Data in support of FSH induction of IRS-2 in human granulosa cells: Mapping the transcription factor binding sites in human IRS-2 promoter

Surleen Kaur, G. Anjali, Priya Bhardwaj, Jyoti Taneja, Rita Singh*

Division of Molecular Endocrinology and Reproduction, Department of Zoology, University of Delhi, Delhi 110007, India

ABSTRACT

Insulin receptor substrate-2 (IRS-2) plays critical role in the regulation of various metabolic processes by insulin and IGF-1. The defects in its expression and/or function are linked to diseases like polycystic ovary syndrome (PCOS), insulin resistance and cancer. To predict the transcription factors (TFs) responsible for the regulation of human IRS-2 gene expression, the transcription factor binding sites (TFBS) and the corresponding TFs were investigated by analysis of IRS-2 promoter sequence using MatInspector Genomatix software (Cartharius et al., 2005 [1]). The ibid data is part of author’s publication (Anjali et al., 2015 [2]) that explains Follicle stimulating hormone (FSH) mediated IRS-2 promoter activation in human granulosa cells and its importance in the pathophysiology of PCOS. Further analysis was carried out for binary interactions of TF regulatory genes in IRS-2 network using Cytoscape software tool and R-code. In this manuscript, we describe the methodology used for the identification of TFBSs in human IRS-2 promoter region and provide details on experimental procedures, analysis method, validation of data and also the raw files. The purpose of this article is to provide the data on all TFBSs in the promoter region of human IRS-2 gene as it has the potential for prediction of the regulation of IRS-2 gene in normal or diseased cells from patients with metabolic disorders and cancer.

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* Corresponding author. Tel.: +91 8527820707, +91 11 27667985/308.
E-mail addresses: ghrika_s@yahoo.com, ritas@zoology.du.ac.in (R. Singh).
### Specifications Table

| Subject area                  | Biology                                      |
|------------------------------|----------------------------------------------|
| More specific subject area   | Gene regulation and TFBS                    |
| Type of data                 | MatInspector data, figures and table         |
| How data was acquired        | In silico analysis of IRS-2 promoter sequence using Genomatix MatInspector software, ChIP assay, qRT-PCR |
| Data format                  | Raw Excel spreadsheet (.xls), filtered and analysed data |
| Experimental factors         | Isolation of human GCs from the follicular fluid aspirates obtained after IVF treatment, grown in culture and FSH treated. Precipitation of protein–DNA complexes with anti-SP1 antibody and PCR |
| Experimental features        | Analysis of known TFBS on IRS-2 promoter using MatInspector software, filtering the TFs that are regulated by FSH, validation of the induction of SP1 binding to IRS-2 gene promoter by FSH in human GCs by ChIP assay |
| Data source location         | Delhi, India                                 |
| Data accessibility           | Data are provided with this article.          |

### Value of the data

- IRS-2 protein is an important signaling component in the regulation of metabolism in human and other organisms. However, specific transcription regulation of IRS-2 has not yet been characterized completely in the existing literature.
- This data exhibits all TFBS and the corresponding TFs that may bind human IRS-2 promoter.
- FSH stimulated TFs and activation of human IRS-2 promoter.
- The data provided in this paper would be extremely relevant for further analysis of the regulation of IRS-2 interactions especially related to cancer progression.

### 1. Data

In order to identify the TF responsible for the activation of IRS-2 promoter activity downstream FSH, all TFBS in the IRS-2 promoter region [3] were explored using the Genomatix MatInspector software (Fig. 1) followed by a search for TFs that are reported to be transcriptional activators of FSH (Table 1, Supplementary material). The data also shows SP1 as a potential key TF downstream of FSH in human GCs as it has SP1 binding sites with a very high similarity (Core similarity = 1) (Table 2). The increased binding of SP1 to IRS-2 promoter by FSH in human GCs was validated by ChIP assay (Fig. 2). Here, we have identified putative TFBS in the IRS-2 promoter region and data thereof was subjected to IRS-2–protein interaction analysis to emphasize the importance of this data. Unweighted binary interactions were analyzed for TF regulatory genes in IRS-2 network using Cytoscape software tool and R-code (Supplementary material)Fig. 3.

### 2. Experimental design-materials and methods

2.1. Transcription factor binding sites analysis

We carried out an in-depth computational analysis of the transcription binding sites on human IRS-2 promoter to identify the potential TFs that are responsible for FSH mediated regulation of IRS-2
Table 1
Putative transcription factor binding sites in IRS-2 promoter region with match $\geq 15$.

| Matrix family | Matrix information | Match total |
|--------------|--------------------|-------------|
| ZF02         | Zinc finger with KRAB and SCAN domains | 3 | 88 |
| ZF5F         | Zinc finger/POZ domain transcription factor | 73 |
| KLFS         | Kruppel-like factor 7 (ubiquitous, UKLF) | 70 |
| E2FF         | E2F transcription factor 1 | 69 |
| PLAG         | Pleomorphic adenoma gene | 56 |
| MZF1         | Myeloid zinc finger protein MZF1 | 50 |
| TF2B         | Transcription factor II B (TFIIB) recognition element | 48 |
| IKZF         | IKAROS family zinc finger 5 (Pegasus) | 44 |
| SP1F         | Specificity protein 1, ubiquitous zinc finger transcription factor | 40 |
| EGRF         | EGR1, early growth response | 39 |
| SPZ1         | Spermatogenic Zip 1 transcription factor | 32 |
| EBOX         | E-box binding factors | 29 |
| AP2F         | Transcription factor AP-2, alpha | 28 |
| MAZF         | Myc associated zinc fingers | 27 |
| IKRS         | Ikaros 2, potential regulator of lymphocyte differentiation | 25 |
| HESF         | Drosophila hairy and enhancer of split homolog 1 (HES-1) | 24 |
| NDPK         | Nucleoside diphosphate kinase | 16 |
| AP1R         | Transcription factor AP-1 | 15 |

Highlighted in yellow are the transcription factors activated by FSH.

Fig. 1. Identification of putative transcription factor-binding sites in the IRS-2 promoter region using Genomatix MatInspector software.
expression in human GCs. Human IRS-2 promoter sequence [3] was analyzed for putative TFBS using MatInspector software version 8.1, Matrix Library 9.1 from the Genomatix suite v3.4 [1]. The parameters for comparing the binding sites with database were set at matrix similarity and core similarity 0.85 (maximum 1.00) (Supplementary material). TFBS in the IRS-2 promoter region were explored for IRS-2–protein interactions using open-access database [4] for experimentally verified human transcriptional regulation interactions (HTRIdb) (Supplementary material).

2.2. Cell culture

Human GCs were isolated from the follicular fluid aspirates obtained after IVF treatment of subjects as described earlier [5]. Briefly, the follicular fluid was centrifuged at 350 × g for 15 min to pellet follicular cells and the GCs were isolated on Percoll gradient. The cells were seeded in DMEM containing 5% FBS and supplemented with 1x antibiotic antimycotic solution in multiwell plates at 37 °C in 5% CO2.

2.3. Chromatin Immunoprecipitation (ChIP) and reverse-transcription quantitative PCR (qRT-PCR)

Cells were serum starved and left either in the basal condition or with FSH (25 ng/mL) for 2 h. ChIP assay was performed as described earlier [6,7]. Briefly, cells were fixed with 1% formaldehyde,
Fig. 2. FSH increases SP1 binding to IRS-2 promoter in human granulosa cells. ChIP assay was performed for verification of SP1 binding to IRS-2 promoter. Input reflects the relative amounts of sonicated DNA fragments before immunoprecipitations. (A) The relative amounts of IRS-2 promoter DNA fragments were determined with semi-quantitative PCR by separating the amplified product on 1.5% agarose gel. (B) The relative amounts of IRS-2 promoter DNA fragments were determined with qRT-PCR. Data are expressed as % input after normalizing the Ct values obtained from SP1 antibody treated samples with the Ct values from input DNA. Values presented are mean ± SD from 3 independent experiments (n = 3). *P < 0.05 vs. untreated. − IgG as negative control; + SP1 Antibody; i Input (Total sonicated DNA) as positive control.

Fig. 3. TF regulatory gene network for IRS-2.
quenched with 0.125 M glycine and washed with cold PBS. After cell lysis, protein–DNA complexes were incubated with 2 μg of anti-SP1 and 2 μg non-specific IgG (normal rabbit IgG, Santa Cruz) antibodies and protein G agarose. 50 μl of cell lysate was used as input control. Protein–DNA complexes were released from the antibodies with elution buffer. After RNase A treatment, each sample was decrosslinked at 67 °C overnight and DNA was purified using chromatin IP DNA purification kit (Active Motif, Carlsbad, CA) and then subjected to semi-quantitative PCR analysis with specific primers for SP1 binding site on promoter of IRS-2 gene. Primer sequences for IRS-2 promoter were 5’-ACAAAGCCTGATTAGAGGC-3’ and 5’-TGACTCGCCTTACGC AGGCAC-3’. Relative real-time qPCR was performed on Applied Biosystems 7500 Fast Real Time PCR System using a Power SYBR® Green PCR Master Mix. PCR amplified products were separated on 1.5% agarose gel in TAE buffer. The quantity of mRNA was calculated based on the cycle threshold (Ct) values which were normalized to the expression of the reference gene (β2M), which served as internal control. Data are expressed as % input after normalizing the Ct values obtained from SP1 antibody treated samples with the Ct values from input DNA. All experiments were performed in triplicates.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.12.001.

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