THE PATHOPHYSIOLOGY OF VASCULAR CALCIFICATION

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DISCLOSURES

• None
PRESENTATION OVERVIEW:

• Vascular smooth muscle cell-mediated calcification
  – VSMC phenotypes
  – Setting in which it occurs
• Processes that promote or drive vascular calcification
  – Hyperphosphatemia, hypercalcemia
  – Loss of inhibitory mechanisms
  – Oxidative stress
• How CKD specifically leads to vascular calcification
• Are patterns of coronary and/or peripheral calcifications – in a clinical and/or pathophysiological way - different in CKD-patients?
Vascular calcification occurs at two sites:

- **MEDIAL**
  - Diabetes
  - Renal Disease
  - Ageing
  - Stiffness

- **INTIMAL**
  - Atherosclerosis
  - Plaque Rupture

- Major cause of cardiovascular mortality in CKD
- Increased risk of myocardial infarction and all cause mortality
- Surgical complications and amputations
- Valve calcification
Vascular Calcification is a Cell-Mediated Process similar to bone calcification
Mechanisms of Vascular Smooth Muscle Cell Calcification

CKD
Diabetes
Ageing
ROS?

DAMAGE/STRESS
LOSS OF INHIBITORS
OSTEOGENIC DIFFERENTIATION
CELL DEATH/VESICLE RELEASE
MINERALIZATION

Shanahan, C. M. (2013)
Ageing is the Strongest Risk Factor for Defects in Kidney-Bone-Vascular Axis Tissues

- Elevated Phosphate/FGF23/Klotho
- Low Vitamin D
- DNA damage
- Oxidative Stress
- Systemic Inflammation
Inhibitors Prevent Vascular Calcification

Mouse gene knockouts develop vascular calcification and bone defects (eg. osteoporosis).

- MGP (matrix Gla protein)
- Fetuin**
- Osteoprotegerin
- Klotho/ FGF23** - Phosphate and Vit D metabolism – premature ageing
- Pyrophosphate metabolism (ENPP1)
- carbonic anhydrase
- Smad 6

Luo et al Nature 1997
Incidence of Vascular Calcification with Age

Cardiovascular mortality in CKD patients

Adolescents and young adult with CKD:
- structural and functional abnormalities in the large vessels
- present even in the second decade of life
- linked to disorders in mineral metabolism

Adapted from Foley et al. Am J Kid Dis 1998

Goodman, NEJM, 2000; Litwin, JASN, 2005; Mitsnefes, JASN 2005; Goldsmith, NDT, 2006
GFR

Renal osteodystrophy

Vascular insults

Disease

- ↑Calcium
- ↑Phosphate
- ↑Ca x PO4
- Oxidized lipids
- Inflammation
- Hypertension
- PTH
- Advanced glycation end-products

VSMC Damage

Treatment

- Vitamin D
- Calcium-based phosphate binders
- Warfarin

- Time on dialysis
- Pre-existing vascular calcification (once present rapidly progresses)
Vessel Rings from Children *in vivo* and *ex vivo*

Studied vessels from children on dialysis who develop rapid medial vascular calcification

- pristine vessels -no atherosclerosis

- Intact - vascular matrix structure maintained

Measured:  
- CALCIUM LOAD
- VESSEL HISTOLOGY

Correlated with:  
- VASCULAR MEASURES
- BIOCHEMICAL DATA
Children on Dialysis develop rapid medial calcification

High Circulating Phosphate Levels, Transient Hypercalcemia?
Calcification correlates with VSMC loss via apoptosis

![Graph showing the number of VSMCs per unit area across Normal, Pre-dialysis, and Dialysis groups.](image)

- **Normal**: n = 4
- **Pre-dialysis**: n = 8
- **Dialysis**: n = 10

*p < 0.001*

**KDIGO**

**α-SM actin**

**TUNEL**
Loss of Calcification Inhibitors
Non-functional Glu-MGP predominates in Dialysis vessels

KDIGO
Dialysis vessels show increased osteogenic differentiation

(Shroff et al 2008, Circulation)
Ca load is associated with increased vesicle deposition by VSMCs

(Shroff et al 2008, Circulation)
Is there Evidence that the VSMCs from Children on Dialysis are Prematurely Aged?

ENVIRONMENTAL TOXINS → DNA DAMAGE → SENESCENCE and CELL DEATH
Vessels from Children on dialysis have increased senescence markers and oxidative DNA damage.

Oxidative DNA damage increases predialysis.

p16 is increased in Dialysis.
Calcium and Phosphate Drive Oxidative DNA damage in Dialysis Vessels
VSMCs from children on dialysis senesce early in culture

SAβG = senescence associated β-galactosidase
Calcium and Phosphate Induce DNA damage

- **γH2AX** and DAPI
- **pATM** and DAPI

No treatment
Ca + P

Graph showing
- % Positive cells
- Baseline, DOXO, H2O2, Ca + P

Bar chart with data points for p ATM / ATR and p H2AX
Phosphate treatment promotes VSMC senescence

Day 0 + P  Day 4 + P

% positive for b-gal

Untreated  Ca  P  CaP

Control

KDIGO

Dialysis

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Senescence Associated Secretory Phenotype (SASP)

Oxidative stress, Ca, P, uraemic toxins

DNA damage

ATM/ATR → DNA repair

Senescence

Senescent VSMC

SASP factors

BMP2, IL-6, OPG

Reinforce growth arrest

Chronic inflammation

Induction of osteogenic differentiation of local VSMCs and stem cells

Shanahan, C. M. (2013)
Array analysis shows VSMCs secrete pro-osteogenic cytokines in response to prelamin A accumulation.

Drugs that block the DDR reduce secretion of SASP factors and calcification.
VSMCs from children on dialysis have increased levels of DNA damage and osteogenic differentiation *in vitro*

Pilar Sanchis et al, KI 2019
ATM inhibition blocks calcification and inflammation in VSMCs from Children on Dialysis

Control

Dialysis

Baseline        Ca/P        Ca/P + iATM

KDIGO
BMP2, IL6 and OPG are elevated in children on dialysis and correlate with vascular stiffness.
Circulating cytokines were significantly increased in children with calcification.

\[ p < 0.0001 \]
Tissue ageing is driven by DNA damage and inflammatory mediators released from senescent tissues.
Progressive Development of Aberrant Smooth Muscle Cell Phenotype in Abdominal Aortic Aneurysm Disease

Kirsten Riches, Emily Clark, Rebecca J. Helliwell, Timothy G. Angelini, Karen E. Hemmings, Marc A. Bailey, Katherine I. Bridge, D. Julian A. Scott, Karen E. Porter

Abstract

Abdominal Aortic Aneurysm Disease

Keywords

Objective:

In this study, we investigated the role of SIRT1 (Sirtuin 1), a class III histone deacetylase, in AAA formation and the underlying mechanisms linking vascular senescence and inflammation.

Methods and Results:

Increased DNA Damage and Senescence in AAA

Age-Associated Sirtuin 1 Reduction in Vascular Smooth Muscle Links Vascular Senescence and Inflammation to Abdominal Aortic Aneurysm

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Association of relative telomere length with cardiovascular disease in a large chronic kidney disease cohort: The GCKD study

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1. Introduction
Chronic kidney disease (CKD) is a highly prevalent disease. Data from the United States and Europe report about 10\textup{e}15\% of the general population have signs of impaired kidney function or structure. This prevalence increases even to 50\% in high-risk subpopulations [1,2]. Patients with CKD are at high risk for cardiovascular disease (CVD). This risk increases with decreasing glomerular filtration rate (GFR) and increasing albuminuria. In patients with advanced CKD the risk for total and cardiovascular mortality[3,4] is higher than the risk for kidney failure requiring dialysis. The high prevalence of CVD in CKD patients can only partly be attributed to traditional CVD risk factors. The CKD phenotype is characterized by age-related characteristics such as atherosclerosis,
Diabetes is Associated with a high Prevalence of Vascular Calcification in Peripheral Arteries

Peripheral Artery Calcification in Diabetes

Associated with increased CV mortality, amputation and ulcers, surgical complications
Mechanisms of Calcification in Diabetes/Charcot Foot

- Diabetes
  - Vascular Calcification
  - Neuropathy
    - Oxidative stress
      - Premature Ageing
    - Inflammation
      - Osteogenesis
    - AGE/RAGE
      - Matrix change + osteogenesis
Neuropathy as a Novel Cause of Vascular Calcification in the Extremities

Mechanisms Unknown:
Loss of Trophic Factors?
Calcium Metabolism?