**In silico** prediction of specific pathways that regulate mesangial cell proliferation in IgA nephropathy

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**Abstract**

IgA nephropathy is one of the most common forms of primary glomerulonephritis worldwide leading to end-stage renal disease. Proliferation of mesangial cells, i.e., the multifunctional cells located in the intracapillary region of glomeruli, after IgA-dominant immune deposition is the major histologic feature in IgA nephropathy. In spite of several studies on molecular basis of proliferation in these cells, specific pathways responsible for regulation of proliferation are still to be discovered. In this study, we predicted a specific signaling pathway started from transferrin receptor (TFRC), a specific IgA1 receptor on mesangial cells, toward a set of proliferation-related proteins. The final constructed subnetwork was presented after filtration and evaluation. The results suggest that estrogen receptor (ESR1) as a hub protein in the significant subnetwork has an important role in the mesangial cell proliferation and is a potential target for IgA nephropathy therapy. In conclusion, this study suggests a novel hypothesis for the mechanism of pathogenesis in IgA nephropathy and is a reasonable start point for the future experimental studies on mesangial proliferation process in this disease.

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**Introduction**

Uncontrolled proliferation of mesangial cells in kidney is a common feature of numerous diseases that can eventually end up with end-stage renal failure. IgA nephropathy (IgAN), a type of mesangio-proliferative glomerulonephritis, is the most frequent primary form of glomerulonephritis worldwide and is considered as the major cause of end-stage renal disease (ESRD) [1,2]. Severe impairment of renal function occurred in ESRD leads to the need of renal replacement therapy including kidney transplantation and dialysis. In long term follow-up, approximately 30–50% of patients with IgAN will register for renal replacement therapy [3,4]. Mesangial proliferation is the major histological characteristics of IgA nephropathy independent from different etiologies of the disease, which occurs in association with IgA dominant or co-dominant immune deposits on these cells [5,6].

Mesangial cells, located between the capillary endothelial cells and the basal membrane of the glomeruli, are involved in blood flow regulation and phagocytosis [7–9]. Intracapillary location and inflammatory molecule synthesis of mesangial cells makes them potential sensitive places for glomerular injury [10]. Occurrence of proliferation could be reinforced and resumed by a revival cycle mediated by growth factors, cytokines, chemokines and oxidant molecules and with the involvement of extracellular matrix deposition and glomerulosclerosis [11,12]. The stepwise mechanism of mesangial cell proliferation and the precise set of the involved signaling molecules are still to be discovered.

Due to technical limitations, identification of the signaling intermediates is typically not a cost effective task. A predictive method by bioinformatics tools based on evidence-based databases could be a more logical start point for identification of candidate intermediate signaling molecules. For example, in order to find pathways involved in IgAN, Cox et al. [13] used Ingenuity Pathway Analysis to assess the biological relationships among differentially expressed genes of peripheral blood mononuclear cell.

Unfolding the puzzle of proliferation mechanism of mesangial cells in IgAN by means of in silico methods may help to predict key regulatory pathways, and consequently, suggest potential targets for efficient therapeutic intervention. In the present study, an attempt was made to predict the specific molecular mechanism of proliferation in the glomerular mesangial cells as the main site of injury in IgAN. The results suggest candidate pathologic pathways...
in disease development and might be considered as potential targets for the future experimental validation.

Hypothesis

Mesangial cell proliferation is a key histological marker in a number of renal diseases including IgA nephropathy, mesangial proliferative lupus glomerulonephritis, membranous proliferative glomerulonephritis and diabetic nephropathy [14]. One of the important functions of mesangial cells is phagocytosis of locally accumulated macromolecules such as immunoglobulins (e.g., IgA and IgG) through specific or non-specific mechanisms [15]. The specific removal is mediated by receptors and triggers specific pathways by which mesangial cells respond to signals like proliferation. Proliferation of mesangial cells might be due to compensation for removal of immunoglobulins or other macromolecules or might be due to stimulatory effect of growth factors including PDGF-C, fibroblast growth factor, hepatocyte growth factor, EGF, connective tissue growth factor, and TGF-β [16–18]. However, while immunoglobulin deposition is suggested as one of the possible causes of proliferation process in mesangial cells, it is not common in glomerulonephritis diseases other than IgA nephropathy. On the other hand, IgA deposition (in IgA nephropathy and not essentially in other forms of proliferative glomerulonephritis) is always associated with proliferation process.

As mesangial cell proliferation is the possible phenomenon which occurs in different types of glomerulonephritis, we speculate that different mechanisms and signaling pathway(s) might contribute in this process based on etiology of the disease. In other words, mesangial proliferation occurs only in some cases of focal segmental glomerulonephritis, membranous glomerulonephritis, membranous proliferative glomerulonephritis and lupus nephritis and other glomerulopathies but always happens in IgA nephropathy. Furthermore, it has been previously observed that in IgAN, IgA1-containing immune complexes differentially influence the proliferation of mesangial cells [19]. Based on these facts, we hypothesized that mesangial proliferation in IgA nephropathy must have a specific mechanism related to IgA which is induced directly by deposition of IgA deposited immune complexes on mesangial cells in the glomeruli. Induction of proliferation process by IgA is likely to be initiated by interaction between IgA and its specific receptor on mesangial cells. As transferrin receptor (TFRC) expression on mesangial cells was strongly correlated with the presence of IgA deposits, based on experimental data [20,16], one may consider it as a major (but not the only) candidate IgA receptor on mesangial cells [10]. We selected TFRC as the mesangial receptor specific for IgA and considered it as the start point of proliferation signaling pathway. Exploration of interacted intermediate proteins between TFRC and proteins of which expression were differentially changed in mesangial cells of IgA nephropathy patients by means of protein-DNA interaction analysis could elucidate the possible pathway(s) responsible for proliferation.

Evaluation of the hypothesis

We analyzed kidney biopsy samples of 98 non-IgA nephropathy patients to investigate the mesangial cell proliferation status. The immunofluorescent staining was used to determine whether IgA deposit occurs on mesangial cells or not and light microscopy imaging was applied to investigate the proliferation of mesangial cells in the glomeruli. The results of this analysis are summarized in Table 1. Based on these data 62.5% of cases with mesangial proliferation were not associated with deposition of immunoglobulins or other immune molecules (26% of all cases). This observation suggests that: (a) removal of immune complex or other macromolecule deposits might not be the major cause of mesangial proliferation; (b) proliferation process in non-IgA nephropathy cases might be either specific (receptor dependent) or non-specific (receptor independent), while it is definitely specific for IgA nephropathy; and (c) mesangial proliferation is a complex process regulated by different pathways in different diseases. Furthermore, based on these preliminary data, while IgA deposition is not necessarily associated with mesangial proliferation in non-IgAN cases (such as lupus nephritis without mesangial proliferation), specific forms of IgA (polymeric form or poor galactosilated form), which is only present in IgAN, might stimulate proliferation in the mesangial cells through a specific pathway.

Based on the literature, receptor candidates which interact with IgA are: (1) IgA Fc receptor (CD89), which binds both IgA1 and IgA2 [21]; (2) the polymeric-lg receptor, which binds polymeric IgA and IgM [22]; (3) FcγR/μR, which binds IgA and IgM [17]; (4) asialoglycoprotein receptor (ASGP-R), which binds IgA1 and IgA2 through terminal galactose moiety; and (5) transferrin receptor (TFRC or CD71) which binds IgA1 [16]. The latter receptor is the only IgA specific receptor overexpressed on the glomerular mesangial cells in IgAN [20], and hence, it is considered as the best candidate for investigation its role on proliferation through IgA induction. Moreover, involvement of transferrin receptors in the cell growth and proliferation has been reported earlier [23–25].

In another study, Moura et al. indicated that interaction between IgA1 and TFRC induces mesangial cell proliferation and secretion of inflammatory cytokines such as IL-6 and TGF-β [10]. Moreover, Tamouza et al. in 2007 showed that TFRC in cultured HMC is overexpressed under the influence of sera from IgAN patients in an IgA-dependent manner [26]. These findings are in favor of activation of specific signaling pathway(s) originated from TFRC induction by IgA1 but the molecular mediators of such signaling pathway(s) are to be determined. In the following sections we review proliferation signaling initiation from TFRC and proliferation signaling in the mesangial cells.

TFRC-related proliferation pathways

According to the literature, there is a known relationship between TFRC and cell proliferation [27–29,16] but the stimulators of cell activation seem to be different in different cell types and hence different pathways might be involved. For instance, Kindrat et al. recently have shown that epigenetic controls of TFRC gene and impaired iron homeostasis influence proliferation in hepatocellular carcinoma [27]. Pham et al. showed the effect of Sphin-gosine kinase 1 (SK1) on the expression of TFRII and transferrin uptake in tumor development [28]. Other researchers believe the fibrosis and renal impairment following TFRC related proliferation in the kidney is consequence of inflammatory response by promoting the release of proinflammatory cytokines, such as IL-1, IL-6 and TNF-α [29,16].

Well defined signaling pathways related to transferrin trafficking and proliferation are: (1) inositol-1,4,5-triphosphate and diacylglycerol signaling pathway, (2) MAPK signaling pathway, and (3) growth factors signaling pathway [30]. In the first pathway, phospholipase C (PLC) is activated and hydrolyzes phosphatidylinositol-4,5-diphosphate (PI-4,5-P2) to inositol-1,4,5-triphosphate (InsP3) and diacylglycerol (DAG). DAG, thus activates protein kinase C (PKC) and InsP3 influences the release Ca2+ stored in the endoplasmic reticulum. The InsP3/Ca2+ signaling can control proliferation [31–33]. The second pathway (MAPK sig-naling) is activated by tyrosine kinase-linked receptors (such as TFRC) resulting in the phosphorylation of extracellular signal–regulated kinases 1/2 (ERK1/2) that then translocate into the nucleus which controls many cellular processes, particularly those related to cell proliferation [30]. The third signaling pathway through growth factors
produce phosphatidylinositol 3-kinase (PI3K) that contributes to sending information to the target of rapamycin (TOR) signaling pathway [30,33]. TOR is a serine/threonine protein kinase that activates cell proliferation under the condition of starvation [30].

**Pathways related to proliferation in the mesangial cells**

A few pathways and mediator molecules that can potentially be involved in mesangial cell proliferation are reviewed here. Tamouza et al. performed a study specifically on proliferation mesangial cells in IgA nephropathy patients [34]. They could recognize a pattern of signaling molecules which was dependent on the level of proteinuria. Accordingly, phosphor (p)-ERK1/2 expression was detected by immunohistochemical analysis in >20% mesangial cells of the renal biopsy from subjects with IgA nephropathy and with >1 g/day proteinuria.

ERK1/2 are known signaling molecules from mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway involved in regulation of cell proliferation, hypertrophy, cytokine secretion, and extracellular matrix synthesis [35]. In the Tamouza et al. study, p-ERK1/2 was observed in the cell nuclei in patients with >1 g/day proteinuria. Furthermore, expression pattern of these signaling molecules was correlated with high blood pressure. Activation of MAPK/ERK signaling pathway was detected in a fraction of the subjects and lack of correlation with serum galactose (Gal)-deficient IgA1 levels, which are often (but not always) elevated in IgAN patients [36], support the hypothesis regarding the possibility of involvement several pathways in the mesangial proliferation depends on different clinical manifestations in IgA nephropathy. Furthermore, those results confirmed that the pathways leading to mesangial proliferation originate from TFRC engagement by plgA1. The details of the involved signaling pathway were not determined in that study.

Gao et al. on the other hand, investigated the proliferative effect of TNF-like weak inducer of apoptosis (TWEAK) on mesangial cells in the kidney [37]. They observed a significant proliferation in vitro and in vivo studies presumably through TWEAK receptor Fn14. The study was performed on a human mesangial cell line and the following signaling molecules remained unclear.

In a recent study on animal models, Lu et al. suggested the involvement of cyclins and p53 in the pathology of mesangial proliferative nephritis. However, they did not evaluate human subjects, and specifically, patients with IgA nephropathy [38].

To sum up, the details of the pathways which mediate the mesangial cell proliferation in IgA nephropathy are to be determined. The goal of this study is to present new hypotheses on the nature of these pathways and their involved intermediates. However, this study does not rule out the other possible pathways implicated in the mesangial proliferation via IgA dominant immune complex.

### Pathway analysis (for Evaluation of the Hypothesis)

**Identification of the novel signaling network**

For uncovering the signaling pathways in the context of interaction network, ANAT (Advanced Network Analysis Tool) was applied [39,40]. ANAT is an interactive tool for inferring functional protein-protein interaction and protein-DNA interaction networks running in Cytoscape platform [41]. Unlike most of PPI network inference tools, the database of ANAT includes only physical interactions. Each interaction in the network is assigned a confidence score.

<table>
<thead>
<tr>
<th>Type of Nephropathy</th>
<th>Number of samples</th>
<th>Percentage of proliferative cases with deposition</th>
<th>Percentage of proliferative cases without deposition</th>
<th>Percentage of non-proliferative cases with deposition</th>
<th>Percentage of non-proliferative cases without deposition</th>
<th>Types of immune deposit components in proliferative cases</th>
<th>Types of immune deposit components in non-proliferative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranous Glomerulonephritis</td>
<td>31</td>
<td>0%</td>
<td>19%</td>
<td>0%</td>
<td>81%</td>
<td>IgG and C3c (along GBM)</td>
<td>IgG, C3c, IgM and C1q (along GBM)</td>
</tr>
<tr>
<td>Focal Segmental Glomerulosclerosis/ Minimal change disease</td>
<td>36</td>
<td>14%</td>
<td>25%</td>
<td>12%</td>
<td>47%</td>
<td>IgM, C3c and IgG (mesangium and GBM)</td>
<td>IgG, C3c, IgM (mesangium)</td>
</tr>
<tr>
<td>Lupus Nephritis</td>
<td>10</td>
<td>60%</td>
<td>10%</td>
<td>30%</td>
<td>0%</td>
<td>IgA, IgG, IgM,C3c, C1q, C4c (along GBM and mesangium)</td>
<td>IgA, IgG, IgM,C3c, C1q, C4c (along GBM and mesangium)</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>8</td>
<td>0%</td>
<td>12%</td>
<td>38%</td>
<td>50%</td>
<td>IgG, IgM, C3c, albumin (along GBM and/or mesangium)</td>
<td>IgG, C3c, albumin (along GBM and/or mesangium)</td>
</tr>
<tr>
<td>Other (sever/moderate chronic tubulointerstitial nephritis, MPGN C3 dominant GN, diffuse proliferative GN, post infectious hypertensive induced nephropathy, chronic sclerosing crescent GN, Deposition glomerular disease)</td>
<td>13</td>
<td>30.8%</td>
<td>53.8%</td>
<td>7.7%</td>
<td>7.7%</td>
<td>IgM, C3c</td>
<td>IgM, C3c</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>15%</td>
<td>26%</td>
<td>11%</td>
<td>48%</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* These components have been observed only in this dataset and it does not necessarily imply the existence of all of them in every case with that certain pathology. For instance, IgM might exist in some patients with membranous glomerulonephritis but not in other patients with the same disease and vice versa.
based on the experimental evidence that support them. Another distinguishing feature of the application is offering anchored PPI analyses tool that connects phenotype related proteins to one or more anchor nodes initiating the signaling pathway.

In this study, we defined TFRC, the specific receptor of glomerular mesangial cells, as the root node which is responsible for initiation of the signaling cascade of cell proliferation. Furthermore, a list of 203 phenotype-related genes, which represent the specifically-expressed genes in the mesangial cells of IgA nephropathy patients, are chosen as the terminal nodes. Contribution of these differentially-expressed genes in IgAN had been confirmed experimentally [42]. The complete list of these proteins is presented in Table S1.

In order to obtain the highest possible amount of information and correctly recognize the transcription factors which regulate the pathways, we selected protein interaction of human as the background network. ANAT uses "Charikar-\(\alpha\) algorithm" for constructing the network. In this algorithm, parameter \(\alpha\) is the sole adjustable parameter, which can be set in the range of 0–0.5. This parameter can control the relative importance of the local and global criteria. The local approach provides reliable pathways between the root and the terminal nodes, by maximization of the likelihood of connecting pathways and includes many extra genes that are not known to be phenotype-related. In contrast the global approach results in a holistic but adjusted network connecting the root to all the terminals by minimization the number of such extra genes. The value for \(\alpha\) was set as 0.25 to consider the equal importance between local and global models in the network and achieving the best results. The Charikar-\(\alpha\) algorithm analyzes all of the available interaction data that connect the root to subsets of the terminals iteratively until the entire set of terminals is covered and then identifies subtrees with a high confidence level. The pathways are then reported with the corresponding gene ontology enrichment [43] results by Cytoscape.

**Evaluation of the constructed network**

The constructed network was filtered in three steps:

1) filtration based on the corrected p-value of Gene Ontology enrichment analysis;
2) evaluation of the specificity of the method; and
3) literature based verification, i.e., only pathways with reasonable direction of interaction (from root toward terminals) are kept in the analysis.

To evaluate the specificity of the results (step 2 above), we performed a permutation-based validation. Briefly, the interaction network was reconstructed 50 times using 50 lists of 200 terminal nodes that randomly selected from the human glomerular gene expression profile reported by Lindenmeyer et al. [44]. The survived pathways from the original list after the first filtration of which confidence level >95% of the confidence level in the random pathways were considered significant.

In this analysis, only minor adjustments were done on the preliminary network. The changes include removing Transferrin protein from the network due to its irrelevance to our disease and forcing directionality from TFRC to its neighboring proteins.

**Analysis of the signaling network**

Fig. 1 shows the view of the interaction network. Overall, ANAT generated 203 pathways, of which 29 pathways were found to be significant according to Gene Ontology enrichment analysis (corrected p-value <0.05). Significant pathways according to the first step of filtration are listed as Table S2. In the second step of filtration, the confidence level of 13 pathways in the original anchored network was >95% of confidence level in the random pathways after 50 times permutations (see the bold pathways in Table S2). In the third step of filtration, direction of the interactions was examined by literature searching and seven annotated pathways containing reverse interactions from terminals toward root were excluded. At the end, six pathways were considered as final significant subnetwork involved in proliferation of mesangial cells in IgAN (see Table 2 and Fig. 2).

We also considered the results of gene ontology enrichment analysis of the ANAT final report to find the biological processes which are involved in this disease. Using gene ontology analysis, “positive regulation of cell killing”, “positive regulation of immune effector process” and “multicellular organismal homeostasis” processes were found to be significantly over-represented in the pathways.

**Suggestion for experimental evaluation of the hypothesis**

Beside statistical methods bioinformatic approaches are safe and affordable technique that helps to introduce valuable candidates for experimental testing based on a computational logical trend. This hypothesis and signaling targets with a reasonable background could be tested in vitro (human mesangial cells) or in vivo (animal models). Suggested in vitro procedure is: (1) purification IgA dominant immune complex from the serum of patients with IgA nephropathy, (2) Treated human mesangial cells (HMC) with appropriate concentration of extracted immune complexes (IC) and comparison with control group (non-treated HMC), (3) proliferation assay of HMC after treated with IC, 4) evaluation the changes of target proteins using western blotting, ELISA or using high resolution mass spectrometric based quantification method such as selective reaction monitoring (SRM).

**Discussion**

Deposition of IgA1 on mesangial cells in the glomeruli followed by mesangial cell proliferation is one of the characteristics of initial phase of IgAN [45,46]. The mechanism for proliferation of mesangial cells is still unclear. However phospholipase C-\(\gamma1\) activation, inositol triphosphate formation, and Ca\(^{2+}\) mobilization, which is linked to activation of tyrosine kinase, were reported earlier as the possible mediators of proliferation via IgA Fc receptor [47]. The characterization of transferrin receptor as a specific IgA1 receptor on renal mesangial cells [16], elucidated the TFRC–IgA1 interaction as a basis for mesangial IgA1 deposition in IgAN, and consequently, functional changes including proliferation in the mesangial cells. However, this finding does not exclude the possibility of involvement of other IgA receptors (e.g., Fc receptor [48–50] or asialoglycoprotein receptor [51]), which are not specifically expressed in glomerular mesangium.

Our computational results propose the signaling pathways by which proliferation is induced. These findings can be seen as a starting point for the future experiments. Based on these results, at the beginning of the signaling cascade, IgA1-activated TFRC binds to HFE (Hereditary hemochromatosis protein), a protein associated in iron homeostasis [10,52]. Nevertheless, activation of proliferation signaling through TFRC and HFE in IgAN is independent from their role in iron homeostasis [10]. HFE, which has been originally identified as a human leukocyte antigen (HLA) class I-like protein, then interacts with B2-microglobulin (\(b2M\)) for the presentation of HFE to the cell surface and correct subcellular distribution [53]. B2-microglobulin is a subunit of all major histocompatibility class (MHC) I molecules that subsequently interacts with HLA-A. Involvement of HLA class I signaling pathways has been...
reported in the proliferation of various cells (e.g. vascular ECs, SMCs, T cells, and B cells) and regulation of apoptosis in activated T and B cells [54–56]. Here, we suggest the possible involvement of this protein in the mesangial proliferation signaling. HLA-A stimulates tyrosine phosphorylation activity of a member of SRC family [57], a non-receptor protein tyrosine kinase participates in a diverse spectrum of signaling pathways, which likely results in activation of ESR1 proteolysis [58]. Negative correlation of SRC and ESR1 levels has been previously reported in uncontrolled proliferative cells like primary breast cancer cells [58]. On the other hand, there is evidence on stimulation of Src/Shc/Ras/Erk pathway via ESR1 [59]. Under proliferative conditions, estradiol activation can lead to increased expression of cyclin D1, nuclear exclusion of the CDK inhibitor p27, and stimulation of G1-S transition in cancer cells [60,61], and therefore, can positively regulate proliferation. In line with the contradictory data, it seems that the relationship between SRC and ESR1 is reciprocal, complex and depend on the conditions. In addition, according to experimental data, ESR1 is the dominant isoform of estrogen receptors expressed in glomeruli and in isolated mesangial cells [62,63] that involves in regulation of numerous target genes, including regulators of proliferation. Some reports suggest a reverse relationship between sig-
naling pathways via estrogen mediators and renal damage, especially in mesangial cells [64,65]. In other words, ECM synthesis stimulated by TGF-β1, angiotensin II, and endothelin-1 leading to fibrosis, as well as the production of growth promoters that induce glomerulosclerosis are inhibited by estrogen metabolites [66,67] that act through estrogen receptors. This observation implies that estrogen metabolites (and consequently, the estrogen receptor) have renoprotective roles [65]. In our results, the complex interaction between SRC and ESR1 suggests activation of the proliferation pathway. This activation results in altered expression of proliferative and apoptotic markers specifically in kidney mesangial cells, independent from estrogen and estrogen metabolite ligands. Notably, this activation is done through a network started from immune complex deposition. Association between polymorphism of ESR1 gene and IgAN also confirms the importance of this protein in the pathogenesis of IgA nephropathy [68]. Additionally, our findings are consistent with other observations about estrogen receptor involvement in the pathogenesis of renal diseases [69].

In our results, all of the six significant pathways share the same interactions from TFRC toward ESR1. Therefore, ESR1 has the highest degree of interactions and acts as a key molecule that could affect a set of proteins and transcription factors.

According to gene ontology (GO) annotations, we suggest that IgA deposition might stimulate down regulation of terminal nodes of the pathways (including CEBPB, CAV1, MGMT, COX5A) under the annotation of “positive regulation of cell killing”. This claim can be confirmed experimentally in the further analysis. In addition, as mentioned earlier, involvement of MAPK/ERK signaling pathway in mesangial proliferation has been proven experimentally [34]. Interaction between ESR1, as a key molecule in the significant subnetwork, and other four nodes (i.e. CAV1, EP300, TAL1 and CEBPB) with elements of MAPK/ERK signaling pathway indicates the cross-talk between these two pathways (Fig. 3). Activation of MAPK signaling by TFRC [30], Strengthen the possibility of interconnection between IgA-specific pathway and known MAPK signaling via ESR1.

Our findings are in accordance with the observation of Tamouza et al. [34] and elucidates the candidate missing molecules by which p-ERK1/2 induce proliferation in mesangial cells in response to IgA deposition. This interaction, also highlights the role of ESR1 as an important player in the pathogenesis of IgA nephropathy, and additionally, as a potential target for its therapy.

**Conclusion**

In this study, we constructed a network of mesangial cell proliferation pathways specific for IgA nephropathy. The final significant subnetwork starts from TFRC, a specific receptor of IgA1 on the glomerular mesangial cells, and ends in six proteins related to proliferation of the mesangial cells, namely COX5A, LTF, CEBPB, CAV1, MGMT, and XBP1. Among the pathway mediators, ESR1 is the most important intermediate node in the significant subnetwork and we suggest it as a potential target for the future experiments. The suggested pathway improves our understanding of the pathogenesis of IgA nephropathy, and suggests the existence of a specific proliferation process in this disease, which is distinct from other proliferative glomerulonephritis. Future interventions to regulate this pathway can be promising for presenting a new therapeutic strategy for IgA nephropathy.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.


