

Decreased vascular endothelial growth factor expression in focal segmental glomerulosclerosis lesions of patients under sirolimus

Helena Viana, Fernanda Carvalho, José Reimão Pinto, Maria João Galvão, Ana Santos, Fernando Nolasco, João Ribeiro Santos.
Serviço de Nefrologia, Hospital de Curry Cabral, Lisboa, Portugal.

A-Vascular endothelial growth factor (VEGF) is essential to the glomerular filtration barrier

- The vascular endothelial growth factor (VEGF) is essential to the vascular permeability, angiogenesis and cellular survival in different tissues.
- In glomerulus (GL), the podocytes (PD) synthesizes VEGF and endothelial cells express receptor to VEGF.
- The VEGF acts on endothelial cells and mediates functions such as vascular permeability and endothelial cell division. In addition to its paracrine role in endothelial cells, VEGF have an autocrine function that is required for podocyte survival and differentiation
- Several studies documented that minimal alterations in glomerular (GL) VEGF expression play a pathogenic role in initiating GL lesion . It is clear that interaction between podocytes and endothelial cells are critical in the filtering GL. [1, 2]
- In diverse clinical issue the reduction of VEGF is associated with proteinuria (PT). Several reports indicate that women with preeclampsia present elevated soluble VEGF receptor 1, an inhibitor of VEGF. In a significant percentage of oncology's patients the anti-VEGF antibody therapy leads to proteinuria. [3]

B- Sirolimus cause proteinuria and FSGS glomerular lesions

- Calcineurin inhibitor (CNI) therapy has been identified to be an important non-immunological cause of Chronic allograft nephropathy (CAN), the most prevalent cause of late kidney transplant failure.
- Switching from CNI to sirolimus (SRL) when CAN is suspected has become a frequent practice but is complicated by proteinuria (PT) in a significant percentage of cases.
- The pathophysiological mechanism behind the proteinuria is still unclear.
- The CNI withdrawal led to an increase of PT and an increased intra-glomerular pressure, witch supports the hypothesis that haemodynamic changes play a significant role. [4]
- FSGS has been related frequently as the GL lesion in these patients with CAN.
- But FSGS has also been related in renal transplant of patients who were treated *de novo* with SRL, without any medical history of FSGS in their native kidneys. [6]
- A recent study reports a reversible increase of PT after conversion from azathioprine to SRL after kidney transplantation.[7]
- These cases suggest a direct effect of SRL as a cause of proteinuria

C- Sirolimus reduce VEGF expression in glomerulus

- Sirolimus forms a complex with the FK-binding protein complex (FKBP-12) that binds with high affinity to the mammalian target mTOR.
- The mTOR controls the phosphorylation of proteins that regulate the cell cycle and is also involved in the regulation of growth factor including VEGF.
- Sirolimus inhibits the production of VEGF in different tumour cell lines in vitro and in vivo.
- This antiangiogenic activity of sirolimus is linked to the decreased VEGF production and markedly inhibited response of vascular endothelial cells to stimulation by VEGF.
- The use of sirolimus may reduce the chance of recurrent or de novo cancer in high-risk transplant patients. [8, 9, 10]

Objectives

According with the described in A+B+C:

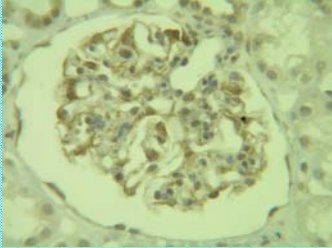
- Sirolimus can cause proteinuria and FSGS lesions by a reduction in VEGF expression in the glomerulus.
- To determine if glomerular lesions and proteinuria in SRL patients could be related to altered VEGF expression

Material and Methods

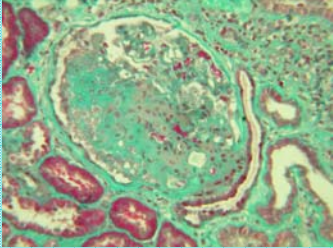
–We employed indirect immunohistochemistry in paraffin-embedded sections using a mouse monoclonal antibody against human VEGF-C1 (Santa Cruz®) to evaluate VEGF expression in 10 biopsies:

- Group A-** allograft kidney in backtable (n=3);
- Group B-** native normal kidney (n=1);
- Group C-** native kidney with FSGS lesions (n=2);
- Group D-** allograft kidney with FSGS lesions from proteinuric patients under sirolimus after conversion from CNI (n=3);
- Group E-** allograft kidney in proteinuric patient under sirolimus with membranous glomerulonephritis (n=1).

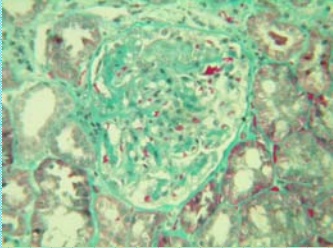
Group A- Normal VEGF expression in GL



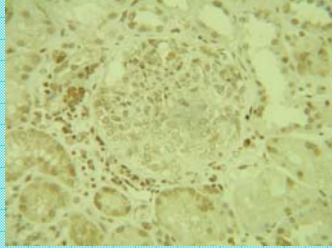
Group C- FSGS lesion in native kidney



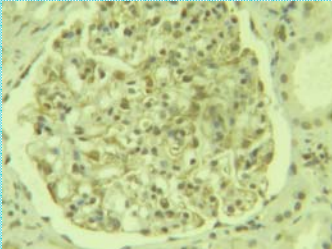
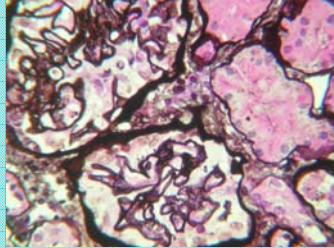
Group D- FSGS lesion in allograft kidney under SRL



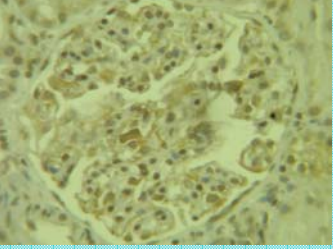
Group D- Reduced VEGF expression in hypertrophied PD



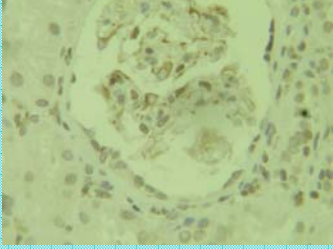
Group E- Membranous GNF with hypertrophied PD



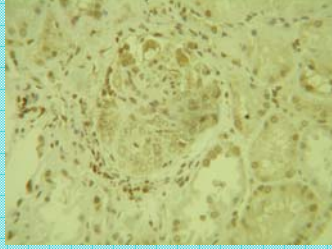
Group B- Normal VEGF expression in normal GL



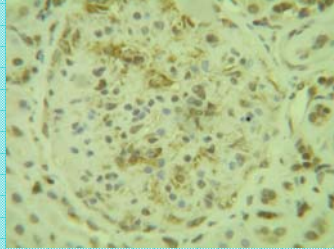
Group C- Normal VEGF expression in hypertrophied PD



Group D- Reduced VEGF expression in detached PD



Group D- Gradual reduction of VEGF expression with progressive dedifferentiation of PD



Group E- Reduced VEGF expression in hypertrophied PD

Results

- We found that the **controls biopsies (A; B)** showed normal global VEGF expression, with strong podocyte staining.
- The VEGF expression in the **group C** was similar to the control groups, although no FSGS lesions were observed in the stained glomerulus.
- Group D** showed normal VEGF expression in the apparently normal glomerulus, hypertrophied podocytes with reduction of VEGF in anomalous glomerulus, and no staining in sclerotic lesions.
- We observed a gradual reduction of VEGF expression with progressive dedifferentiation of podocytes.
- In the **group E** the VEGF was globally reduced, with some hypertrophied podocytes expressing decreased VEGF.

Conclusions

- We confirmed the diminished VEGF expression in injured podocytes of sirolimus patients.
- This decreased expression may result from a direct effect of sirolimus and precede the appearance of FSGS lesions and proteinuria.
- Further studies are needed with greater number of cases and controls, including early biopsies of patients under sirolimus.

References:

1- **Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal disease.** Eremina V, Sood M, Haigh J, Nagy A, Lajolo G, Ferrara N, Gerber HP, Kikawa P, Minet J, Quaggin S. J Clin Invest 111:707-716, 2003; 2- **Vascular permeability factor or mRNA and protein expression in human kidney.** Brown L, Berse B, Ogazzi K, Manseau E, Water L, Songer D, Dvorak H, Rosen S. Kidney Int 42:1457-1461, 1992; 3- **Proteinuria in a patient receiving anti-VEGF therapy for metastatic renal cell carcinoma.** Ronconi D, Sotoukar A, Nadasdy D, Monk JP, Rovin B. Nature Clinical Practice 3(3):287-293, 2007; 4- **Conversion from calcineurin inhibitors to sirolimus in chronic allograft nephropathy: benefits and risks.** Dickinson P, Campistol J. Nephrol Dial Transplant 21: 560-568, 2006; 5- **Conversion from calcineurin inhibitors to sirolimus in chronic allograft dysfunction: changes in glomerular haemodynamics and proteinuria.** Saurina A, Campistol J, Piera C, Diekmann F, Campos B, Campos N, Cuevas X, Oppenheimer F. Nephrol Dial Transplant 21:488-492, 2006; 6- **High Sirolimus levels may induce focal segmental glomerulosclerosis is de novo.** Leda vernier E, Buncel P, Mandet H, Van Huyen JP, Peraldi MN, Holal I, Noël LH, Legendre C. Clin J Am Nephrol 2: 326-333, 2007; 7- **Proteinuria following conversion from azathioprine to sirolimus in renal transplant recipients.** JM Akker, JFM Wetzel and AJ Hoibma. Kidney Int 70: 1355-1357, 2006; 8- **Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor.** Markus Guba, Philipp Von Breitenbuch, Markus Steinhaub, Gudrun Koehl, Christiane J. Bruns, Carl Zedlitz, Stefan Sarkas, Matthias Anthofer, Karl-Walter Jauch, Edward Gelscher. Nature Med. Vol 8: 128-135, 2002; 9- **Rapamycin is an effective inhibitor of human renal cancer metastasis.** Pu Lian, Ruchang Ding, Vijay Sharma, W Chon, Milagros Lagman, Manikann Suthanthiran. Kidney International, Vol. 63: 917-926, 2003; 10- **Sirolimus-induced thrombotic microangiopathy is associated with decreased expression of vascular endothelial growth factor in kidneys.** Hervé Sarradet, Olivier Toupanec, Marianne Lorenzato, Ronad Fédet, Laure Hélène Nod, Eymérie Lagoutte, Philippe Bimbaut, Jacques Chanard, Philippe Riou. Am J Transplant, 5: 2441-2447, 2005