THE GEORGE WASHINGTON UNIVERSITY
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Stem Cell Transplantation in Diabetic Kidney Disease (DKD)

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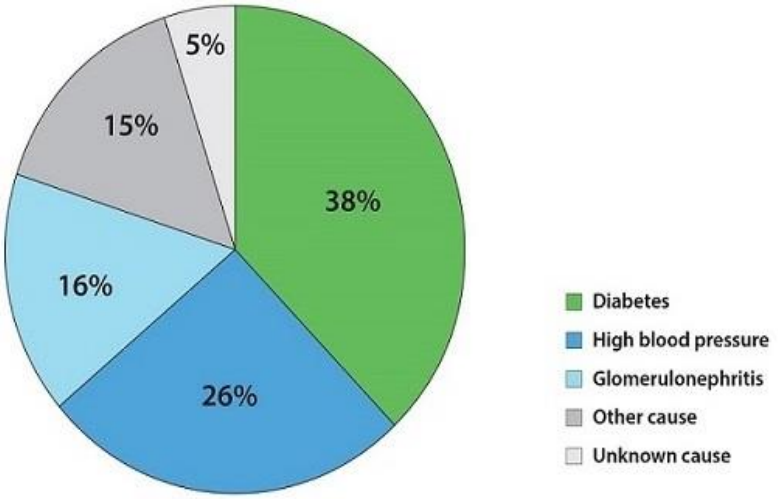
Diabetic kidney Disease (DKD)

- Chronic kidney disease (CKD): Gradual loss of **kidney** function over time
- 15% of US adults—37 million people—are estimated to have chronic kidney disease (CKD)
- Diabetes is one of the major causes of CKD in adults.



CDC

N=726,331 (all ages, 2016)
Source: US Renal Data System
*Includes polycystic kidney disease, among other causes.

- DKD is associated with sclerotic glomerulus and poor perfusion.

Treatments

STAGES OF CHRONIC KIDNEY DISEASE		GFR*	% OF KIDNEY FUNCTION
Stage 1	Kidney damage with **normal** kidney function	90 or higher	
Stage 2	Kidney damage with **mild loss** of kidney function	89 to 60	
Stage 3a	**Mild to moderate** loss of kidney function	59 to 45	
Stage 3b	**Moderate to severe** loss of kidney function	44 to 30	

- Control high blood pressure
- Manage high blood sugar
- Lower high cholesterol
- Control protein in urine.

Treatment for advanced diabetic kidney disease

Stage 4	**Severe** loss of kidney function	29 to 15	
Stage 5	Kidney **failure**	Less than 15	

- Kidney dialysis
- Transplant

Future treatment

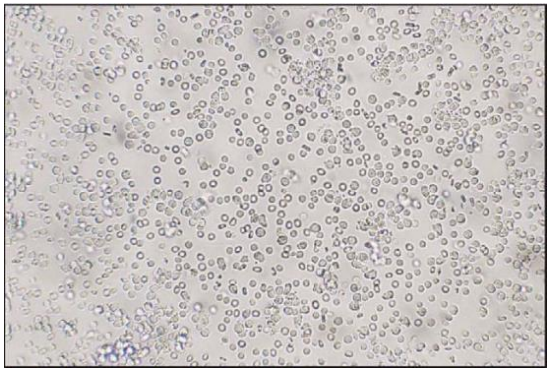
regenerative medicine: These techniques may help reverse or slow kidney damage

- Pancreas islet cell transplant
- Stem cell therapy

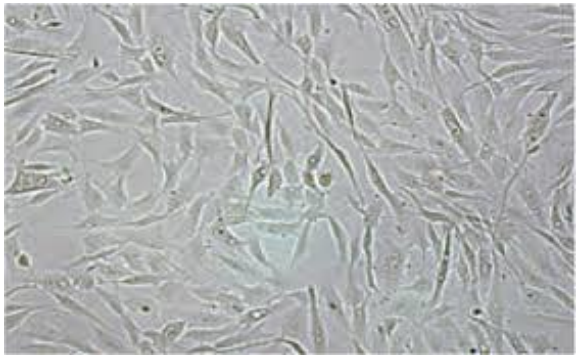
MSC **EPC**

Two Major Types of Adult Stem Cells from BM

- Short term such as Endothelial Progenitor Cells- (part of hemopoetic stem cells) necessary for **acute repair** of ischemic injury,
- CD34+
- KDR+ve
- Mostly in marrow
- 1% in periphery



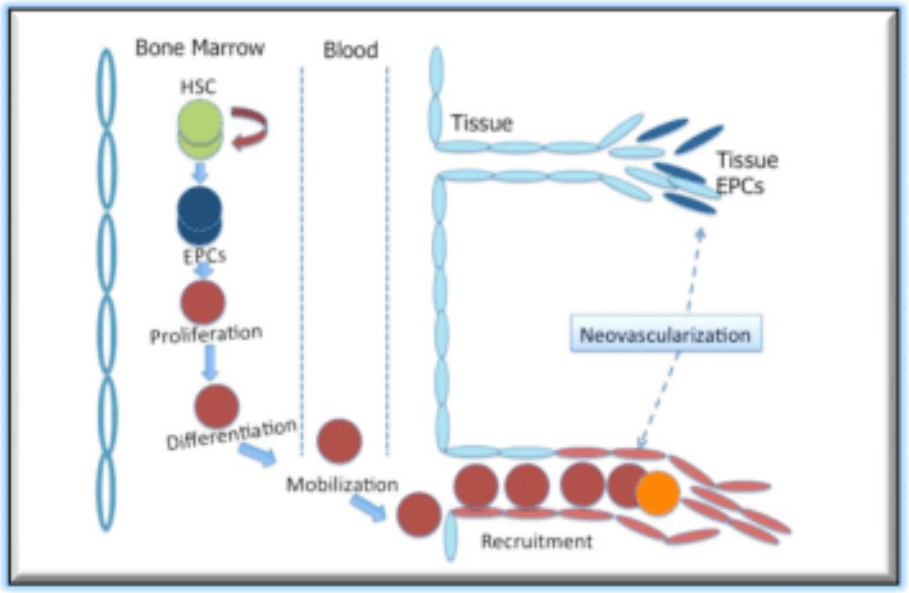
- Long term such as Mesenchymal Stem Cells-for body structural tissue repair, or **chronic repair**
- CD73, CD105, CD90 +ve
- Source could be any mesenchymal tissue



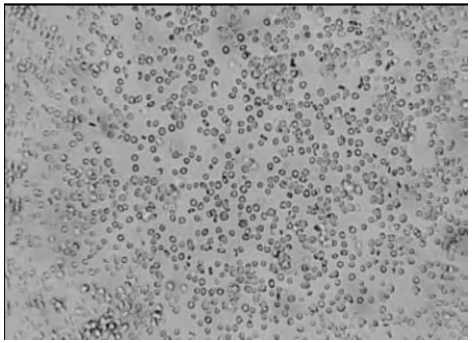
Endothelial progenitor cells (EPC)

- Endothelial progenitor cells augments neovascularization of tissue after ischemia endothelial injury

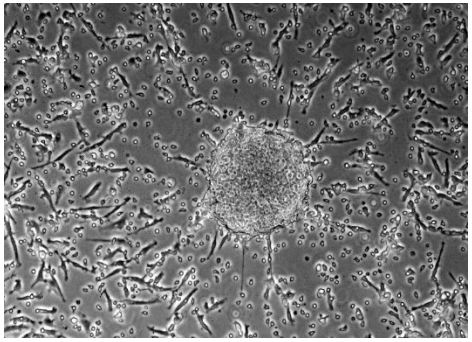
- Provide a novel therapeutic option



Mononuclear Cells at D0



Colony Formation at Day 14

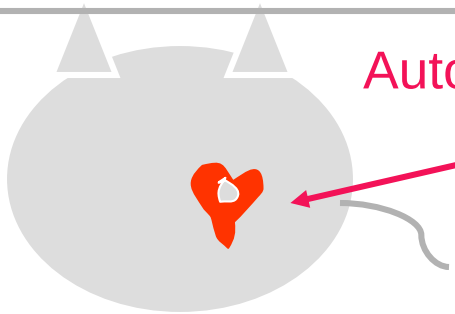


Our Aim Was To Note…

- If EPCs or modified endothelial progenitor cells augments neovascularization

- Compare EPC therapy with another possible stem cell therapy such as MSC as a therapy in DKD

EPCs have regenerative property in heart



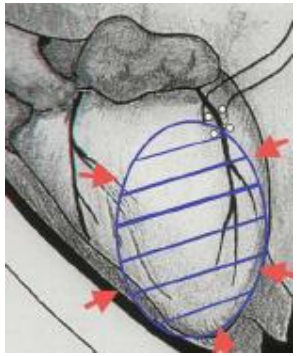
Autologous transplantation of EPCs Post-MI

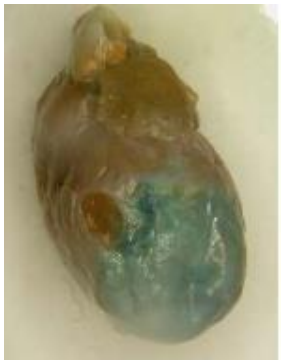
5~6-week old S-D rats

Take peripheral blood (1000 μl)

↓

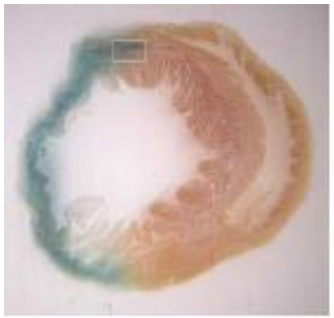
EPCs expansion





Intramyocardial injection (5 sites)

Saline or EPC

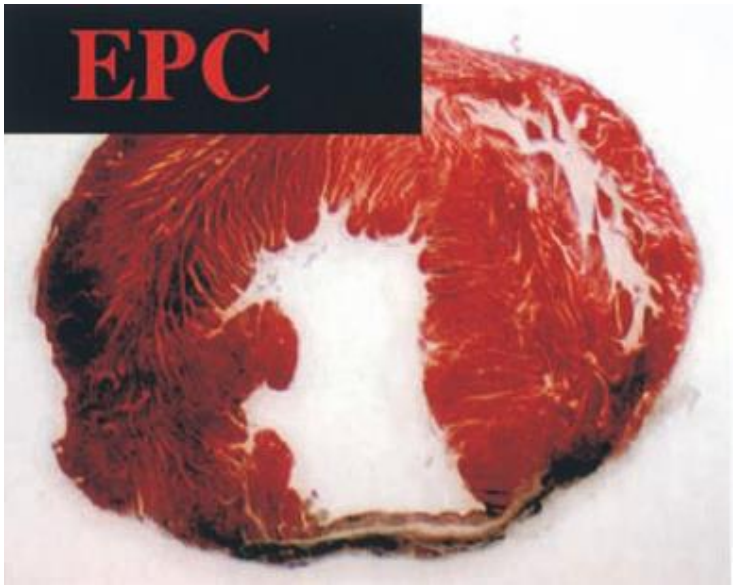


Echocardiography
Immunohistochemical analysis

1 week **12 weeks**

Sen et al, HGT 2010

Comparison of Myocardial Infarction with or without EPC



Representative findings of elastic tissue trichrome staining of the left ventricle samples

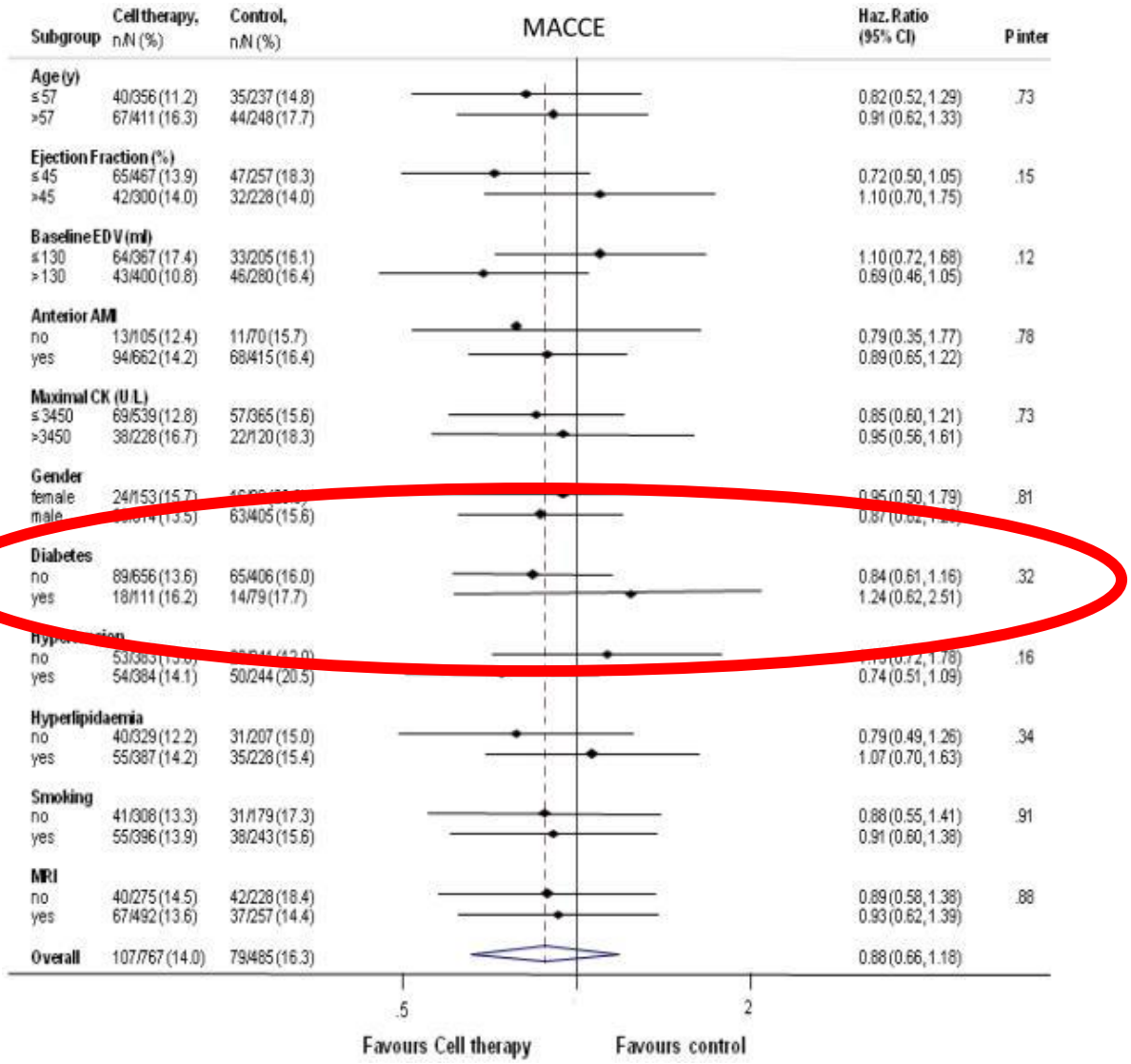
Human trials using EPCs

- Losordo DW, Henry T, Schatz RA et al. **Autologous CD34 cell therapy for refractory angina: 12 Month results of the Phase II ACT34-CMI study**. *Circulation*. 2009;120:S1132.

- Losordo DW, Henry TD, Davidson C, et al, **Intramyocardial, autologous CD34+ cell therapy for refractory angina**. Circ Res. 2011 Aug 5;109(4):428-36. Epub 2011 Jul 7. (Phase III)

- Other forms of cell therapy in cardiac ischemia has also been used such as iPSc and cardiomyocytes stem cells (Anaversa et al)

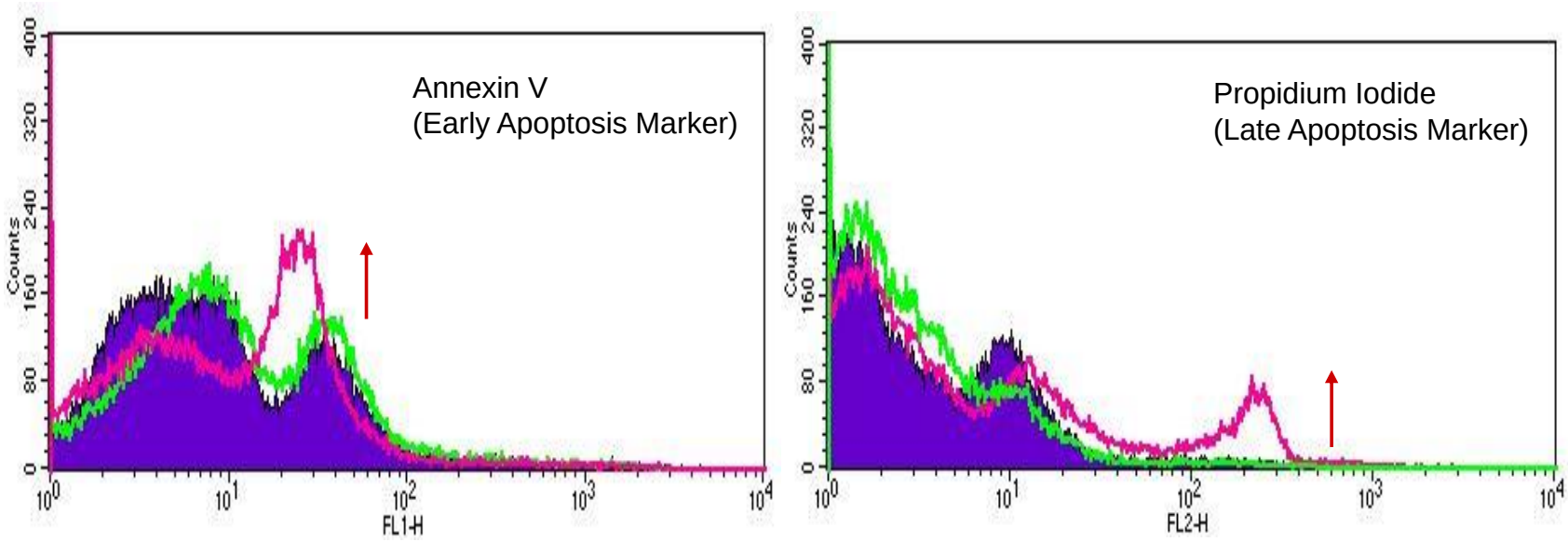
- **Losordo** DW, Kibbe MR, Mendelsohn F, et al; **A randomized, controlled pilot study of autologous CD34+ cell therapy for critical limb ischemia.** Autologous CD34+ Cell Therapy for Critical **Limb** Ischemia Investigators. Circ Cardiovasc Interv. 2012 Dec;5(6):821-30. (Phase II/III)



Gyongyosi M, Circ Res, 116; 1346, 2015

FACS Analysis of Human EPCs on exposure to HG

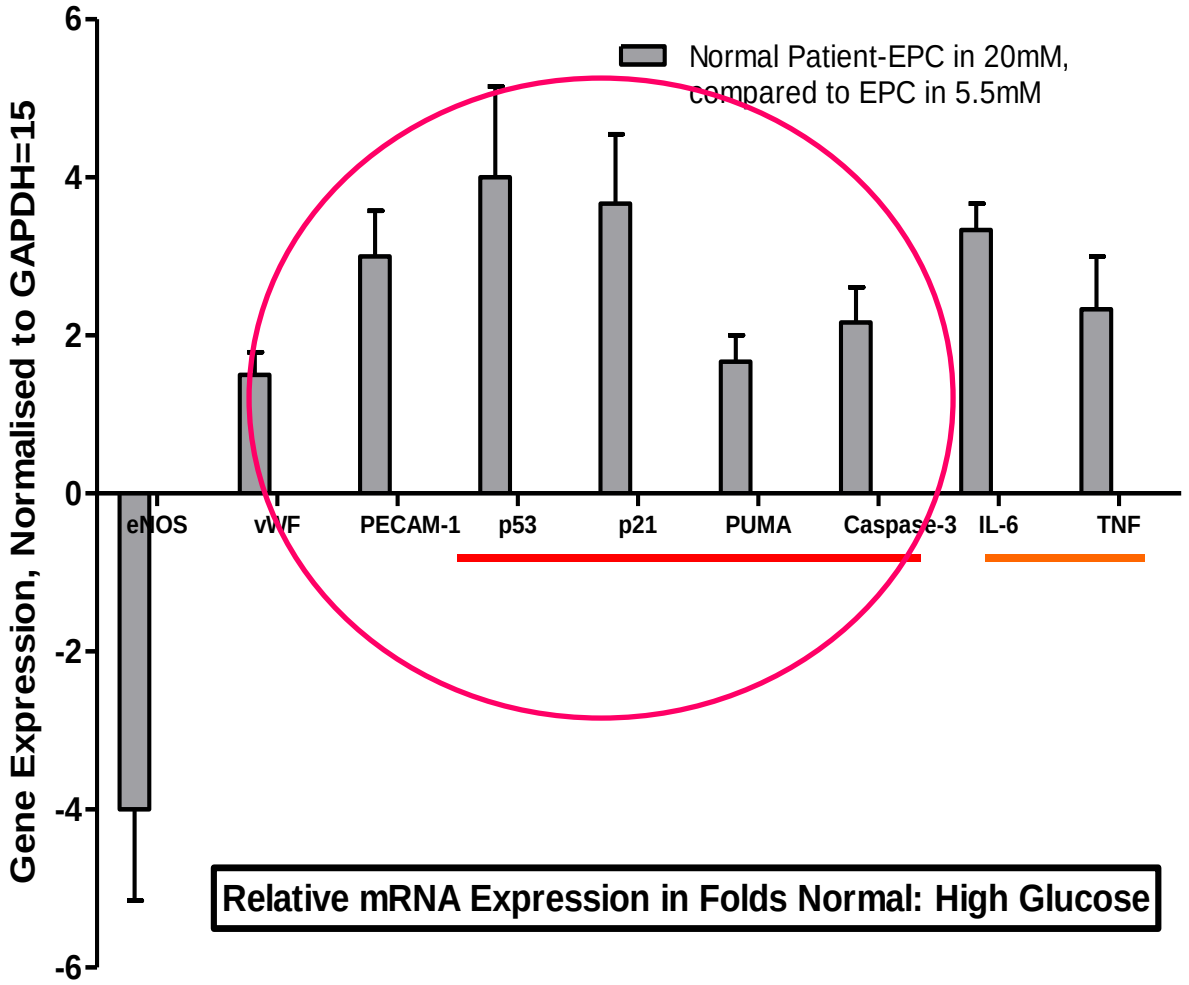
Human EPC, Post 48hrs (2 days) of High Glucose Exposure by FACS Analysis



Purple in solid- Isotype control
Green=Normal Glucose, 100mg%
Red= High Glucose, 360mg%

Within 48hrs there is significant apoptosis and death of EPCs in high glucose

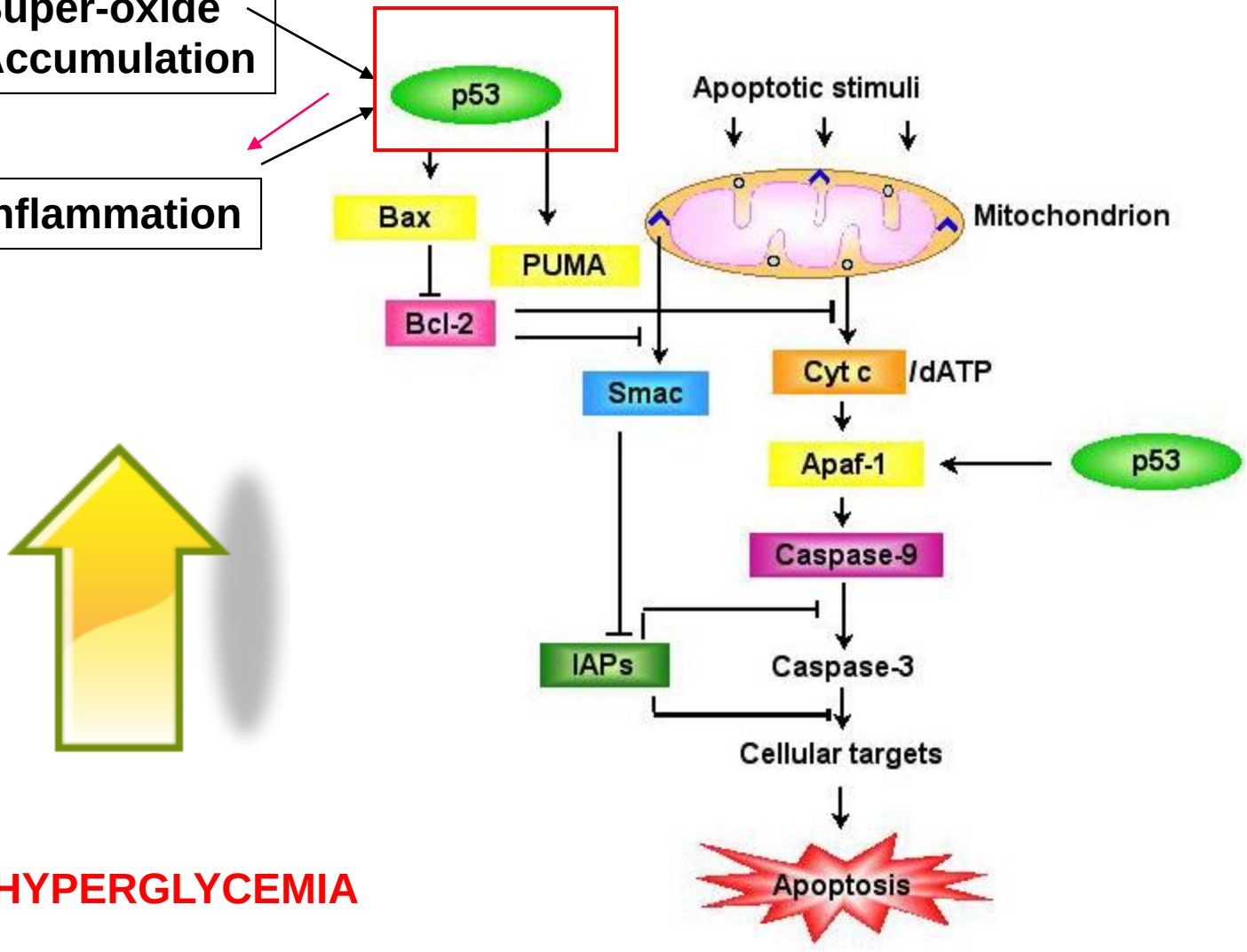
Genes Responsible for the Apoptosis in Hyperglycemia



P53 Gene is activated by multiple factors

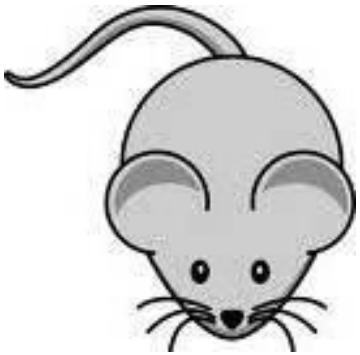
Super-oxide Accumulation

Inflammation



HYPERGLYCEMIA

P53 KO Mice

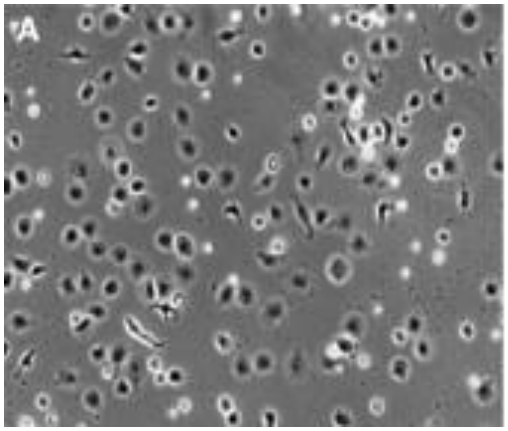


Knocking out or silencing p53 may prevent apoptosis and result in EPC survival

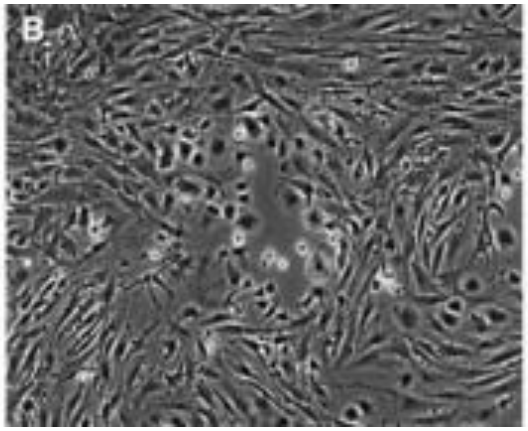
Increased survivability in p53 knockout EPC

EPC in HG

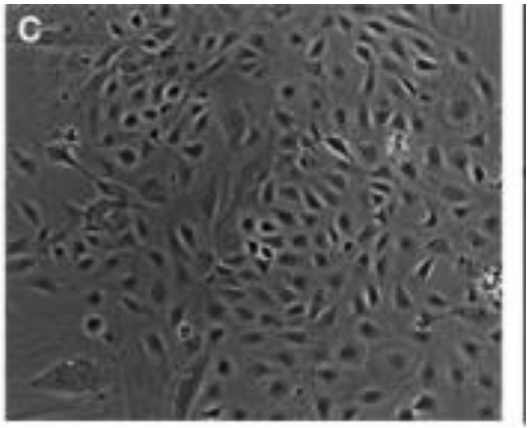
p53KO -EPCs in HG



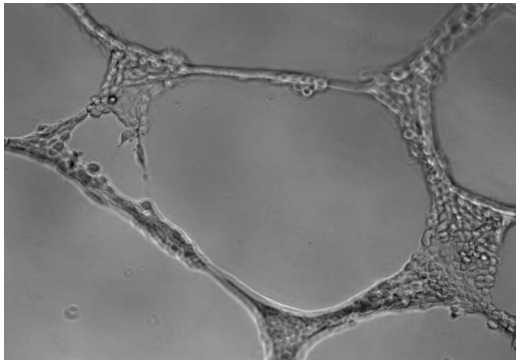
day 14



day 14



day 28



- Permanent Knocking down p53 could be translationally detrimental
- **Transient silencing of p53 by adenovirus**

Kundu et al, J Am Heart Assoc. 2017 Apr; 6(4): e005146.

American Heart Association- Journal of AHA (JAHA) Scientific Development Grant-12050535 Grant

ORIGINAL RESEARCH

American Heart Association | American Stroke Association.

Use of p53-Silenced Endothelial Progenitor Cells to Treat Ischemia in Diabetic Peripheral Vascular Disease

Nabanita Kundu, PhD; Cleyton C. Domingues, PhD; Cyril Chou, BS; Neeki Ahmadi, BS; Sara Houston, PhD; D. Joseph Jerry, PhD; Sabyasachi Sen, MD

Background—Peripheral vascular disease is a major diabetes mellitus-related complication. In this study, we noted that expressions of proapoptotic p53 gene and its downstream cascade gene such as p21 are upregulated in hyperglycemia. Therefore, we investigated whether p53- and p21-silenced endothelial progenitor cells (EPCs) were able to survive in hyperglycemic milieu, and whether transplantation of either p53 knockout (KO) or p21KO or p53- and p21-silenced EPCs could improve collateral vessel formation and blood flow in diabetic vaso-occlusive peripheral vascular disease mouse models.

Methods and Results—We transplanted p53 and p21KO mouse EPCs (mEPCs) into streptozotocin–induced diabetic (type 1 diabetes mellitus model) C57BL/6J and db/db (B6.BKS(D)-Leprdb/J) (type 2 model) post–femoral artery occlusion. Similarly, Ad-p53–silenced and Ad-p21–silenced human EPCs (CD34+) cells were transplanted into streptozotocin-induced diabetic NOD.CB17-Prkdcscid/J mice. We measured blood flow at 3, 7, and 10 days and hindlimb muscles were obtained postsacrifice for mRNA estimation and CD31 staining. Enhanced blood flow was noted with delivery of p53 and p21KO mEPCs in streptozotocin-induced diabetic C57BL/6J mice. Similar results were obtained when human Ad-p53shEPCs(CD34+) and Ad-p21shEPCs(CD34+) were transplanted into streptozotocin-induced nonobese diabetic severe combined immunodeficiency mice. Gene expression analysis of p53 and p21KO EPCs transplanted hindlimb muscles showed increased expression of endothelial markers such as endothelial nitric oxide synthase, vascular endothelial growth factor A, and platelet endothelial cell adhesion molecule 1. Similarly, quantitative reverse transcriptase polymerase chain reaction of human Ad-p53shEPCs (CD34+)– and Ad-p21shEPCs (CD34+)–transplanted hindlimb muscles also showed increased expression of endothelial markers such as vascular endothelial growth factor A, noted primarily in the p53-silenced EPCs group. However, such beneficial effect was not noted in the db/db type 2 diabetic mouse models.

Conclusions—Transient silencing of p53 using adenoviral vector in EPCs may have a therapeutic role in diabetic peripheral vascular disease. **(*J Am Heart Assoc.* 2017;6:e005146. DOI: 10.1161/JAHA.116.005146.)**

Key Words: adenovirus vector • apoptosis • diabetes mellitus • endothelial progenitor cells • gene therapy

Diabetes mellitus affects more than 11% of US adults and is projected to nearly double by 2025.[1] The presence of moderate hyperglycemia in addition to mild and moderate obesity may confer significant cardiovascular risk.[2] The American Diabetes Association has reported that coronary artery disease and stroke are 3 times more common in prediabetic compared with nondiabetic patients,[3] and overt diabetes mellitus increases this risk 5-fold. Many patients with prediabetes are either overweight or obese. Both diabetes mellitus and obesity are associated with cardiovascular complications such as endothelial dysfunction, oxidative stress, endothelial cell inflammation, and cardiovascular prothrombotic states.[4] These complications are primary reasons of cardiovascular disease. Cardiovascular disease includes cerebral, cardiac, and peripheral vascular diseases (PVDs). In this study, our main focus will remain on PVD as a consequence of diabetes mellitus.

One of the major consequences of diabetes mellitus is PVD. PVD is the inadequate perfusion of blood in the peripheral arteries often triggered by atherosclerosis, one of the major consequences of diabetes mellitus. Every day, 230 patients undergo diabetes mellitus-related amputation[5] due to

From the Department of Medicine, The George Washington University, Washington, DC (N.K., C.C.D., N.A., S.H., S.S.); Pioneer Valley Life Science Institute, Baystate Medical Center, Springfield, MA (C.C., D.J.J., S.S.).

Accompanying Figures S1 through S4 are available at http://jaha.ahajournals.org/content/6/4/e005146/DC1/embed/inline-supplementary-material-1.pdf

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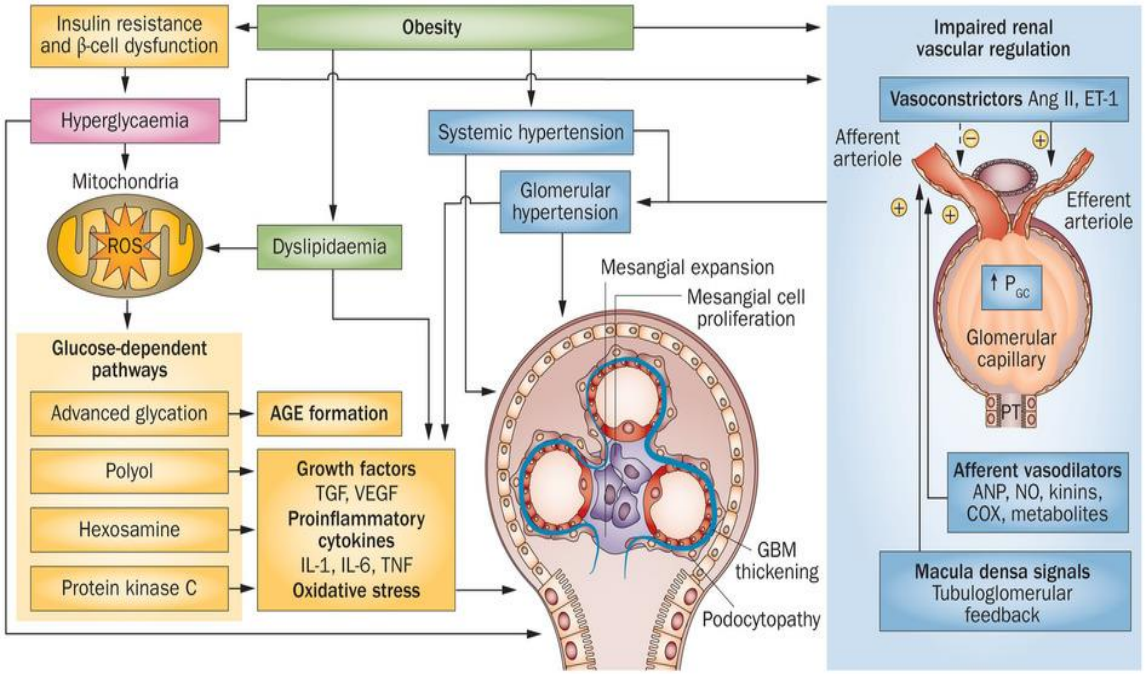
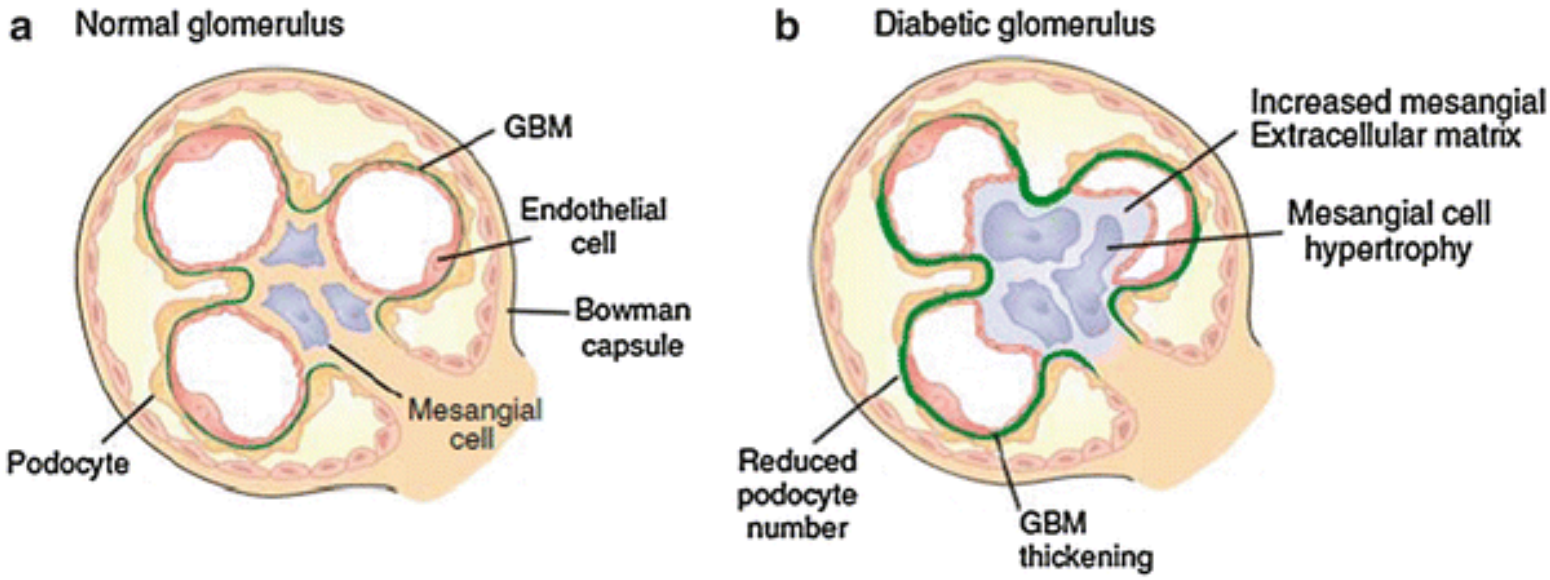
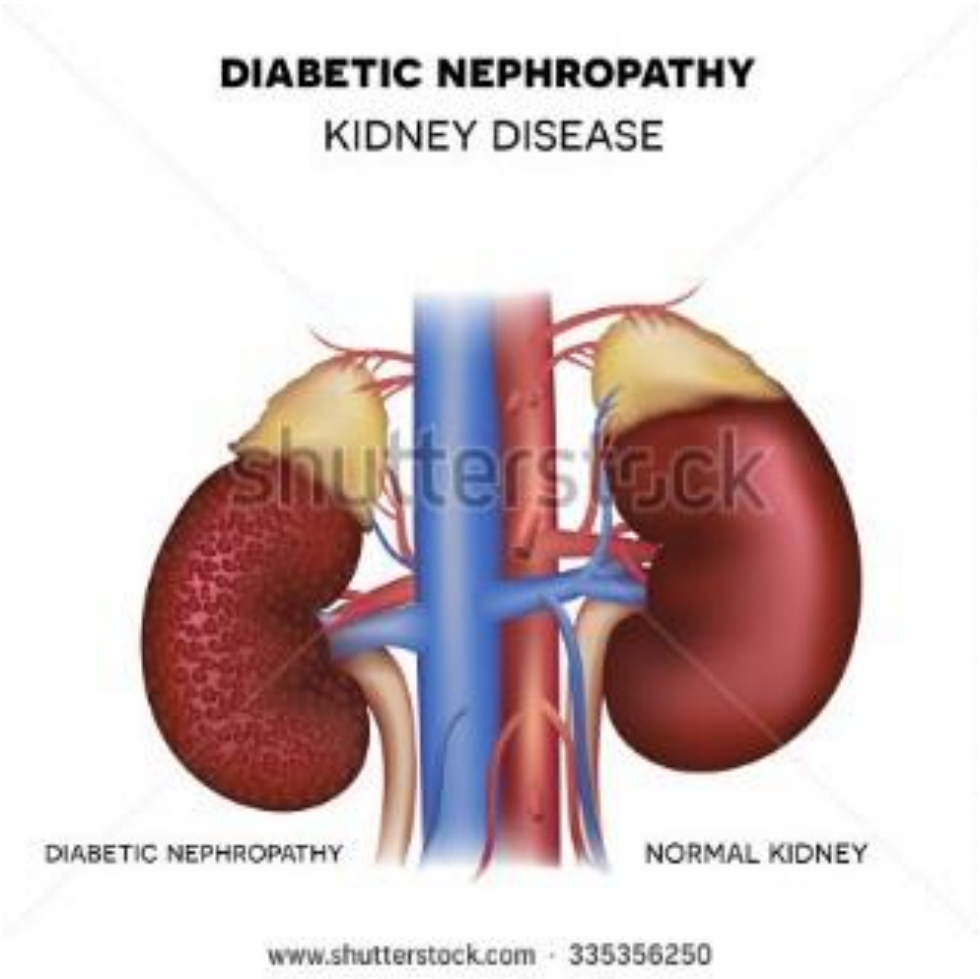

Insulin resistance and β-cell dysfunction
Obesity
Impaired renal vascular regulation
Hyperglycaemia
Vasoconstrictors Ang II, ET-1
Systemic hypertension
Afferent arteriole
Mitochondria
Glomerular hypertension
Efferent arteriole
ROS
Dyslipidaemia
Mesangial expansion
Mesangial cell proliferation
Glucose-dependent pathways
Glomerular capillary
PT
Advanced glycation
AGE formation
Afferent vasodilators
ANP, NO, kinins, COX, metabolites
Polyol
Growth factors
TGF, VEGF
Proinflammatory cytokines
IL-1, IL-6, TNF
Oxidative stress
Hexosamine
GBM thickening
Protein kinase C
Podocytopathy
Macula densa signals
Tubuloglomerular feedback


a Normal glomerulus
GBM
Endothelial cell
Bowman capsule
Mesangial cell
Podocyte
b Diabetic glomerulus
Increased mesangial Extracellular matrix
Mesangial cell hypertrophy
Reduced podocyte number
GBM thickening

Model: Type 1 Diabetes



MSC **EPC**
Modified EPC

The Promise of Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease: Current Diabetes Reports: May 2016, 16:42|

Clinical Translation of Mesenchymal Stromal Cell Therapies in Nephrology

Norberto Perico, [1] Federica Casiraghi,1 and Giuseppe Remuzzi

J Am Soc Nephrol. 2018; 29(2): 362–375

Potential and Therapeutic Efficacy of Cell-based Therapy Using Mesenchymal Stem Cells for Acute/chronic Kidney Disease

Chul Won Yun[1] and Sang Hun Lee. Int J Mol Sci. 2019 Apr; 20(7): 1619.

Results from animal models suggest distinct potential for systemic MSC infusion to favourably modulate DKD progression.

However, only a few early phase clinical trials have been initiated and efficacy in humans remains to be proven.

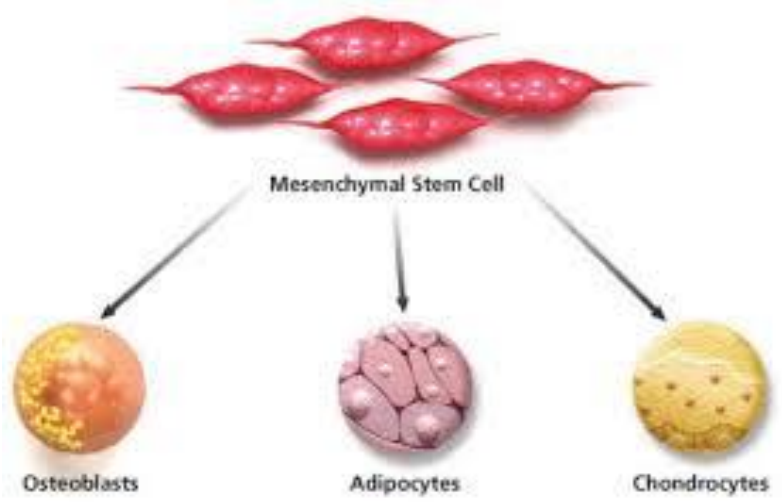
Key knowledge gaps and research opportunities exist in this field.

Recent PubMed search shows 21 publications on MSC transplant with only 1 on PBMNC ell therapy in DKD in last 3 years

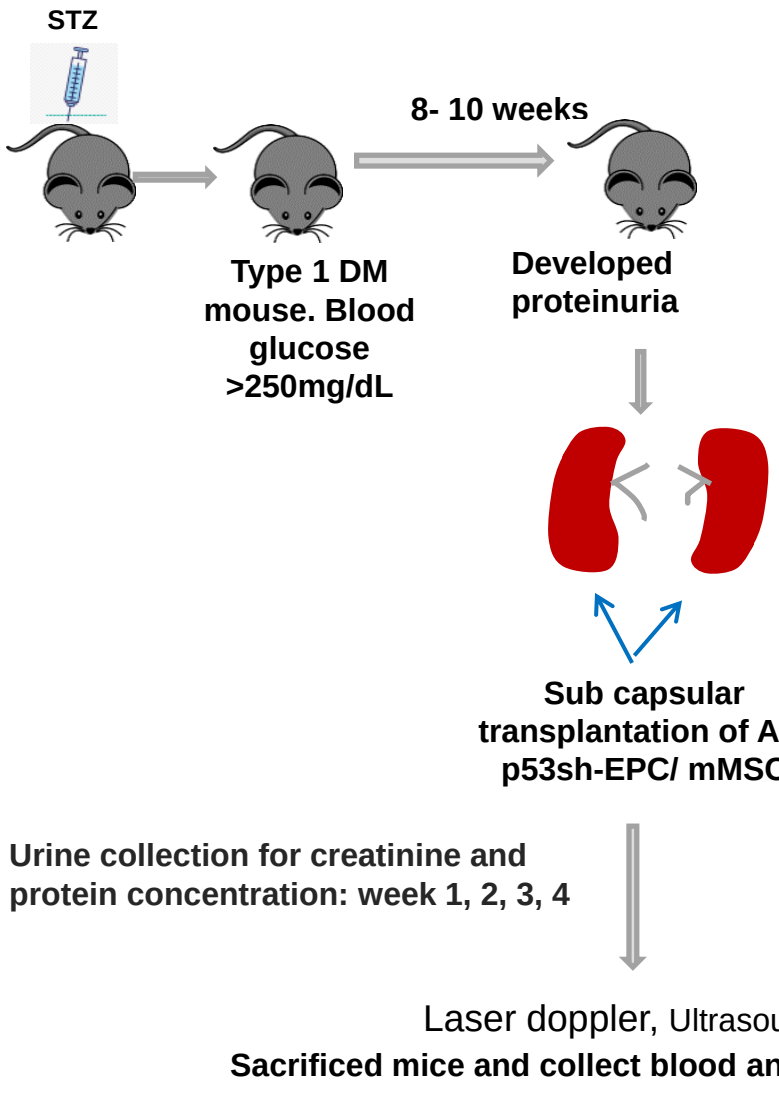
Use of Mesenchymal Stromal Cells (MSC) as a therapy

Mesenchymal stromal cells (MSCs) has multipotent differentiation capacity

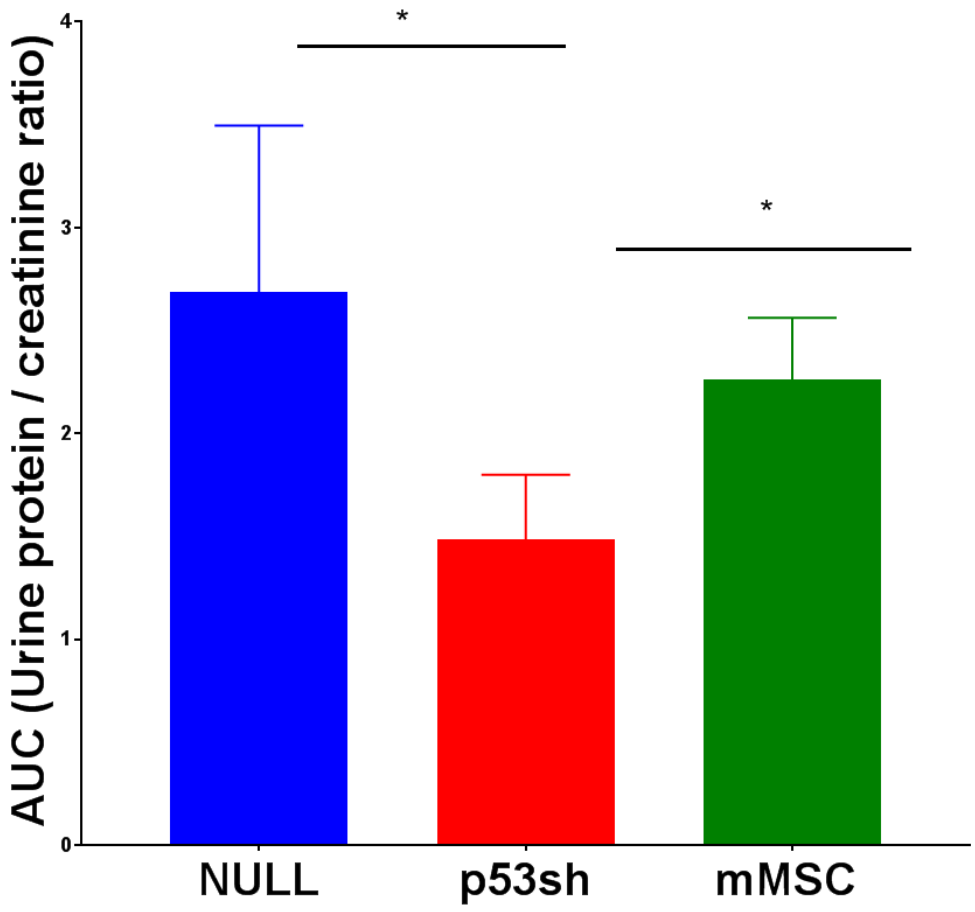
However, epithelial to mesenchymal cell transformation is of concern post MSC therapy



Transplantation of P53-Silenced Endothelial Progenitor Cells (EPCs) Under Renal Capsule

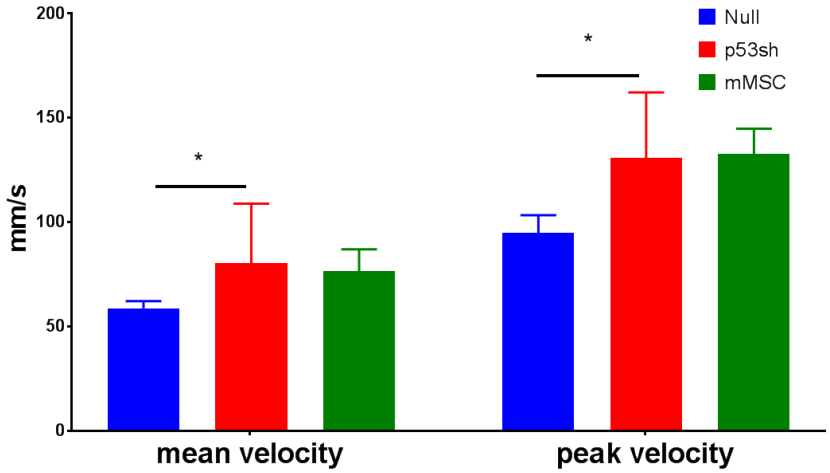


Results

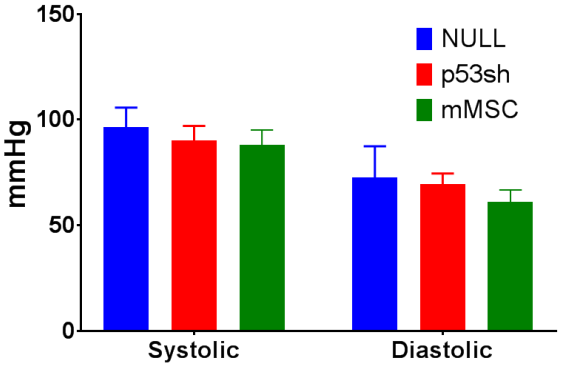


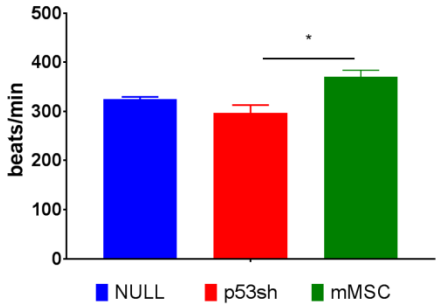
Area under the curve (AUC) of urine protein/ creatinine concentration ratio decreased with p53sh EPC transplantation

Results



Ultrasound analysis (Visual Sonics)

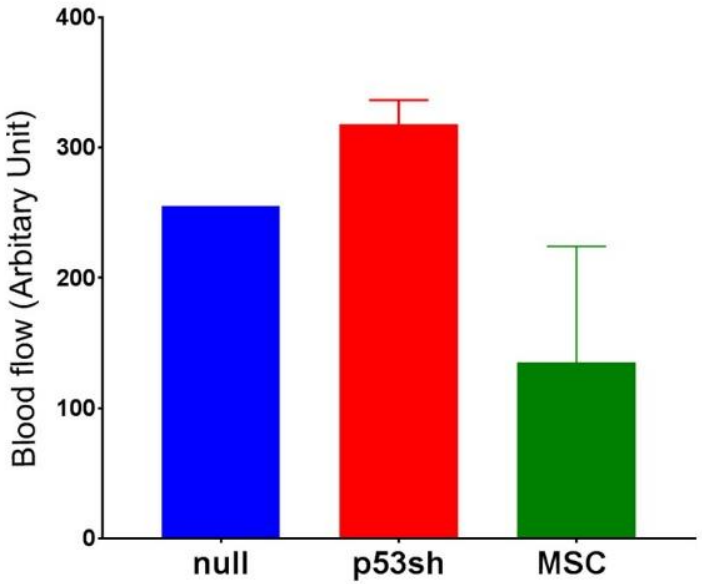




Measurements of blood pressure and heart rate

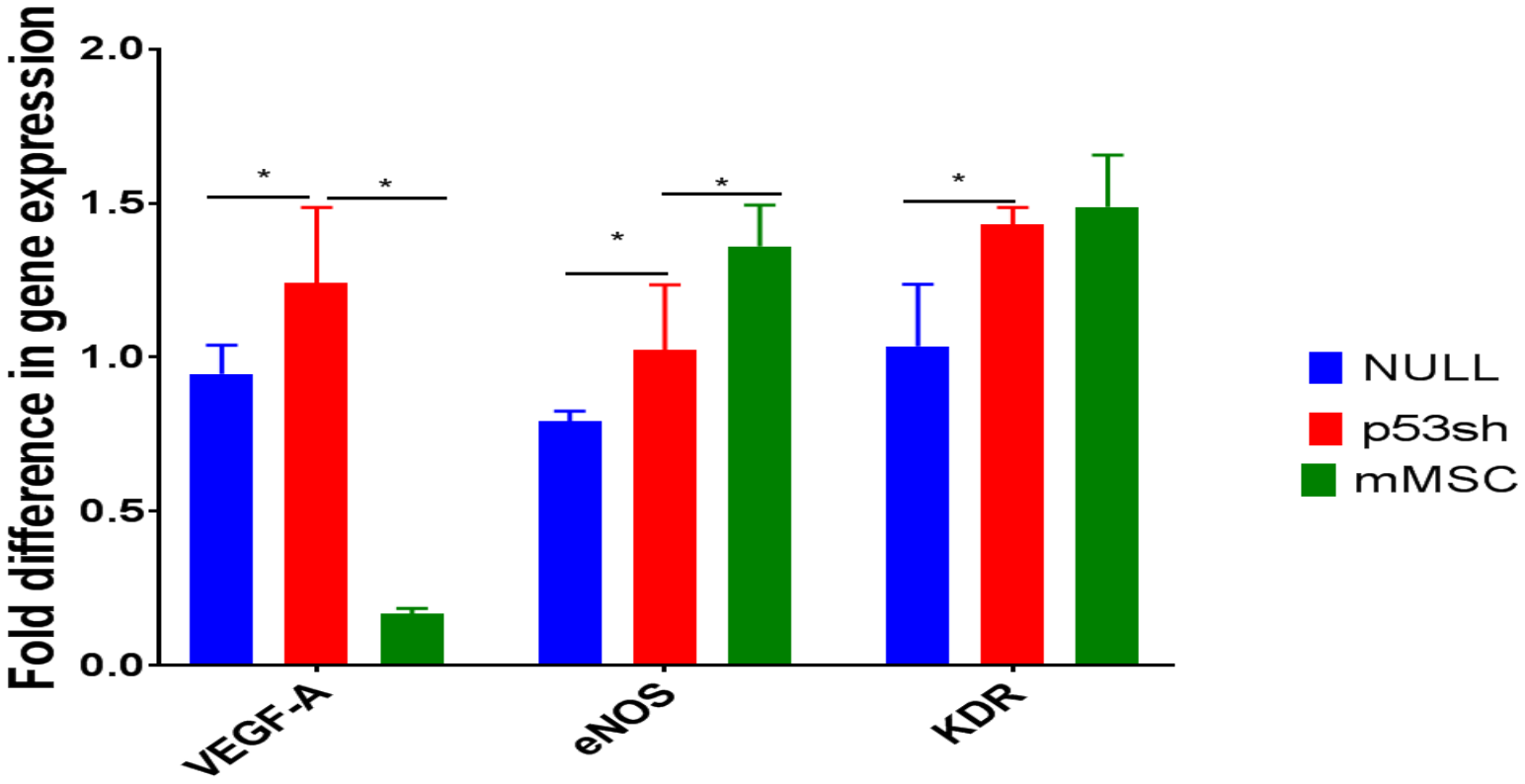
Blood flow (perfusion) in
transplanted kidney increased with p53sh EPCs

Blood flow (perfusion) in transplanted kidney increased with p53sh EPCs



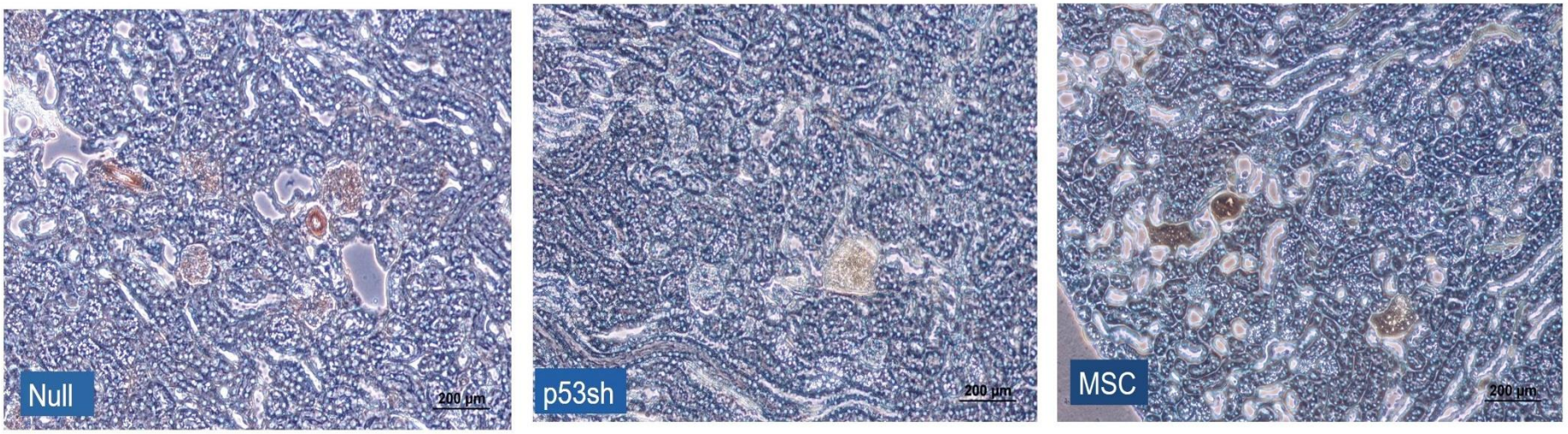
Laser doppler analysis

Results



Gene expression analysis showed markers of angiogenesis upregulated with p53sh EPC transplantation

Histology



Renal Histology: H&E AND Isolectin-β4 staining showing improved glomerular architecture, with p53sh EPC delivery

- Transplanting p53 silenced mouse EPCs under renal capsule improves proteinuria, renal blood flow and improves renal histology.

- Transplanting p53 silenced mouse EPCs is more beneficial compared to MSC transplantation, taking all outcome measurements into consideration

Conclusion

- Modified EPC transplantation may have a prominent cell therapy role in Diabetic Vascular Complications such as DKD.

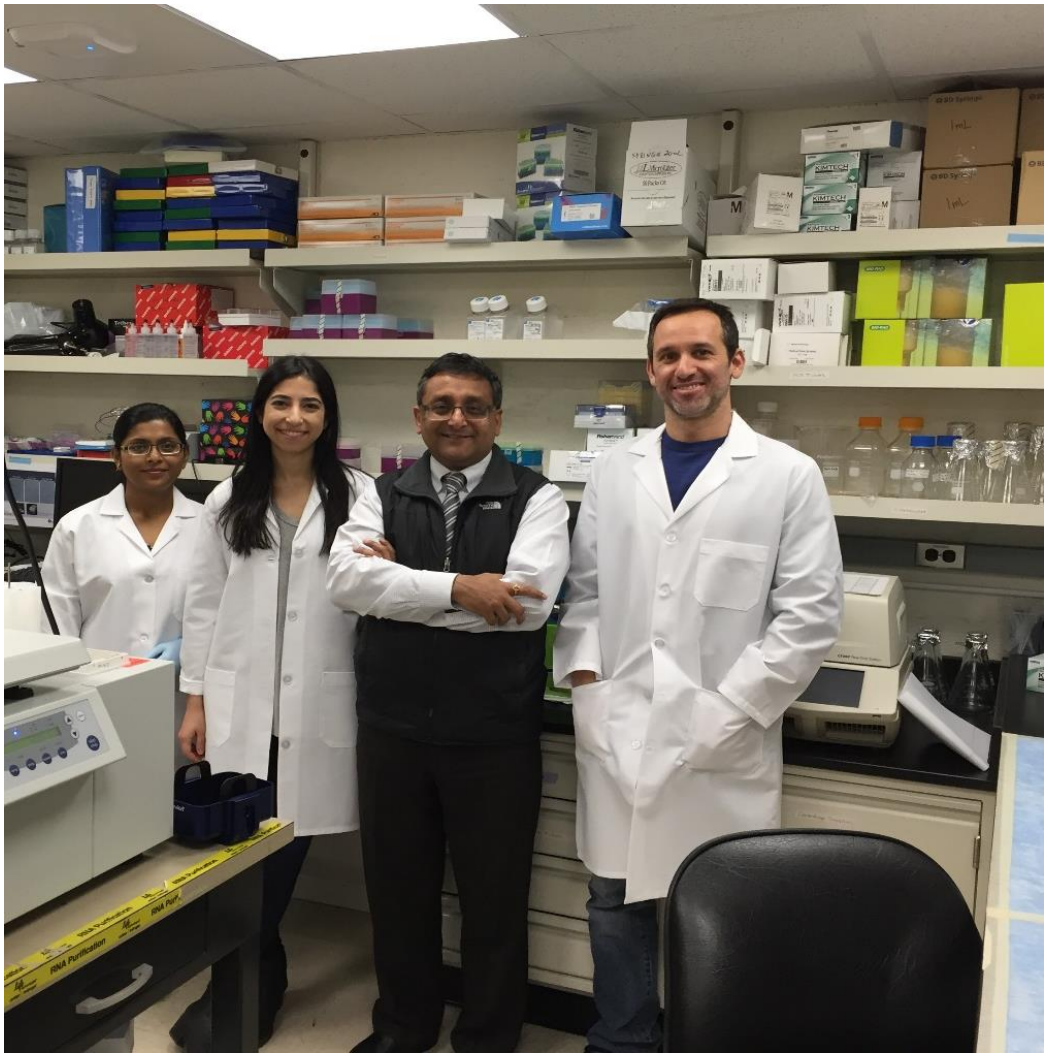
Stem Cells - Is it the Holy Grail (Chalise of Life) for Kidney Regeneration?

The Damsel of the Sanct Grael **by** **Dante Gabriel Rossetti**

Some Progress but Lots more to achieve in order to understand stem cell behavior in Hyperglycemia

The process of scientific discovery is, in effect, ***a continual flight****….. from wonder.* …….**Albert Einstein**

Team



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Kristina Rother, NIDDK
Colin Young, Pharmacology-Physiology, GW
Paul Marvar, Pharmacology-Physiology, GW
Vivek Jain, Pulmonary Medicine, GW

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