape [12]. Albeit the molecular mechanisms of CD25-mediated signaling pathways has been revealed, some inquiries are still unanswered. Using systems biology approach, the molecular interactors including lncRNAs, miRNAs and proteins for significant protein of CD25 will be unraveled. Revealing the interactions between CD25 and other molecules in TME may be promised to overcome the cancer immune escape and gain the cancer successful treatment [13].

With this aim, we found that the CD25 protein interactors were included IL2, IL2RB, IL2RG, STAT5B, FOXP3, STAT5A, CSF2, JAK3, STAT3, IL5, JAK1, LCK, IL3, CD8A, CD3E, STAT1, AKT1, JAK2, CSF2RA and SOCS1. Also, the enrichment analysis showed that the top five significant signaling pathways were included JAK-STAT signaling pathway, Th17 cell differentiation, Th1 and Th2 cell differentiation, Measles and pathways in cancer. Indeed, JAK-STAT signaling pathway and pathways in cancer were related with high score based on the tree illustration in ShinyGO v0.60 web server (Fig. 2d). Among the evaluated miRNAs targeting CD25 mRNA, starBase v3.0 revealed that hsa-miR-30a-5p, hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-30d-5p, hsa-miR-30e-5p, hsa-miR-140-5p, hsa-miR-143-3p, hsa-miR-211-5p and hsa-miR-324-3p were engaged in lncRNA interaction with OIP5-AS1. SFPEL-LPI demonstrated that the lncRNA had the interaction with the following proteins: ELAVL1, IGF2BP3, IGF2BP2, IGF2BP1, RNA-binding protein FUS (FUS), TARDBP, AGO2, TIA1, PTBP1, AGO3, AGO4, SRSF1, Putative helicase MOV-10 (MOV10), AGO1, TNRC6A, RBFOX2, Transcriptional repressor protein YY1 (YY1), Transcription factor Sp1 (SP1), PTEN, Polycomb protein SUZ12 (SUZ12), SF1 and REST (Table 3, Fig. 3). Generally, SFPEL-LPI utilizes the sequence features of lncRNAs and proteins. Also, this web server calculates multiple similarities of protein-protein and lncRNA-lncRNA using protein and lncRNA sequences and recognized lncRNA-protein

interactions. Next, SFPEL-LPI merges multiple features and similarities with a feature projection ensemble learning frame [10].

This lncRNA is an important non-coding RNA involved in many cellular processes. In fact, the lncRNA with NONCODE ID: NONHSAT041930 or OIP5-AS1 abbreviated from OIP5 antisense RNA 1, is a mammalian lncRNA functioning in the cytoplasm [14]. The OIP5-AS1 has been focused for its role in the development of brain and eye [15]. Kim et al. (2016) reported that the lncRNA could inhibit HuR binding to target mRNAs. Therefore, it repressed the HuR-elicited proliferative phenotypes. In fact, they reported as the study of HeLa cells that OIP5-AS1 sponges ELAVL1 [16]. Also, Kim et al. (2017) illustrated that the lncRNA had the interaction with GAK mRNA, advancing GAK mRNA decay and sodecreasing GAK protein levels and reducing cell proliferation [17]. Also, Zhang et al. (2019) concluded OIP5-AS1 played as a ceRNA to make proliferation, migration and invasion of primary HemECs via regulating miR-195-5p/NOB1 axis. Indeed, ceRNAs perform as the molecular sponges for a particular miRNA via their miRNA binding sites [18]. Because of this function, also known as MREs, they de-repressed all target genes from the respective miRNA family [19].

In another point of view, the present co-expression analysis showed that OIP5-AS1 was co-expressed in normal vs. tumor bladder cancer based on the RNA-seq data. Clearly, this lncRNA was co-expressed in aminoacyl-tRNA biosynthesis pathway with p-value= $3.33\text{E-}15$ and Bonferroni correction= $6.02\text{E-}13$. Also, this cancer had the co-expressed OIP5-AS1 in other four top predicted pathways such as DNA replication, RNA degradation, cell cycle and spliceosome (Table 4). This analysis in breast cancer showed that pathways including pathways in cancer, neurotrophin signaling pathway, spliceosome, purine metabolism and aminoacyl-tRNA biosynthesis possessed the co-expressed OIP5-AS1 in the normal vs. tumor tissue (Table S4). This characteristic in head and neck cancer was engaged in these cellular processes including endocytosis, T cell receptor signaling pathway, neurotrophin signaling pathway, MAPK signaling pathway and lysosome (Table S5). In case of kidney cancer, purine metabolism, prostate cancer pathways, insulin signaling, endocytosis and RNA degradation were involved by p-value of $1.32\text{E-}12$, $5.94\text{E-}12$, $1.89\text{E-}11$, $3.66\text{E-}10$ and $3.87\text{E-}10$, respectively (Table S6). The liver cancer showed that the regulation of actin cytoskeleton, neurotrophin signaling pathway, focal adhesion, pancreatic cancer pathway and ribosome were engaged in OIP5-AS1 co-expression (Table S7). For lung cancer, MAPK signaling pathway, purine metabolism, lysosome, huntingtons disease and pyrimidine metabolism demonstrated the co-expressed OIP5-AS1 (Table S8). For prostate cancer, focal adhesion, MAPK signaling pathway, chemokine signaling pathway, regulation of actin cytoskeleton apoptosis and apoptosis were shared the co-expression of OIP5-AS1 (Table S9). Notably, the thyroid cancer illustrated that neurotrophin signaling pathway, RNA degradation, lysine degradation, aminoacyl-tRNA biosynthesis and renal cell carcinoma pathways had the shared feature from the point of view of OIP5-AS1 co-expression by p-value of $5.55\text{E-}16$, $3.75\text{E-}14$, $2.82\text{E-}11$, $1.06\text{E-}10$ and $1.48\text{E-}10$, respectively (Table S10).

As a recent report, OIP5-AS1 lncRNA could adjust cell proliferation and apoptosis by miR-410 and its target KLF10/PTEN/AKT [20]. In addition, the researchers recognized a putative ceRNA network for lncRNAs of AC008124.1, OPI5-AS1 and NEAT1 in breast tumors [21]. Also, it was studied in two human

osteosarcoma cell lines, MG63 and SaOS2, that OIP5-AS1 led cisplatin resistance via provoking the LPAAT β /PI3K/AKT/mTOR signaling pathway as the sponge for miR-340-5p [22]. In another study in osteosarcoma tissues and cells, the silencing of OIP5-AS1 inhibited the proliferation and also speeded up the apoptosis, and G0/G1 cycle arrest. Indeed, OIP5-AS1/miR-223/CDK14 performed the modulation on the [tumorigenesis](#) of osteosarcoma [23]. It was resulted that the over-expression of miR-367-3p, piR-30188 and PIWIL3 or knockdown of OIP5-AS1 effected on the suppression of glioma progression [24]. In undifferentiated oral tumors, the over-expression of OIP5-AS1 could be proposed for the poor clinical result and elevated cancer stemness [25]. Also, OIP5-AS expression was significantly reduced in non-small cell lung cancer tissues against adjacent non-cancerous tissues in whole samples and in male patients [26].

The obtained results pertained to the interaction of OIP5-AS1 with CD25 and its interactors-targeting miRNAs revealed that OIP5-AS1 acts possibly as reported function of molecular sponge in the regulation of CD25 expression and its protein interactors by the relative miRNAs. OIP5-AS1 showed the interaction with CD25-targeting miRNAs. In fact, hsa-miR-152-3p (with medium score of mirDIP), hsa-miR-137 (with medium score of mirDIP), hsa-miR-148a-3p (with medium score of mirDIP), hsa-miR-143-3p (with very high score of mirDIP), hsa-miR-92a-3p (with medium score of mirDIP), hsa-miR-4659a-3p (with medium score of mirDIP), hsa-miR-4659b-3p (with medium score of mirDIP), hsa-miR-1305 (with high score of mirDIP), hsa-miR-92b-3p (with medium score of mirDIP), hsa-miR-3606-3p (with medium score of mirDIP), hsa-miR-1277-5p (with high score of mirDIP) and hsa-miR-32-5p (with medium score of mirDIP), ranging from 1 to 0.996 score in LncBase Predicted v.2, targeted CD25 mRNA and interacted with OIP5-AS1, possibly. Also, from the view point of miRNAs targeting protein interactors of CD25 including hsa-miR-152-3p (with very high score of mirDIP targeting JAK1), hsa-miR-137 (with high score of mirDIP targeting STAT3), hsa-miR-148a-3p (with high score of mirDIP targeting STAT1), hsa-miR-143-3p (with high score of mirDIP targeting AKT1), hsa-miR-92a-3p (with medium score of mirDIP targeting JAK3), hsa-miR-4659a-3p (with medium score of mirDIP targeting CSF2), hsa-miR-4659b-3p (with medium score of mirDIP targeting CSF2), hsa-miR-1305 (with high score of mirDIP targeting IL2RB), hsa-miR-92b-3p (with medium score of mirDIP targeting JAK3), hsa-miR-3606-3p (with medium score of mirDIP targeting JAK2), hsa-miR-1277-5p (with high score of mirDIP targeting CSF2) and hsa-miR-32-5p (with medium score of mirDIP targeting JAK2), ranging from 1 to 0.996 score in LncBase Predicted v.2, had probably the interaction with OIP5-AS1. Consequently, OIP5-AS1 can possibly control the signaling pathways in which CD25 were engaged in as the mentioned top five signaling pathways for CD25 function including JAK-STAT signaling pathway, Th17 cell differentiation, Th1 and Th2 cell differentiation, Measles and pathways in cancer resulted from the enrichment and network analyses. Also, the above considered cancers including bladder, breast, head and neck, kidney, liver, lung, prostate and thyroid possessed probably the clue of the involvement of OIP5-AS1 in CD25-pertained signaling pathways which were analyzed as the co-expressed lncRNA in this research.

Conclusion

Taken together, OIP5-AS1 had the important role in CD25-mediated signaling pathways in a whole systems biology. This role was pertained to CD25 and CD25 protein interactors-targeting miRNAs. According to our analysis, it was shown that OIP5-AS1 had no direct interaction with CD25 protein. However, this lncRNA may play a role as molecular sponge for CD25 and CD25 protein interactors-targeting miRNAs. Also, in the current study, the co-expression of OIP5-AS1 in bladder, breast, head and neck, kidney, liver, lung, prostate and thyroid cancers and their signaling pathways was identified. Possibly, OIP5-AS1 can effect on CD25 expression in all relative signaling pathways of these cancers. These systems biology data may be useful to find out the interactions between CD25 and other molecules in TME to overcome the cancer immune escape and gain the cancer successful treatment. However, the *in vitro* and *in vivo* investigations should be performed to verify these bioinformatic data.

Limitations

The validation of these bioinformatic analyses should be done both *in vitro* and *in vivo*. Also, These analyses should be carried out about other cancers.

Abbreviations

Treg cell: Regulatory T cell; lncRNA: Long non-coding RNA; miRNA: MicroRNA; IL-2: Interleukin-2; IL-2RA: Alpha subunit of the interleukin-2 receptor; MAPK: Mitogen-activated protein kinase; ERK: Extracellular signal-regulated kinase; PI(3)K: Phosphatidylinositol 3-kinase; STAT5: Signal transducer and activator of transcription 5; ORA: Over-representation analysis; CLIP: Cross-linking immunoprecipitation; ELAVL1: ELAV-like protein 1; IGF2BP3: Insulin-like growth factor 2 mRNA-binding protein 3; IGF2BP2: Insulin-like growth factor 2 mRNA-binding protein 2; IGF2BP1: Insulin-like growth factor 2 mRNA-binding protein 1; TARDBP: TAR DNA-binding protein 43; AGO2: Protein argonaute-2; TIA1: Nucleolysin TIA-1 isoform p40; PTBP1: Polypyrimidine tract-binding protein 1; AGO3: Protein argonaute-3; AGO4: Protein argonaute-4; SRSF1: Serine/arginine-rich splicing factor 1; AGO1: Protein argonaute-1; TNRC6A: Trinucleotide repeat-containing gene 6A protein; RBFOX2: RNA binding protein fox-1 homolog 2; PTEN: Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase; SF1: Steroidogenic factor 1; REST: RE1-silencing transcription factor; TI-Treg: Tumor-infiltrating Treg; TME: Tumor microenvironment; ceRNAs: Competing endogenous RNAs; HemECs: Human hemangioma endothelial cell; MREs: miRNA response elements.

Declarations

Authors' contributions

Bioinformatic analyses: Moein Dehbashi, Study design: Moein Dehbashi, Zohreh Hojati, C. S. Cho, Mazdak Ganjalikhani-Hakemi, Akihiro Shimosaka and Majid Motovali-bashi, Study conduct: Zohreh Hojati, Mazdak Ganjalikhani-Hakemi and Akihiro Shimosaka, Data collection: Moein Dehbashi, Data interpretation: Moein Dehbashi, Zohreh Hojati, Mazdak Ganjalikhani-Hakemi, C. S. Cho and Akihiro

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and supplementary files.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Tables

Table 1. Enrichment analysis using ShinyGO v0.60 and KEGG web servers.

Enrichment FDR	Genes in list	Total genes	Functional Category
6.00E-30	16	162	JAK-STAT signaling pathway
5.30E-25	13	106	Th17 cell differentiation
2.00E-23	12	91	Th1 and Th2 cell differentiation
6.70E-19	11	138	Measles
4.70E-18	14	528	Pathways in cancer
7.70E-17	11	218	Human T-cell leukemia virus 1 infection
1.30E-12	7	70	Prolactin signaling pathway
5.00E-12	8	162	Hepatitis B
1.40E-11	7	101	T cell receptor signaling pathway
9.00E-11	6	64	Inflammatory bowel disease (IBD)
9.90E-11	6	66	Acute myeloid leukemia
3.00E-10	7	162	Necroptosis
6.20E-10	5	37	Primary immunodeficiency
7.80E-10	6	96	Hematopoietic cell lineage
9.30E-10	6	100	AGE-RAGE signaling pathway in diabetic complications
1.30E-09	8	353	PI3K-Akt signaling pathway
1.60E-09	6	111	Toxoplasmosis
9.00E-09	5	66	Non-small cell lung cancer
1.20E-08	7	293	Cytokine-cytokine receptor interaction
3.00E-08	6	186	Kaposi sarcoma-associated herpesvirus infection
3.10E-08	6	189	Chemokine signaling pathway
4.20E-08	6	200	Epstein-Barr virus infection
1.90E-07	5	126	Osteoclast differentiation
2.90E-07	5	139	Signaling pathways regulating pluripotency of stem cells
8.30E-07	4	68	Fc epsilon RI signaling pathway
1.20E-06	4	75	Pancreatic cancer
1.40E-06	4	79	EGFR tyrosine kinase inhibitor resistance

1.50E-06	5	200	Viral carcinogenesis
1.90E-05	4	155	Hepatitis C
2.50E-05	4	166	Influenza A

Table 2. TargetScan 7.2 showed the conserved sites in miRNA-CD25 interaction.

miRNA	Position in the UTR	seed match	context++ score	context++ score percentile	weighted context++ score	conserved branch length	Pct
hsa-miR-302c-3p.2	412-418	7mer-1A	-0.19	89	-0.19	3.926	0.61
hsa-miR-520f-3p	412-418	7mer-1A	-0.16	87	-0.16	3.926	0.61
hsa-miR-30c-5p	849-856	8mer	-0.09	60	-0.09	1.943	< 0.1
hsa-miR-30b-5p	849-856	8mer	-0.09	60	-0.09	1.943	< 0.1
hsa-miR-30a-5p	849-856	8mer	-0.08	59	-0.08	1.943	< 0.1
hsa-miR-30e-5p	849-856	8mer	-0.09	59	-0.09	1.943	< 0.1
hsa-miR-30d-5p	849-856	8mer	-0.08	59	-0.08	1.943	< 0.1

Table 3. SFPEL-LPI web server results for OIP5-AS1-protein interactions.

Index	Protein ID	Uniprot ID	Protein Name	Score
1	9606.ENSP00000385269	Q15717	ELAV-like protein 1	0.999
2	9606.ENSP00000258729	O00425	Insulin-like growth factor 2 mRNA-binding protein 3	0.72
3	9606.ENSP00000371634	Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2	0.6036
4	9606.ENSP00000290341	Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1	0.5697
5	9606.ENSP00000254108	P35637	RNA-binding protein FUS	0.5661
6	9606.ENSP00000240185	Q13148	TAR DNA-binding protein 43	0.3894
7	9606.ENSP00000220592	Q9UKV8	Protein argonaute-2	0.3408
8	9606.ENSP00000381031			0.3103
9	9606.ENSP00000401371	P31483	Nucleolysin TIA-1 isoform p40	0.2765
10	9606.ENSP00000349428	P26599	Polypyrimidine tract-binding protein 1	0.245
11	9606.ENSP00000362287	Q9H9G7	Protein argonaute-3	0.2319
12	9606.ENSP00000362306	Q9HCK5	Protein argonaute-4	0.2219
13	9606.ENSP00000258962	Q07955	Serine/arginine-rich splicing factor 1	0.2003
14	9606.ENSP00000350028	Q9HCE1	Putative helicase MOV-10	0.1953
15	9606.ENSP00000362300	Q9UL18	Protein argonaute-1	0.1924
16	9606.ENSP00000309558			0.1699
17	9606.ENSP00000338371			0.1005
18	9606.ENSP00000379144	Q8NDV7	Trinucleotide repeat-containing gene 6A protein	0.0976
19	9606.ENSP00000354951			0.0743
20	9606.ENSP00000413035	O43251	RNA binding protein fox-1 homolog 2	0.0133
21	9606.ENSP00000262238	P25490	Transcriptional repressor protein YY1	0.0032
22	9606.ENSP00000329357	P08047	Transcription factor Sp1	0.0025
23	9606.ENSP00000329029			0.0022
24	9606.ENSP00000361021	P60484	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	0.0021
25	9606.ENSP00000316578	Q15022	Polycomb protein SUZ12	0.0017

26	9606.ENSP00000362690	Q13285	Steroidogenic factor 1	0.0011
27	9606.ENSP00000311816	Q13127	RE1-silencing transcription factor	0.001

Table 4. Co-LncRNA results for OIP5-AS1 co-expression in bladder cancer (tumor vs. normal) based on the RNA-seq data in The Cancer Genome Atlas (TCGA).

Accession	Name	Genes	CEGs	Overlap	ePvalue	BH corr.	Bonferroni corr.
hsa00970	Aminoacyl Trna Biosynthesis	41	2885	23	3.33E-15	6.02E-13	6.02E-13
hsa03030	Dna Replication	36	2885	21	1.35E-14	1.22E-12	2.45E-12
hsa03018	Rna Degradation	59	2885	27	4.56E-11	2.61E-09	8.25E-09
hsa04110	Cell Cycle	128	2885	49	5.77E-11	2.61E-09	1.04E-08
hsa03040	Spliceosome	128	2885	48	1.03E-10	3.74E-09	1.87E-08
hsa03430	Mismatch Repair	23	2885	12	1.42E-08	4.30E-07	2.58E-06
hsa00100	Steroid Biosynthesis	17	2885	10	2.66E-08	6.90E-07	4.83E-06
hsa03440	Homologous Recombination	28	2885	12	3.01E-07	6.81E-06	5.45E-05
hsa05200	Pathways In Cancer	328	2885	58	3.60E-07	7.24E-06	6.52E-05
hsa03420	Nucleotide Excision Repair	44	2885	15	7.80E-07	1.41E-05	1.41E-04
hsa00900	Terpenoid Backbone Biosynthesis	15	2885	8	1.31E-06	2.16E-05	2.37E-04
hsa04114	Oocyte Meiosis	114	2885	26	3.17E-06	4.78E-05	5.74E-04
hsa00240	Pyrimidine Metabolism	98	2885	23	5.92E-06	8.24E-05	0.001
hsa03450	Non Homologous End Joining	14	2885	7	8.71E-06	1.12E-04	0.001
hsa00230	Purine Metabolism	159	2885	31	1.62E-05	1.96E-04	0.002
hsa04120	Ubiquitin Mediated Proteolysis	138	2885	27	4.59E-05	5.19E-04	0.008
hsa00670	One Carbon Pool By Folate	17	2885	7	5.48E-05	5.84E-04	0.009
hsa00030	Pentose Phosphate Pathway	27	2885	9	7.82E-05	7.86E-04	0.014
hsa00310	Lysine Degradation	44	2885	12	1.03E-	9.78E-	0.018

					04	04	
hsa00480	Glutathione Metabolism	50	2885	13	1.08E-04	9.78E-04	0.019
hsa00270	Cysteine And Methionine Metabolism	34	2885	10	1.42E-04	0.001	0.025
hsa03410	Base Excision Repair	35	2885	10	1.90E-04	0.001	0.034
hsa04914	Progesterone Mediated Oocyte Maturation	86	2885	18	2.32E-04	0.001	0.042
hsa03022	Basal Transcription Factors	36	2885	10	2.51E-04	0.001	0.045
hsa04115	P53 Signaling Pathway	69	2885	15	3.96E-04	0.002	0.071
hsa01040	Biosynthesis Of Unsaturated Fatty Acids	22	2885	7	4.75E-04	0.003	0.086
hsa04330	Notch Signaling Pathway	47	2885	11	8.28E-04	0.005	0.149
hsa00280	Valine Leucine And Isoleucine Degradation	44	2885	10	0.001	0.009	0.294
hsa00450	Selenoamino Acid Metabolism	26	2885	7	0.001	0.009	0.301
hsa00010	Glycolysis Gluconeogenesis	62	2885	13	0.001	0.007	0.214
hsa03050	Proteasome	48	2885	11	0.001	0.006	0.183
hsa00290	Valine Leucine And Isoleucine Biosynthesis	11	2885	4	0.001	0.01	0.331
hsa04310	Wnt Signaling Pathway	151	2885	24	0.002	0.013	0.466
hsa00510	N Glycan Biosynthesis	46	2885	10	0.002	0.012	0.431
hsa00630	Glyoxylate And Dicarboxylate Metabolism	16	2885	5	0.002	0.011	0.374
hsa00330	Arginine And Proline Metabolism	54	2885	11	0.002	0.015	0.54
hsa03020	Rna Polymerase	29	2885	7	0.003	0.017	0.647
hsa04340	Hedgehog Signaling Pathway	56	2885	11	0.004	0.019	0.742
hsa04360	Axon Guidance	129	2885	20	0.006	0.03	1

hsa00020	Citrate Cycle Tca Cycle	32	2885	7	0.006	0.031	1
hsa05120	Epithelial Cell Signaling In Helicobacter Pylori Infection	68	2885	12	0.007	0.035	1
hsa05222	Small Cell Lung Cancer	84	2885	14	0.008	0.037	1
hsa00051	Fructose And Mannose Metabolism	34	2885	7	0.01	0.04	1
hsa04146	Peroxisome	78	2885	13	0.01	0.04	1
hsa05016	Huntingtons Disease	185	2885	26	0.01	0.04	1
hsa00650	Butanoate Metabolism	34	2885	7	0.01	0.04	1
hsa05217	Basal Cell Carcinoma	55	2885	10	0.01	0.04	1
hsa00740	Riboflavin Metabolism	16	2885	4	0.011	0.044	1
hsa05020	Prion Diseases	35	2885	7	0.012	0.044	1
hsa04810	Regulation Of Actin Cytoskeleton	216	2885	29	0.013	0.05	1
hsa05130	Pathogenic Escherichia Coli Infection	59	2885	10	0.016	0.059	1
hsa00250	Alanine Aspartate And Glutamate Metabolism	32	2885	6	0.023	0.08	1
hsa05210	Colorectal Cancer	62	2885	10	0.023	0.08	1
hsa04710	Circadian Rhythm Mammal	13	2885	3	0.025	0.084	1
hsa00620	Pyruvate Metabolism	40	2885	7	0.026	0.084	1
hsa00380	Tryptophan Metabolism	40	2885	7	0.026	0.084	1
hsa04144	Endocytosis	183	2885	24	0.027	0.087	1
hsa00860	Porphyrin And Chlorophyll Metabolism	41	2885	7	0.029	0.091	1
hsa04666	Fc Gamma R Mediated Phagocytosis	97	2885	14	0.029	0.091	1
hsa05216	Thyroid Cancer	29	2885	5	0.044	0.132	1
hsa04510	Focal Adhesion	201	2885	25	0.044	0.133	1
hsa04142	Lysosome	121	2885	16	0.048	0.142	1

Figures

Figure 1

(a) The PPI results for CD25 protein using STRING. (b) The list of protein interactors for CD25 obtained from STRING. (c) JAK-STAT signaling pathway obtained from ShinyGO v0.60 web server using the relative KEGG data. (d) The depicted network for CD25 signaling pathways picked up ShinyGO v0.60.

Figure 3

The interaction of OIP5-AS1 with the considered proteins based on SFPEL-LPI results.

Supplementary Files

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