Systems Biology Unraveled the Relationship of IncRNA 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 form 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-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 IncRNA 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 IncRNA 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 IncRNAs and proteins. Also, this web server calculates multiple similarities of protein-protein and IncRNA-IncRNA using protein and IncRNA sequences and recognized IncRNA-protein
interactions. Next, SFPEL-LPI merges multiple features and similarities with a feature projection ensemble learning frame [10].

This IncRNA is an important non-coding RNA involved in many cellular processes. In fact, the IncRNA with NONCODE ID: NONHSAT041930 or OIP5-AS1 abbreviated from OIP5 antisense RNA 1, is a mammalian IncRNA 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 IncRNA 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 IncRNA 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 IncRNA was co-expressed in aminoacyl-tRNA biosynthesis pathway with p-value=3.33E-15 and Bonferroni correction=6.02E-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 1.32E-12, 5.94E-12, 1.89E-11, 3.66E-10 and 3.87E-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 5.55E-16, 3.75E-14, 2.82E-11, 1.06E-10 and 1.48E-10, respectively (Table S10).

As a recent report, OIP5-AS1 IncRNA 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 IncRNAs 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 LPAATβ/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 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 IncRNA 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 IncRNA 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 |
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| 000425     | 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| 19295     | Protein argonaute-3                                       | 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| 19295     | Protein argonaute-3                                       | 0.1699 |
| 17    | 9606.ENSP00000338371| 19295     | Protein argonaute-3                                       | 0.1005 |
| 18    | 9606.ENSP00000379144| Q8NDV7     | Trinucleotide repeat-containing gene 6A protein           | 0.0976 |
| 19    | 9606.ENSP00000354951| 19295     | Protein argonaute-3                                       | 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| 19295     | Polycomb protein SUZ12                                     | 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 |
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).

|   | ENSP00000362690 | Q13285 | Steroidogenic factor 1 | 0.0011 |
|---|----------------|--------|------------------------|--------|
| 26| 9606.ENSP00000362690 | Q13285 | Steroidogenic factor 1 | 0.0011 |
| 27| 9606.ENSP00000311816  | Q13127 | RE1-silencing transcription factor | 0.001  |
| 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-08 | 9.78E-08   | 0.018           |
| Gene Ontology ID | Pathway Description                                      | Gene Count | q-value 04 | Benjamini 04 | q-value 04 | Benjamini 04 |
|-----------------|----------------------------------------------------------|------------|-------------|--------------|-------------|--------------|
| hsa00480        | Glutathione Metabolism                                   | 50         | 1.08E-04    | 9.78E-04     | 0.019       |
| hsa00270        | Cysteine And Methionine Metabolism                       | 34         | 1.42E-04    | 0.001        | 0.025       |
| hsa03410        | Base Excision Repair                                     | 35         | 1.90E-04    | 0.001        | 0.034       |
| hsa04914        | Progesterone Mediated Oocyte Maturation                  | 86         | 2.32E-04    | 0.001        | 0.042       |
| hsa03022        | Basal Transcription Factors                             | 36         | 2.51E-04    | 0.001        | 0.045       |
| hsa04115        | P53 Signaling Pathway                                   | 69         | 3.96E-04    | 0.002        | 0.071       |
| hsa01040        | Biosynthesis Of Unsaturated Fatty Acids                  | 22         | 4.75E-04    | 0.003        | 0.086       |
| hsa04330        | Notch Signaling Pathway                                  | 47         | 8.28E-04    | 0.005        | 0.149       |
| hsa00280        | Valine Leucine And Isoleucine Degradation                | 44         | 0.001       | 0.009        | 0.294       |
| hsa00450        | Selenoamino Acid Metabolism                             | 26         | 0.001       | 0.009        | 0.301       |
| hsa00010        | Glycolysis Gluconeogenesis                               | 62         | 0.001       | 0.007        | 0.214       |
| hsa03050        | Proteasome                                               | 48         | 0.001       | 0.006        | 0.183       |
| hsa00290        | Valine Leucine And Isoleucine Biosynthesis               | 11         | 0.001       | 0.01         | 0.331       |
| hsa04310        | Wnt Signaling Pathway                                    | 151        | 0.002       | 0.013        | 0.466       |
| hsa00510        | N Glycan Biosynthesis                                    | 46         | 0.002       | 0.012        | 0.431       |
| hsa00630        | Glyoxylate And Dicarboxylate Metabolism                  | 16         | 0.002       | 0.011        | 0.374       |
| hsa00330        | Arginine And Proline Metabolism                          | 54         | 0.002       | 0.015        | 0.54        |
| hsa03020        | Rna Polymerase                                           | 29         | 0.003       | 0.017        | 0.647       |
| hsa04340        | Hedgehog Signaling Pathway                              | 56         | 0.004       | 0.019        | 0.742       |
| hsa04360        | Axon Guidance                                            | 129        | 0.006       | 0.03         | 1           |
| ID     | Name                                              | Gene Count | Benjamini's P | FDR                   |
|--------|---------------------------------------------------|------------|---------------|------------------------|
| hsa00020 | Citrate Cycle Tca Cycle                           | 32         | 2885          | 0.006                  |
| hsa05120 | Epithelial Cell Signaling In Helicobacter Pylori Infection | 68         | 2885          | 0.007                  |
| hsa05222 | Small Cell Lung Cancer                            | 84         | 2885          | 0.008                  |
| hsa00051 | Fructose And Mannose Metabolism                   | 34         | 2885          | 0.01                   |
| hsa04146 | Peroxisome                                        | 78         | 2885          | 0.01                   |
| hsa05016 | Huntingtons Disease                               | 185        | 2885          | 0.01                   |
| hsa00650 | Butanoate Metabolism                              | 34         | 2885          | 0.01                   |
| hsa05217 | Basal Cell Carcinoma                              | 55         | 2885          | 0.01                   |
| hsa00740 | Riboflavin Metabolism                             | 16         | 2885          | 0.011                  |
| hsa05020 | Prion Diseases                                    | 35         | 2885          | 0.012                  |
| hsa04810 | Regulation Of Actin Cytoskeleton                  | 216        | 2885          | 0.013                  |
| hsa05130 | Pathogenic Escherichia Coli Infection             | 59         | 2885          | 0.016                  |
| hsa00250 | Alanine Aspartate And Glutamate Metabolism        | 32         | 2885          | 0.023                  |
| hsa05210 | Colorectal Cancer                                 | 62         | 2885          | 0.023                  |
| hsa04710 | Circadian Rhythm Mammal                           | 13         | 2885          | 0.025                  |
| hsa00620 | Pyruvate Metabolism                               | 40         | 2885          | 0.026                  |
| hsa00380 | Tryptophan Metabolism                             | 40         | 2885          | 0.026                  |
| hsa04144 | Endocytosis                                       | 183        | 2885          | 0.027                  |
| hsa00860 | Porphyrin And Chlorophyll Metabolism              | 41         | 2885          | 0.029                  |
| hsa04666 | Fc Gamma R Mediated Phagocytosis                  | 97         | 2885          | 0.029                  |
| hsa05216 | Thyroid Cancer                                    | 29         | 2885          | 0.044                  |
| hsa04510 | Focal Adhesion                                    | 201        | 2885          | 0.044                  |
| hsa04142 | Lysosome                                          | 121        | 2885          | 0.048                  |
Figures

(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 2

(a) miRTargetLink Human outcomes for CD25 mRNA. (b) starBase v3.0 resulted that hsa-miR-30b-5p, as a TargetScan conserved miRNA, had the interaction with OIP5-AS1. (c) starBase v3.0 illustrated hsa-miR-324-3p, as a mirDIP miRNA, had the interaction with OIP5-AS1. (d) JAK-STAT signaling pathway and pathways in cancer were related with high score based on the tree illustration in ShinyGO v0.60 web server. Other CD25-mediated signaling pathways were also depicted.
Figure 3

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

Supplementary Files

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