A Brief Insight about Role of YKL-40, EGF, suPAR as Biomarkers of Kidney Diseases

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Abstract

Background: In recent decades, the identification of new biomarkers of AKI has been the subject of interest by scientists worldwide. However, the predictive, diagnostic and prognostic ability of biomarkers in the context of iodinated contrast administration, has been less studied. In recent years, many additional potential biomarkers have been newly described for the early detection of tubular dysfunction/lesion associated with CM administration, to reliably measure CI-AKI, and thus prevent patient outcomes. Some of them are well characterized and categorized and could be divided into different groups in response to different physiological conditions: some involved in glomerular filtration e.g cyststin c, others related to the inflammatory response e.g. MCP-1,YKL-40,IL-18 and tubular cell injury e.g KIM-1,NGAL and LFABP or with a not well-defined relationship with the disease and new emergent biomarkers under study e.g CTGF and suPAR.

Keywords: YKL-40, EGF, suPAR, kidney diseases

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Introduction:

Glycoside hydrolase family 18 includes chitinases and nonenzymatic chitinase-like proteins (CLPs), both of which bind chitin, a polysaccharide chain composed of N-acetylglucosamine repeats and present in arthropods and other taxa as a major structural polymer. While chitinases cleave chitin, CLPs do not possess this enzymatic activity. chitinase-3 like-protein-1 (CHI3L1), one of the CLPs, also has been named YKL-40 in humans. It was detected in human chondrocytes, synoviocytes, and vascular smooth muscle cellsIn fact, CHI3L1 is produced by a multitude of cells,

including macrophages, neutrophils, fibroblastlike cells, hepatic stellate cells, endothelial cells, and cancer cells. *(1).*

YKL-40 (CHI3L1) structure:

Crystal diffraction studies revealed that the three-dimensional structure of CHI3L1 (YKL-40) consisted of an (β/α)8-barrel domain with a second domain composed of six antiparallel β-strands, with one α- helix (α + β) domain inserted after strand β7.14,15 Additionally, a 43-residue carbohydrate-binding cleft was found exposed at the C-terminal side of the β-strands in the (β/α)8 barrel Essentially, the protein–carbohydrate interactions are dominated by stacking of the sides. This structure suggests that CHI3L1 acts as a sensor to turn on innate defenses and regulates inflammatory responses as a consequence of infection, which can also contribute to tumorigenesis *(2).*

BIOLOGICAL ACTIVITIES OF YKL-40:

YKL-40 facilitates the growth of synovial cells, articular chondrocytes, skin, and fetal lung fibroblasts through the phosphorylation of MAPK and Akt signaling. During bronchial asthma, YKL-40 increases bronchial smooth-muscle cell growth and proliferation through PAR-2-dependent, Akt-dependent, Erk-dependent, and p38-dependent mechanisms *(3).*

YKL-40 has a chemotactic effect on vascular endothelium and smooth-muscle cells during tissue injury and remodeling, inflammation, and fibrosis. It regulates the morphology of vascular endothelial cells by stimulating endothelium tubulogenesis and vascular smooth muscle cell migration and adhesion *(1).*

CHI3L1 promotes cell survival CHI3L1 has been found to protect cardiomyocytes from apoptosis during ischemia-reperfusion injury. It also has a pro-inflammatory function in reducing inflammatory cell apoptosis and death, by inhibiting Fas expression through the phosphorylation of protein kinase B (PKB)/Akt *(4).*

YKL-40 regulates the synthesis and degradation of the ECM. Increased ECM degradation promotes cell migration, invasion, and tumorigenesis. The ECM barrier represents the first obstacle for invasive tumor migration and establishment of metastases.It has been shown that YKL-40 inhibits the degradation of type I collagen and hyaluronic acid. It also affects the enzymatic activity of matrix metalloproteinases (MMPs), thereby influencing the extent of cell adhesion and migration, influencing tissue remodeling, fibrosis, and tumorigenesis *(5).*

Role of YKL-40 in Diseases:

***Cardiovascular Diseases*:**

YKL-40 was proposed as a predictor of atherosclerosis development. Due to its pathophysiologic role in embolus formation rather than in the atherosclerosis development, elevated plasma YKL-40 concentrations bear a 2-fold increased risk of ischemic stroke and venous thromboembolism *(6).*

Furthermore, a Chinese study on patients with an acute MI with a ST-elevation (STEMI) demonstrated statistically significant, 2 times higher YKL-40 concentrations in STEMI patients compared to controls, arguing for YKL-40 as a potential biomarker for STEMI diagnosis *(7)*.

Liver Fibrosis and Cirrhosis :

Novel biomarkers for noninvasive liver fibrosis/cirrhosis assessment, including YKL-40, are evolving, with the aim for better screening and management of patients. YKL-40 has been studied as a predictor of chronic liver disease. Three to four times higher baseline serum concentrations of YKL-40 measured in cirrhotics compared to healthy controls are associated with increased risk of alcoholic liver disease, and when used in combination with heavy alcohol drinking, calculated 10-years risk of alcoholic liver cirrhosis is up to 7% *(6)*.

Alzheimer's Disease (AD):

The most common neuroinflammatory and neurodegenerative conditions in which the role of YKL-40 was investigated in humans AD. A longitudinal investigation of cognitively healthy individuals at risk for AD indicated an ageassociated increase of YKL-40 in plasma with higher concentrations in men than women as well as positive correlation with memory (82). YKL-40 correlates positively with CSF sphingomyelin and Galectin 3 concentrations, both molecules being key pathological biomarkers of microglial activation in AD, but surprisingly correlates negatively with brain amyloid-β deposition *(8)*.

Acute Kidney Injury:

YKL-40 in serum and urine was investigated in the AKI. Two times increased YKL-40 concentrations were identified both in urine and plasma of the patients with AKI compared with controls, predominantly referring to hospitalized patients or those with preexisting CKD. Plasma YKL-40 concentration was in the linear correlation to the severity of AKI and proinflammatory markers, with a cut-off concentration > 142 μg/L being predictor of adverse outcomes and mortality *(9)*.

Hall et al. showed that increased levels of urinary YKL-40 of up to 5 ng/ml were moderately correlated with AKI progression and/or mortality among patients. Moreover, apparent increases in YKL-40 levels in urine were observed in cases of kidney transplantation among patients hospitalized within 24 hours of developing AKI *(10)*.

Epidermal Growth Factor (EGF):

The epidermal growth factor (EGF) is a globular protein (MW: 6,045 Da). Which is found in many human tissues and body fluids, including the kidneys (specifically the Henle's loop and distal convoluted tubules) and urine, saliva, parotid glands, milk, tears, and only in a minimal concentration in plasma. Platelets are the principal source of blood EGF in humans, and the concentration steadily increases during coagulation following sample collection *(11)*.

EGF Structure and Functions:

EGF is a nondialyzable, compact, heat-stable human polypeptide of 53 amino acids with three intramolecular disulfide bonds. All the amino acids are present except phenylalanine, alanine, and lysine, and it has a C-terminal arginine residue. The *EGF* gene has 24 exons and 23 introns and is found on chromosome 4q25–q27 ***(12)***.

EGF promotes cellular proliferation, differentiation, and survival by binding to 1:1 to the soluble extracellular domain of the EGF receptor, resulting in a complex of 2EGF:2 soluble extracellular domain of the EGF receptor. This ligand-induced dimerization activates the receptor's intrinsic protein-tyrosine kinase activity, triggering a signaling cascade within the cell and resulting in a variety of biochemical changes. This eventually leads to DNA synthesis and cell proliferation ***(14)***.

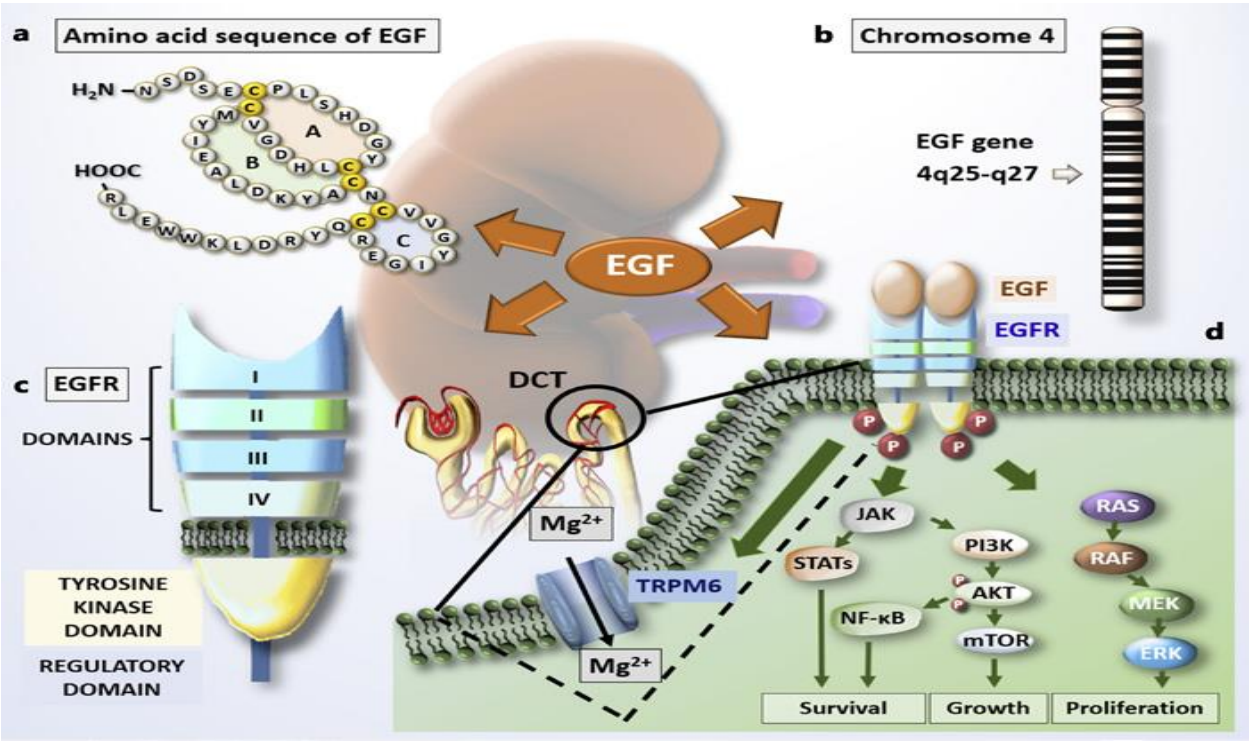

Figure (1): a. EGF is a 53-amino acid singlechain polypeptide with a relative MW of approximately 6,000 Da. b. hromosome 4 (q25–q27) includes the *EGF* gene, which is roughly 120 kb in size. c. The structure of the EGFR, a member of the ErbB receptor family: the extracellular domain with four subdomains, the lipophilic transmembrane region, and an intracellular domain make up the EGFR. d. Signaling pathways downstream of EGFR activation include the JAK-STAT pathway, the PI3K/Akt/ NF-B pathway, the PI3K/Akt/PTEN/ mTOR pathway, and the RAS-RAF-MEKERK MAPK network. EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinase; JAK-STAT, Janus kinase-signal transducer and activator of transcription; ERK, extracellular signal regulated kinase *(13)*.

Functions and Role of EGF in Diseases:

In the kidney, EGF exerts several biological functions such as regulation of cellular metabolism and glomerular hemodynamics, modulation of cell growth, and renal repair after injury ***(15)***.

Moreover, EGF is highly expressed along the distal convoluted tubule (DCT), which is an important site for regulating urinary magnesium excretion and thus magnesium homeostasis. It stimulates magnesium reabsorption by the transient receptor potential cation channel 6 (TRMP6), located at the apical membrane of the DCT cells ***(16)***.

An experimental model of acute renal failure showed that EGF assisted with tubular injury recovery by activating regeneration pathways, resulting in reepithelialization of the injured tubules. Through crosstalk with phosphoinositide 3-kinase, Janus kinase-signal transducer and activator of transcription, and extracellular signal-regulated kinase pathways, higher urinary levels of EGF (uEGF) are thought to reflect functional tubular mass and regeneration potential. In contrast, lower uEGF concentrations are associated with interstitial fibrosis and tubular atrophy ***(17)***.

In previous studies, Lower uEGF levels in children with type 1 DM (T1DM) may indicate a decreased kidney's regenerative capacity, making uEGF a possible biomarker of early diabetic nephropathy. Unlike microalbuminuria, which is a symptom of glomerular disease, a decrease in renal EGF production indicates tubulointerstitial kidney injury and increasing kidney injury. uEGF/Cr was considerably lower in T1DM when compared to age-matched healthy controls in a case-control study ***(18)***.

In addition to its role in magnesium homeostasis, EGF disturbances have been described in patients suffering from oncological pathologies and it is furthermore involved in the proliferation and growth of neurons and glia of the central nervous system (CNS). High levels of EGF are present in the CNS and play a critical role in controlling proliferation and differentiation of the nervous tissue during neurogenesis ***(19)***.

Soluble Urokinase Plasminogen Activator Receptor (suPAR)

Soluble urokinase-type plasminogen activator receptor (suPAR) is the soluble form of urokinase plasminogen activator receptor (uPAR) which is a membrane bound receptor; this cell surface uPAR can be shed by several proteases leaving it devoid of glycosylphosphotidylinositol anchors to generate a soluble form. ***(20)***.

Structure of suPAR:

uPAR and suPAR share the same overall structure, aside from the glycosyl phosphatidylinositol (GPI) anchor that tethers uPAR to the cellmembrane. Both have three homologous domains, D1-D3, connected by a linker region between D1 and D2. uPAR has cleavage sites for several proteases in the linker region (chymotrypsin, elastase, matrix metalloproteases, cathepsin G, plasmin, uPA) and the GPI anchor (phospholipase C and D,

cathepsin G, plasmin), which upon cleavage can result in three suPAR isoforms (suPARI-III [full-length isoform], suPARI, suPARII-III) *(21)*.

The molecular weight of suPAR varies between 24–66 kDa due to variations in posttranslational glycosylation. Additional isoforms generated by alternative splicing have been described on the RNA level, but whether these are transcribed and their possible roles remain unclear *(22)*.

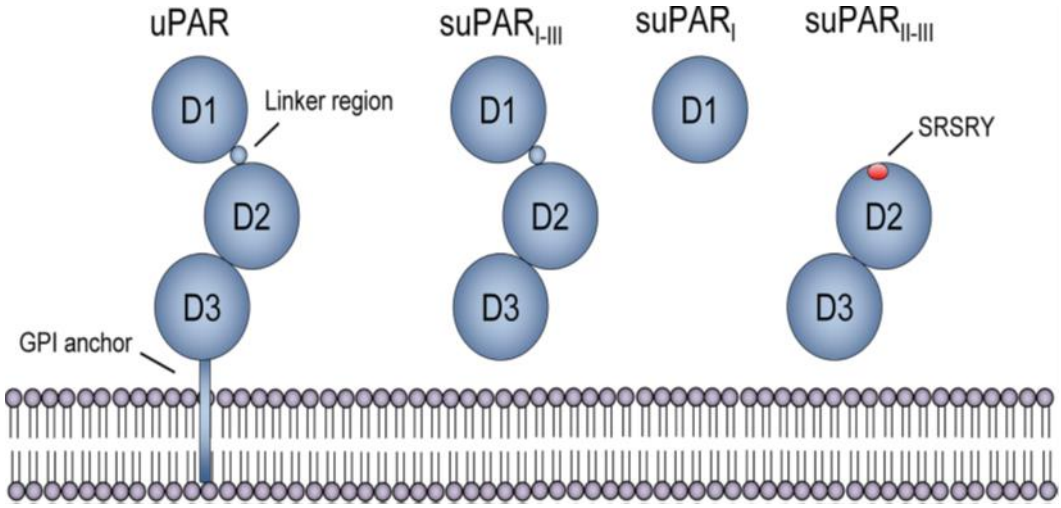

Figure (2): Structure of uPAR and suPAR isoforms.

Soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of the membrane-bound receptor uPAR, which is tethered to the membrane by a glycosyl phosphatidylinositol (GPI) anchor. The protein consists of three domains, D1-D3, that are connected with a linker region between D1 and D2D3. Several cleavage sites exist, both in the linker region and the GPI anchor, and proteolytic cleavage generates three suPAR isoforms: full-length suPARI-III, suPARI, and suPARII-III. Cleavage of uPAR/suPAR in the linker region exposes an SRSRY sequence, which is involved in chemotaxis *(23)*.

Urokinase plasminogen type activator receptors is expressed in different cell types including neutrophils, monocytes, macrophages, activated T- lymphocytes, endothelial cells, and kidney podocytes. uPAR regulates the plasminogen activation system by binding urokinase (uPA) and its zymogen form *(24)*.

suPAR has many types of receptors; the most common known receptor is avb3 integrin; activation of avb3 integrin on podocytes leads to activation of guanosin triphosphate hydrolyzing enzyme (GTPase RacI), which causes podocytes foot process (FP) motility and effacement. In addition to interaction with integrins, suPAR initiates signaling transduction in cooperation with other trans-membrane proteins such as caveolin and G-protein-coupled receptors *(25)*.

Role of suPAR in Diseases:

suPAR levels are thought to reflect the state of immune activation of the individual. This is substantiated by findings of increased suPAR levels in individuals suffering from viral, bacterial or

parasitical infections as well as autoimmune diseases and cancer. Interestingly, in all of these conditions the higher the concentration of suPAR, the worse the prognosis of the disease. Initial studies of soluble uPAR (suPAR) as a potential biomarker were performed in various forms of cancer where increased suPAR levels were associated with worse prognosis *(26)*.

Later, a substantial prognostic value of suPAR was demonstrated for infectious diseases such as HIV. Sidenius *et al.*, first investigated the correlation between serum suPAR levels and HIV-1 disease prognosis in 2000 and found the by now well documented increase of suPAR levels in HIV-1 infected individuals and its power as a prognostic marker, with strength similar to, and independent of, CD4 counts and viral load. Also,it was documented that suPAR is elevated by active TB disease, suPAR levels at time of TB treatment initiation is prognostic for survival during the 8-month treatment period, and in those who successfully complete the treatment, suPAR levels decrease to the level of non-infected individuals *(27)*.

Until today, suPAR has been evaluated as a biomarker of inflammation, organ damage and clinical outcome in numerous disorders including cardiovascular disease, hepatitis, renal disorders and rheumatic diseases *(27)*.

suPAR and AKI:

Recent investigation has suggested Increased suPAR level contribute to deteriorated renal function and poor prognosis in patients with CKD. In addition, suPAR has emerged as a promising biomarker in AKI in identification and prediction *(28)*.

Hayek et al.(29) have found that elevated suPAR is associated with an increased risk of AKI in patients undergoing coronary angiography or cardiac surgery and in those hospitalized in intensive care units. Targeting suPAR has therapeutic promise as it is pathogenic. In experimental mouse models, the use of anti-suPAR MABs eliminated the adverse effects of suPAR on the kidney, indicating that suPAR is a promising therapeutic target for alleviating AKI. Therefore, eliminating suPAR from circulation or neutralizing its biological effects may be a reasonable strategy to reduce the incidence, morbidity, and mortality in AKI. In clinical trials, biomarkers such as suPAR have the advantage of identifying AKI at an early stage and predicting AKI which include also contrast induced acute kidney injury *(30)*.

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