Molecular Mechanism of Vascular Calcification

2010. 4. 17.

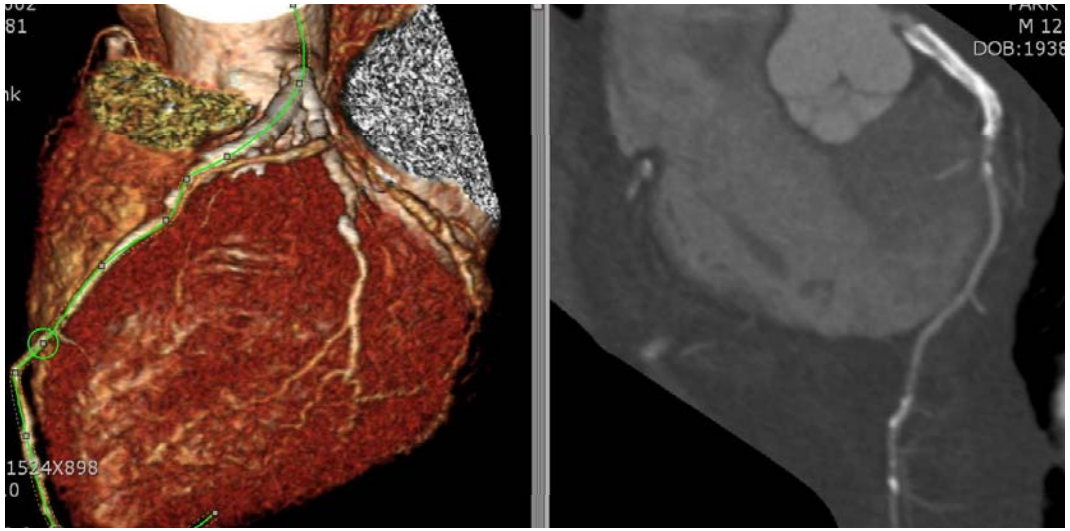
조 현 재

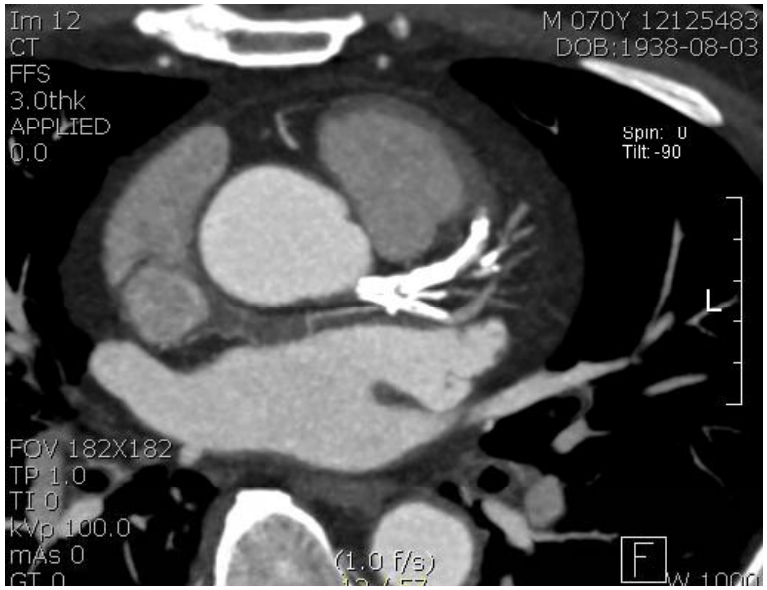
서울대학교병원 순환기내과, 심혈관연구실,
서울대학교병원 심혈관센터

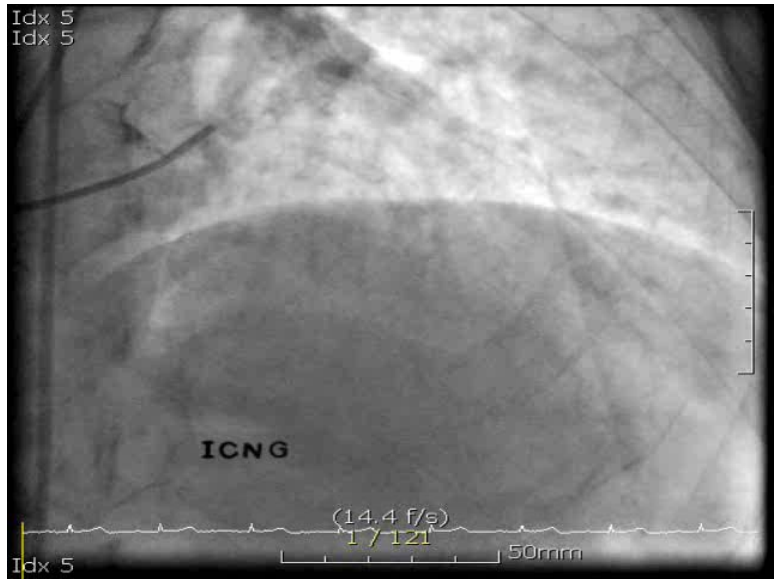
서울대학교병원
SEOUL NATIONAL UNIVERSITY HOSPITAL

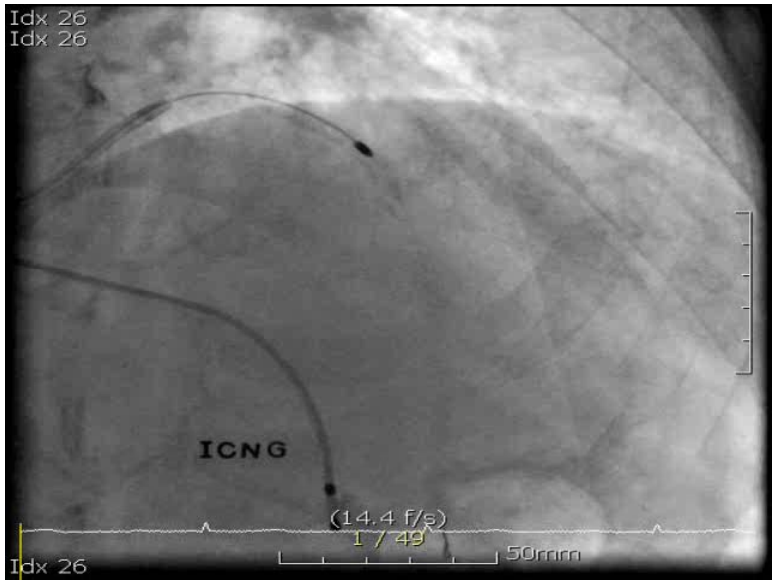
IRICT
Innovative Research Institute for Cell Therapy

Vascular calcification : clinical hurdles









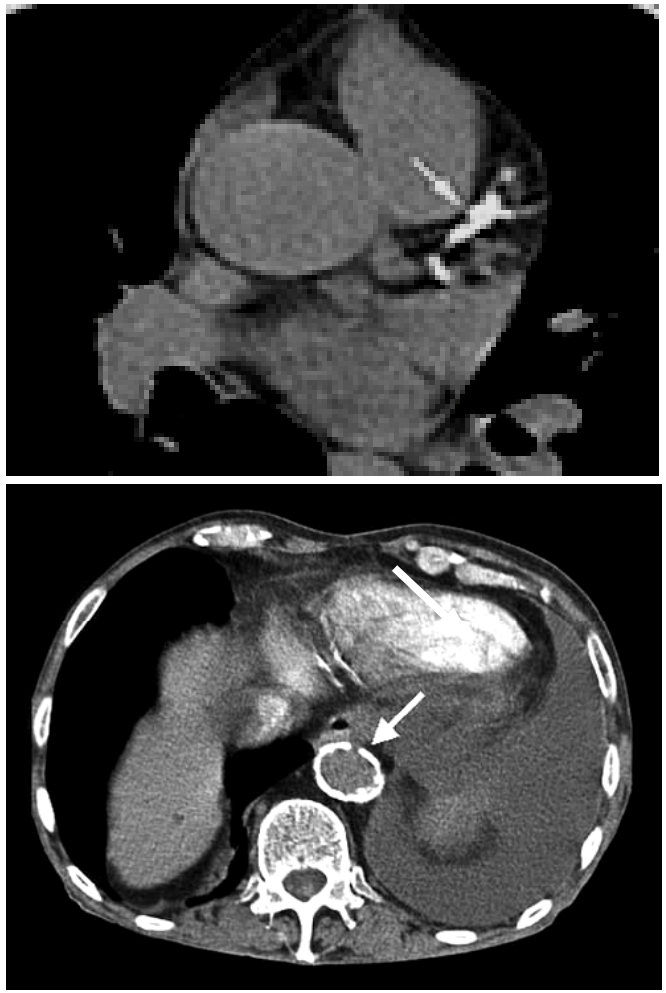
Vascular calcification : clinical significance !

- Clinical consequences in atherosclerosis
- Diabetes, and end-stage renal disease
- Prognostic indicator of future adverse cardiovascular events
  - Increased CAC correlates severity of CAD

    Paolo R et al, Circulation 2000;101:850-855

  - Increased rate of progression of CAC correlates adverse cardiac event

    Schmermund A et al, Cardiol Clin 2003;21(4):521-34



CAC score: coronary artery calcium score, CAD :coronary artery disease

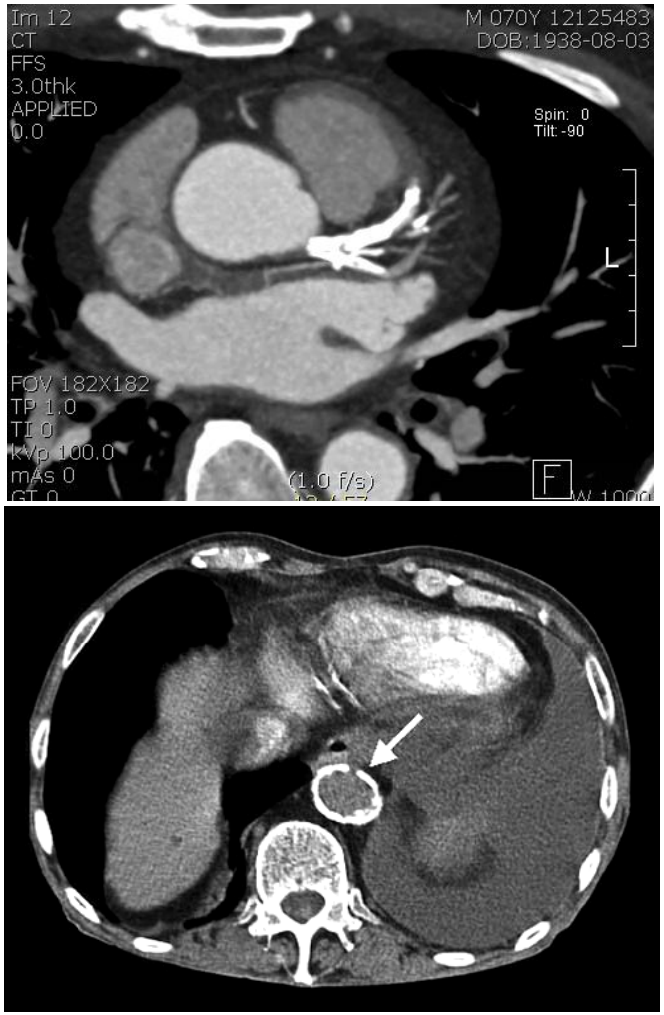
Vascular calcification : current concept

Passive consequence of aging

Actively regulated process

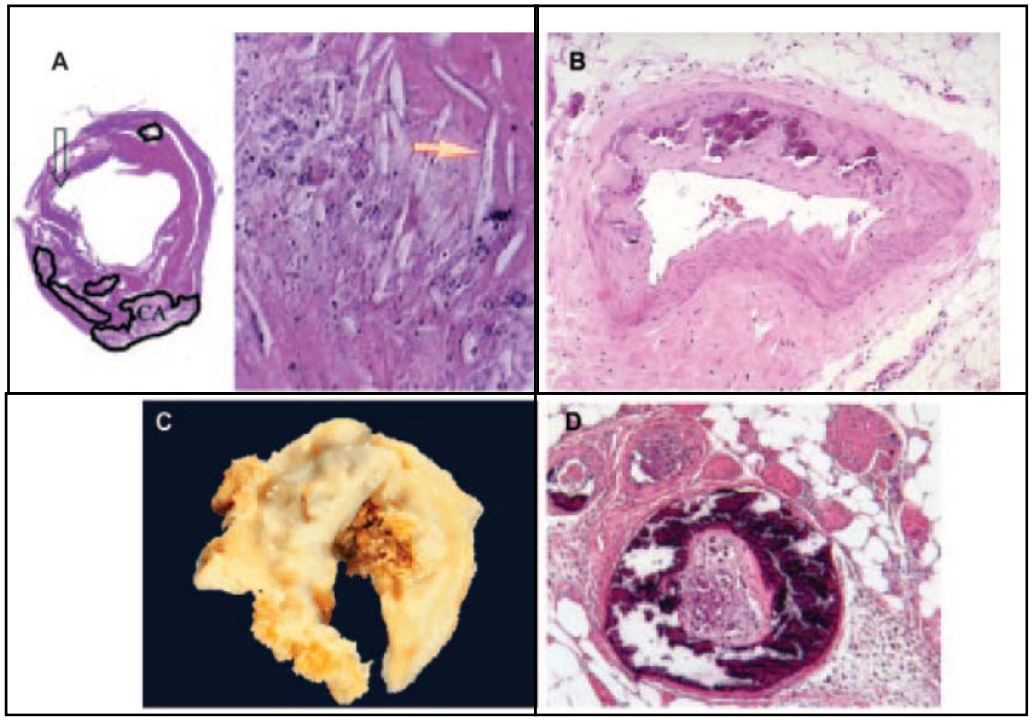
resembling the mineralization

of endochondral and membranous bone



Future therapeutic strategy !

Vascular calcification: type



(A) Atherosclerotic calcification

- intimal and subintimal calcification

(B) Medial calcification

- diabetes, ESRD, non-atherosclerotic?

(C) Valvular calcification

(D) Calciphylaxis

- calcific uremic arteriopathy

Johnson RC *et al . Circ Res.* 2006

Vascular calcification : type

A. Atherosclerotic Calcification

- focal coronary calcification : plaque rupture, coronary dissection under PCI

B. Medial Artery Calcification

- increased arterial stiffness, PWV, Pulse pressure
- strong correlation with CAD, future CV event in CKD/DM, future amputation

C. Cardiac Valve Calcification

- main mechanism of valve failure
- correlate with stroke, atrial fibrillation ,CV event

D. Calciphylaxis

- 1 % of hemodialysis patient
- grave prognosis, high mortality due to tissue necrosis

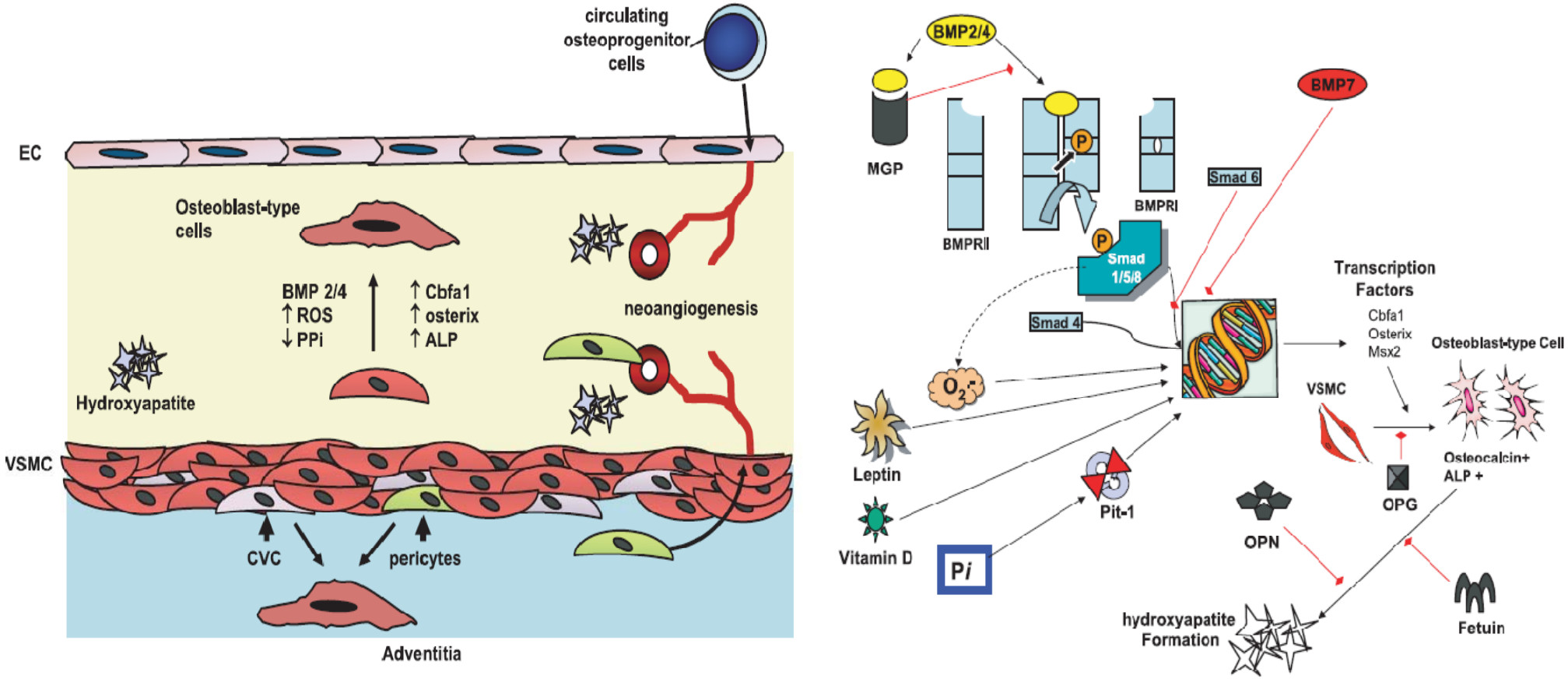
CONTENTS

Cellular determinants – origin of calcifying cells

Origin of osteoblastic or osteogenic cells in vasculature is a great topic of debate.

- vascular smooth muscle cell (VSMC): contractile → proliferative, synthetic form
- calcifying vascular cell (CVC): may be a certain clone or subpopulation of VSMC
- pericyte: a kind of mesenchymal progenitors
- mesenchymal stem cell (circulating or resident), adventitial myofibroblast (resident ?) and fibrocyte (circulating)
- calcifying vascular progenitor cells (circulating, resident)

Cellular determinants – origin of calcifying cells



Cbfa1, Osterix, Msx: transcription factor / ALP, Osteocalcin: functional phenotypic marker

BMP 2/4: osteogenic cytokine

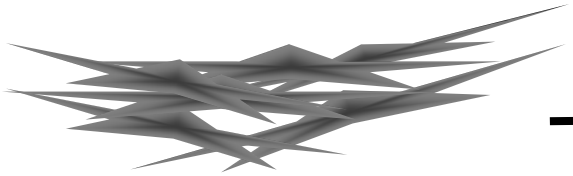
Johnson RC *et al* . *Circ Res.* 2006

Cellular determinants – origin of calcifying cells

VSMC preparation

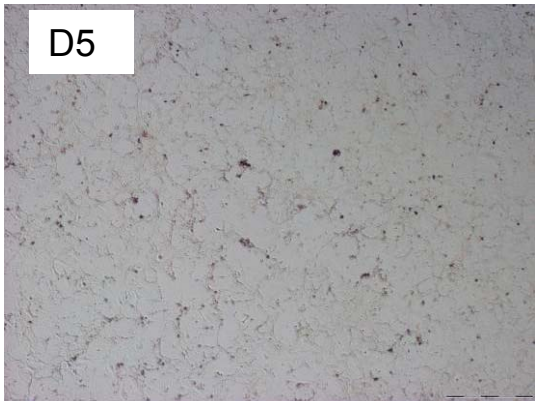
C57BL6 mice

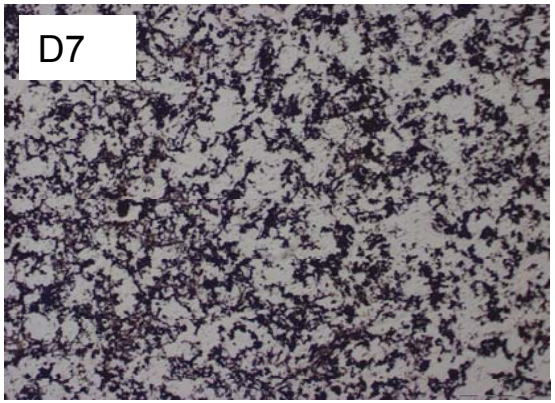
Aorta

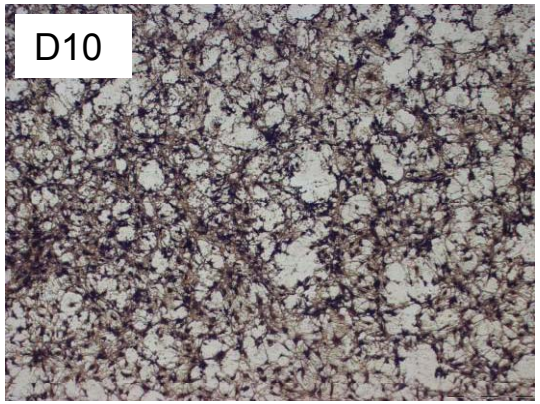


VSMC Passage 6~7

6 well plate







Von Kossa stain

Calcification media : DMEM, 15% FCS, CaCl2 6mM, β-glycerophosphate 10mM

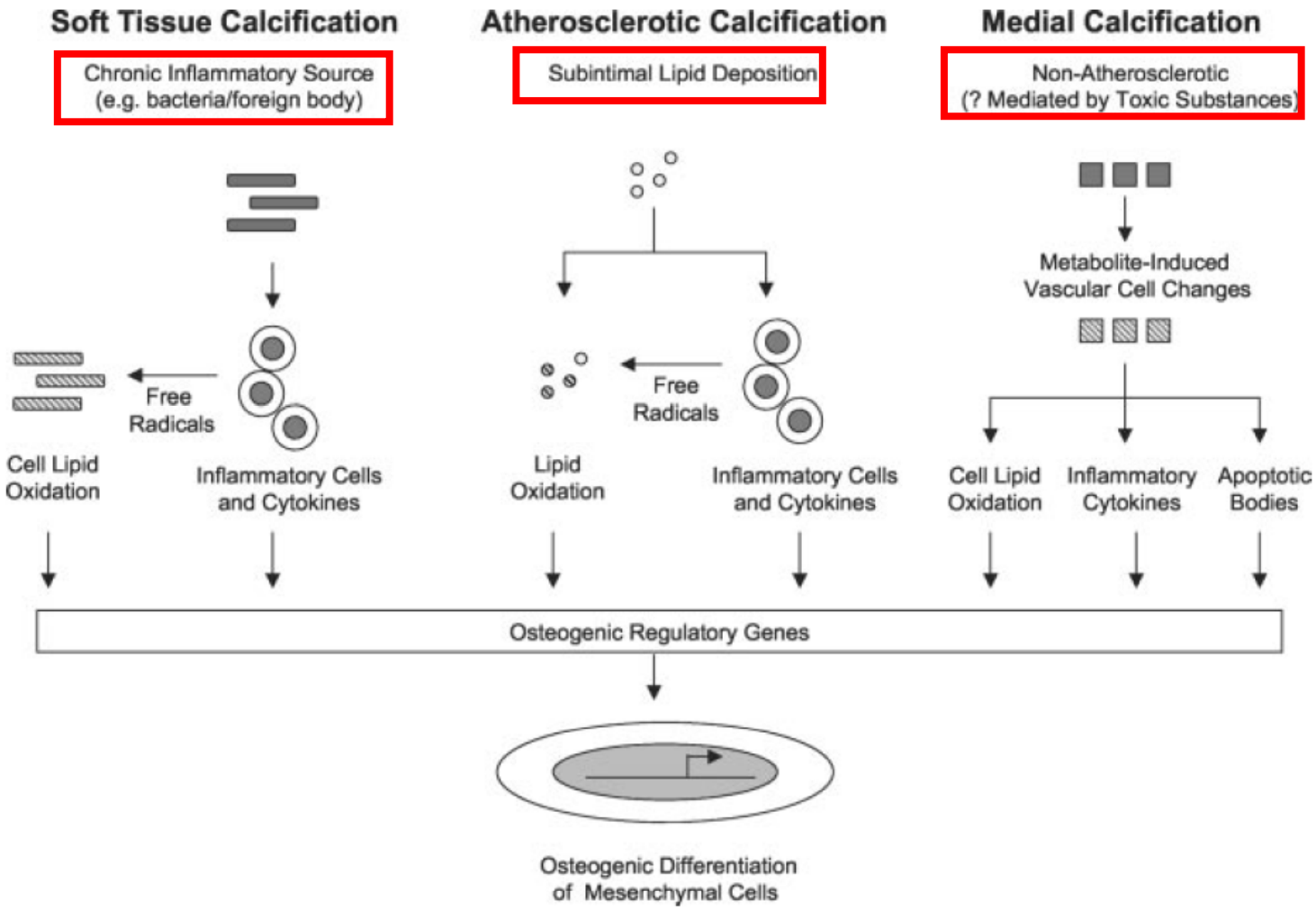
Yoon YE *et al . Unpublished data*

Molecular determinants and inducers (or inhibitors)

1) Inflammatory signal: Oxidized LDL, TNF-α and oxidative stress, etc
2) BMP-2
3) Msx2
4) Runx2/Cbfa1 (core binding factor α1)
5) Osteoclacin
6) Alkaline phosphatase (ALP)
7) RANK/RANKL/Osteoprotegerin (OPG)
8) Osteopontin (OPN)

*Angiotensin II

Inflammatory signal and chronic inflammation

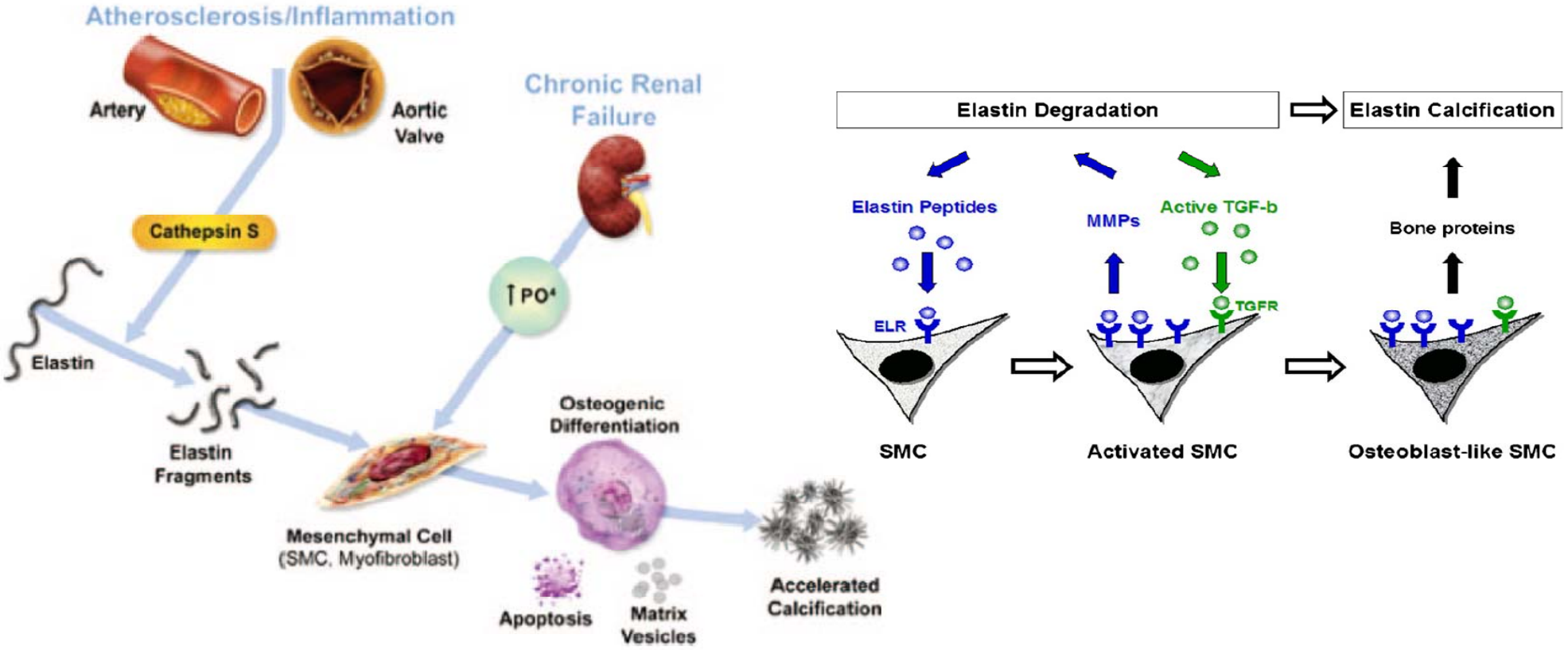


Parallel mechanisms in soft tissue versus atherosclerotic and non-atherosclerotic vascular calcification

Abedin M. et al ATVB. 2004

Inflammatory signal and chronic inflammation

Inflammation → plaque macrophage-derived proteinases [MMP-2/9, Cathepsin B/S etc.]



1. Release of biologically active, soluble elastin-derived peptides: promote osteogenic differentiation of VSMCs
2. Degraded medial elastin: favors calcification through an increase of elastin polarity that in turn enhances elastin affinity for calcium

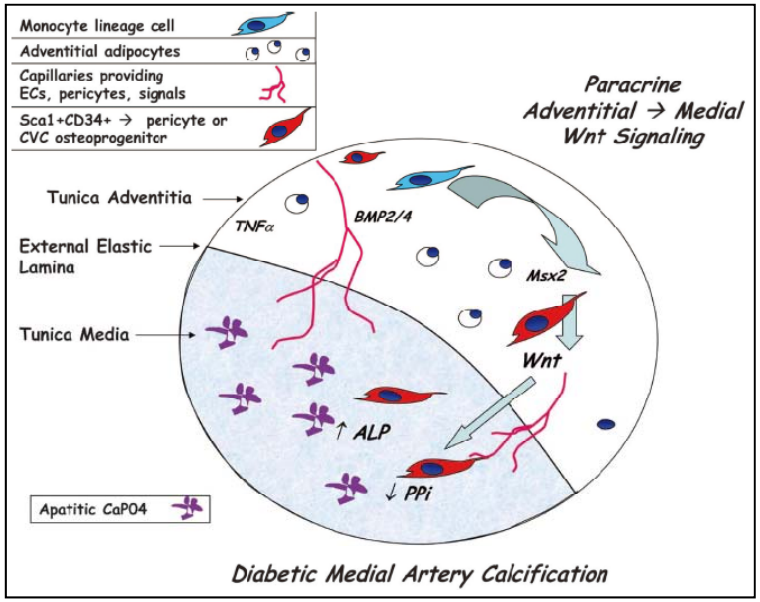
Simionescu A et al BBRC 2005 / Aikawa E. et al Circulation 2009

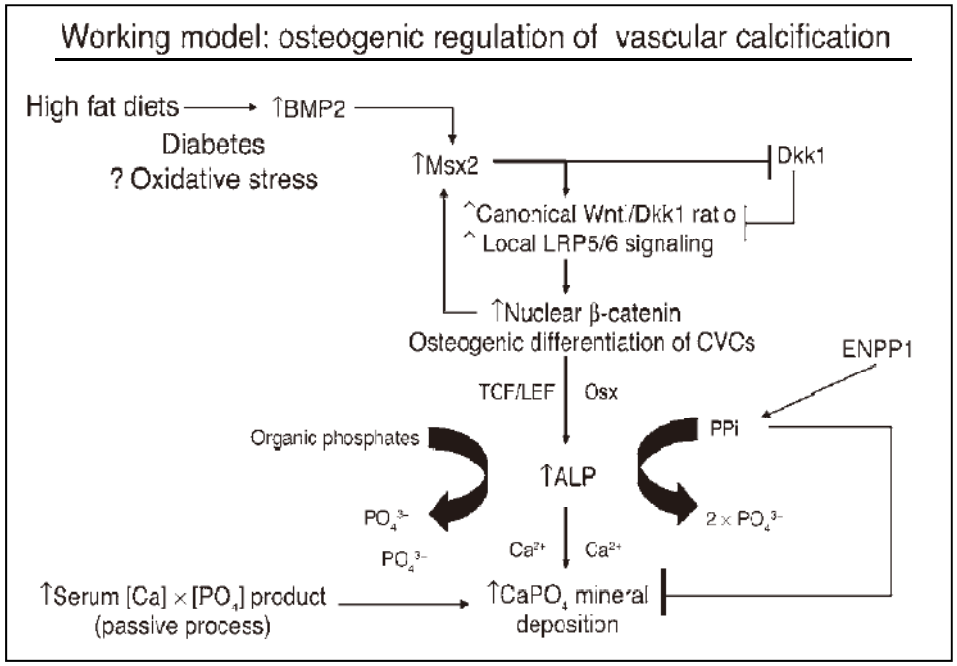
BMP-2 (bone morphogenetic protein 2)

- the largest subclass of the TGF-β superfamily
- a well-known **inducer** for bone and cartilage formation
- expressed by a variety of cells in atherosclerotic lesions, including endothelial cells, foam cells, and VSMCs.
- BMP-2 and BMP-4: both mineralization and local induction of inflammation
- BMP-7: induce osteogenic potential, but retard vascular calcification.

Msx2

- a BMP-2-inducible transcription factor
- a controller of craniofacial mineralization
- BMP-2/Msx/Wnt signaling cascade is upregulated in the context of type 2 diabetes, obesity and hypercholesterolemia
- plays a key role in early stages of medial calcification.





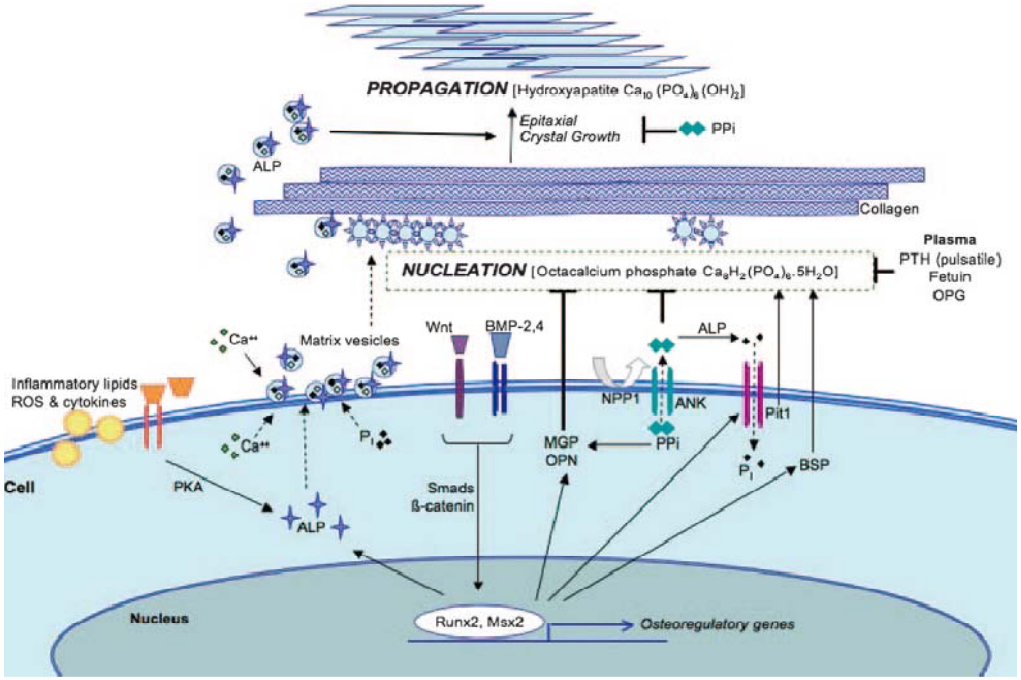
Expression of Msx2 in adventitial cells

Shao JS *et al* . JCI. 2005

Shao JS et al ATVB. 2006

Runx2/Cbfa1 (core binding factor α1), Osx (osterix)

- master regulator of osteoblastic differentiation
- a key transcription factor of various osteogenic and osteoblastic differentiation related genes (osteoclacin, type I collagen, bone sialoprotein, alkaline phosphatase)
- the full effect of Runx2/Cbfa1 is required activation of a downstream transcription factor, Osterix.
- Runx2/Cbfa1 expression in VSMCs serves as an early, definitive marker of osteoblastic differentiation and the initial step in vascular calcification.



BSP (bone sialoprotein), OPG (osteoprotegerin), OPN (osteopontin), OCN (osteocalcin)

Demer LL *et al* . Circulation. 2008

Osteocalcin and alkaline phosphatase (ALP)

Osteoclacin:

1) most osteoblast specific gene. noncollagenous protein
2) secreted by osteoblast
3) clinically used as biomarker for the bone formation process

Alkaline phosphatase (ALP):

1) a functional phenotypic marker of osteoblasts
2) enzyme that induces tissue biomineralization.
3) ALP degrades inorganic pyrophosphate as a necessary step of calcification and ALP activity is crucial to hydroxyapatite formation.
4) ALP activity is often used as a molecular marker for vascular calcification, as it is an early indicator of ECM deposition.

Osteocalcin and alkaline phosphatase (ALP)

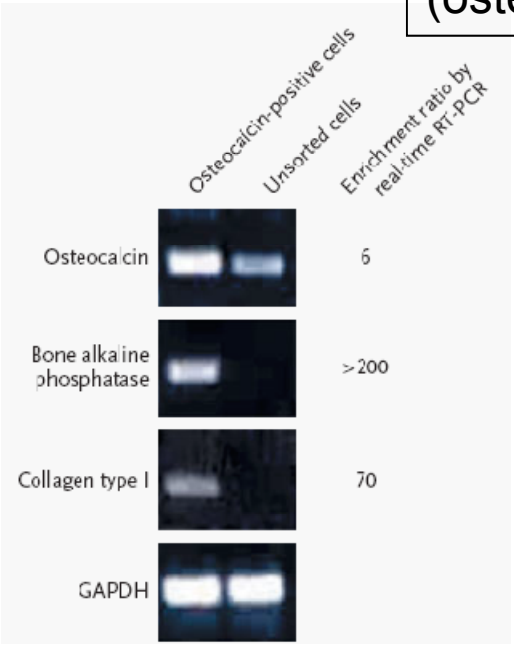
The NEW ENGLAND JOURNAL of MEDICINE

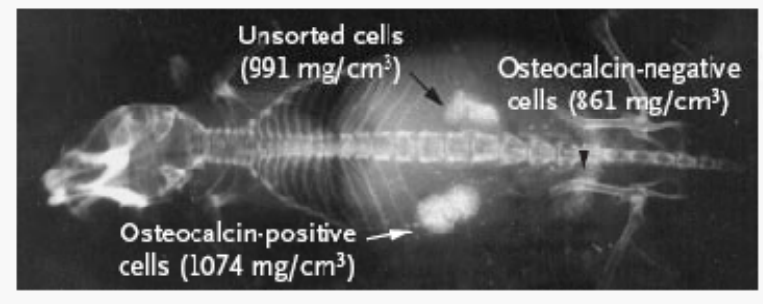
Circulating Osteoblast-Lineage Cells in Humans

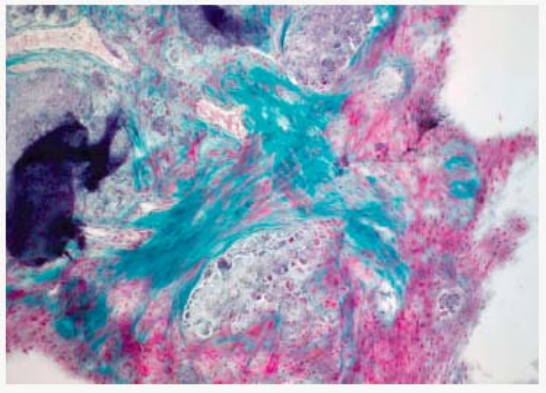
Guiti Z. Eghbali-Fatourechi, M.D., Jesse Lamsam, M.S., Daniel Fraser, Ph.D., David Nagel, A.B., B. Lawrence Riggs, M.D., and Sundeep Khosla, M.D.

N Engl J Med 2005;352:1959-66.

FACS using antibodies to bone-specific proteins (osteocalcin and alkaline phosphatase)

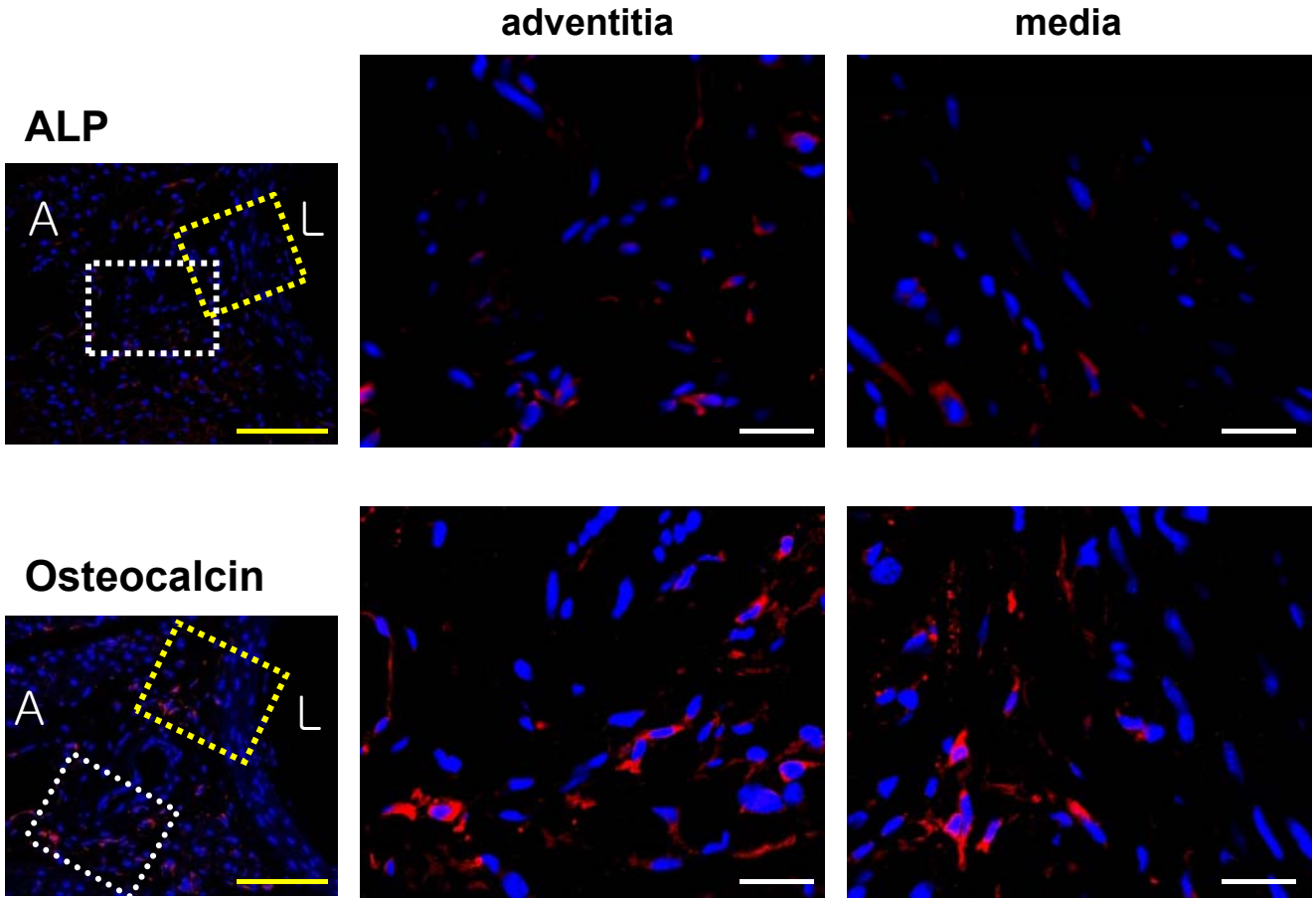






cells were implanted into immunocompromised mouse histologic sections

Osteocalcin and alkaline phosphatase (ALP)

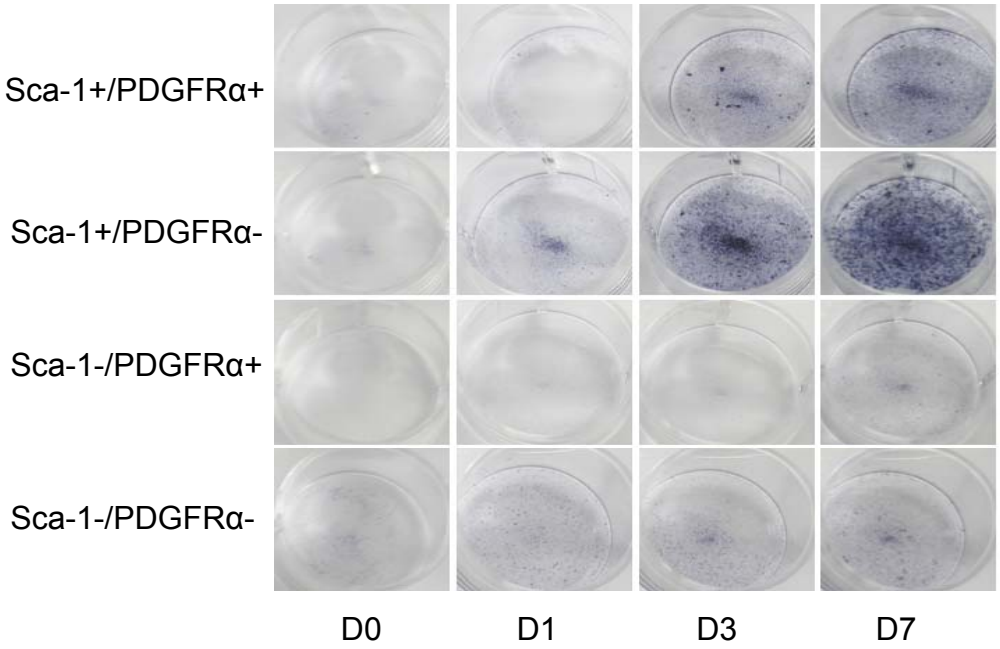


Calcifying progenitor cell in the vasculature

Cho HJ *et al . Unpublished data*

Osteocalcin and alkaline phosphatase (ALP)

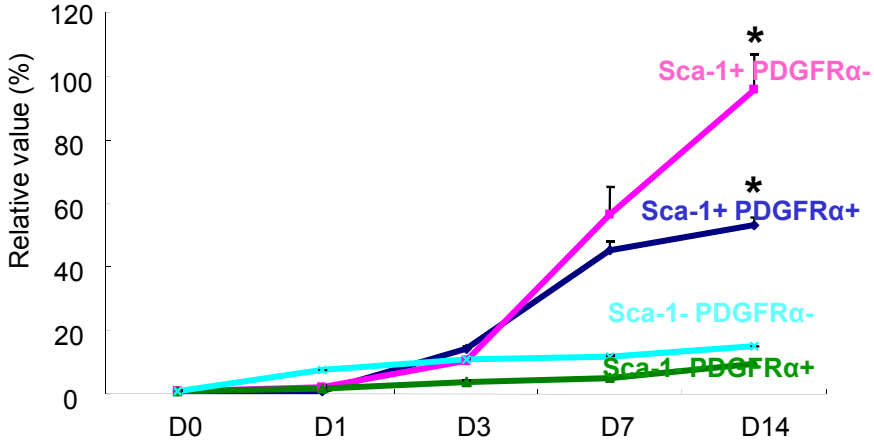
ALP staining



Charcoal stripped FBS 10%

ALP activity

(cell lysate, normalized by protein conc.)



Calcifying progenitor cell in the vasculature

Cho HJ *et al . Unpublished data*

Osteopontin (OPN)

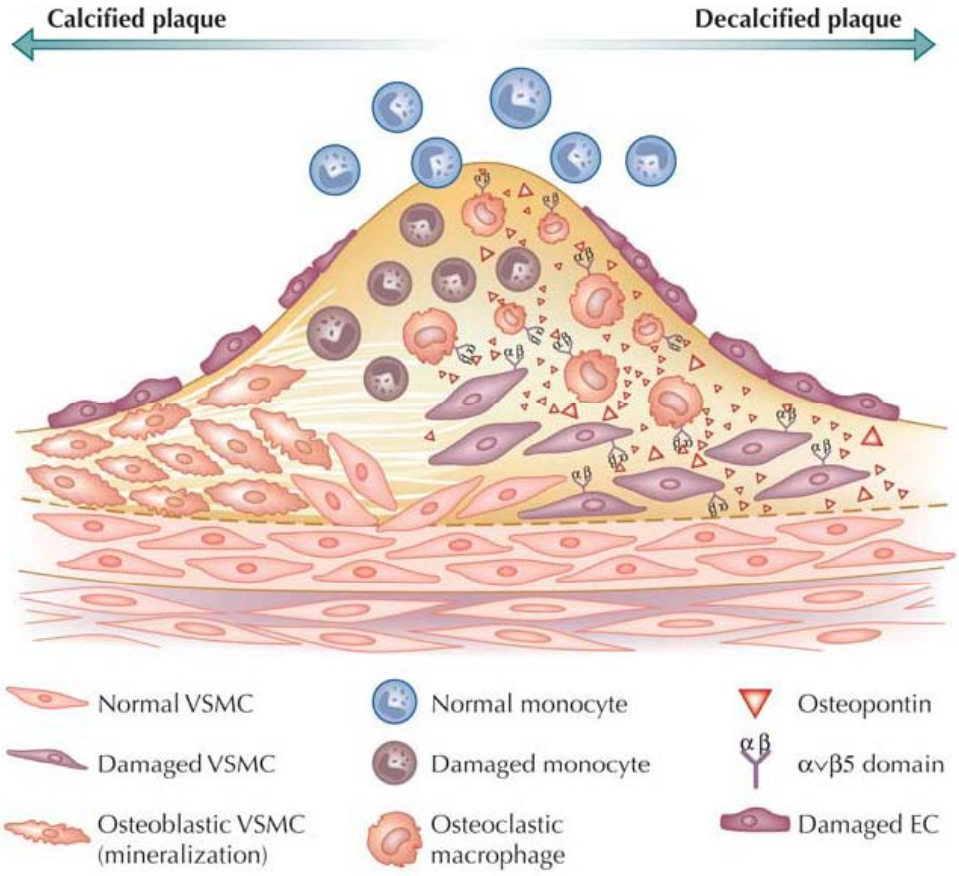
❖ highly expressed at sites with atherosclerotic plaques, especially those associated with macrophages and foam cells.

❖ pro-inflammatory and pro-atherogenic molecule ??, Feedback mechanism?

❖ a negative regulator in vascular calcification: an inhibitor of calcification and an active inducer of decalcification.

OPN-TG mice: atherogenic
OPN-KO mice: less atherogenic,
high vascular calcification

→ OPN expression, function, and regulation are not clearly understood in the context of atherosclerosis and vascular pathophysiology.



Cho HJ *et al* . Curr Atheroscler Rep. 2009

RANK/RANKL/Osteoprotegerin

Key regulator of bone metabolism

- ❖ Receptor Activator of Nuclear factor-κB (RANK): expressed on osteoclast
- ❖ RANK ligand (RANKL): from osteoblast, osteoclastogenesis stimulatory factor
- ❖ Osteoprotegerin (OPG): 1) a member of TNF receptor superfamily

  2) produced by various cells (EC, VSMC, osteoblast, dendritic cell)

  3) soluble receptor: competing with RANK, neutralizing RANKL, preventing RANKL-RANK interaction→ osteoclastogenesis inhibitory activity

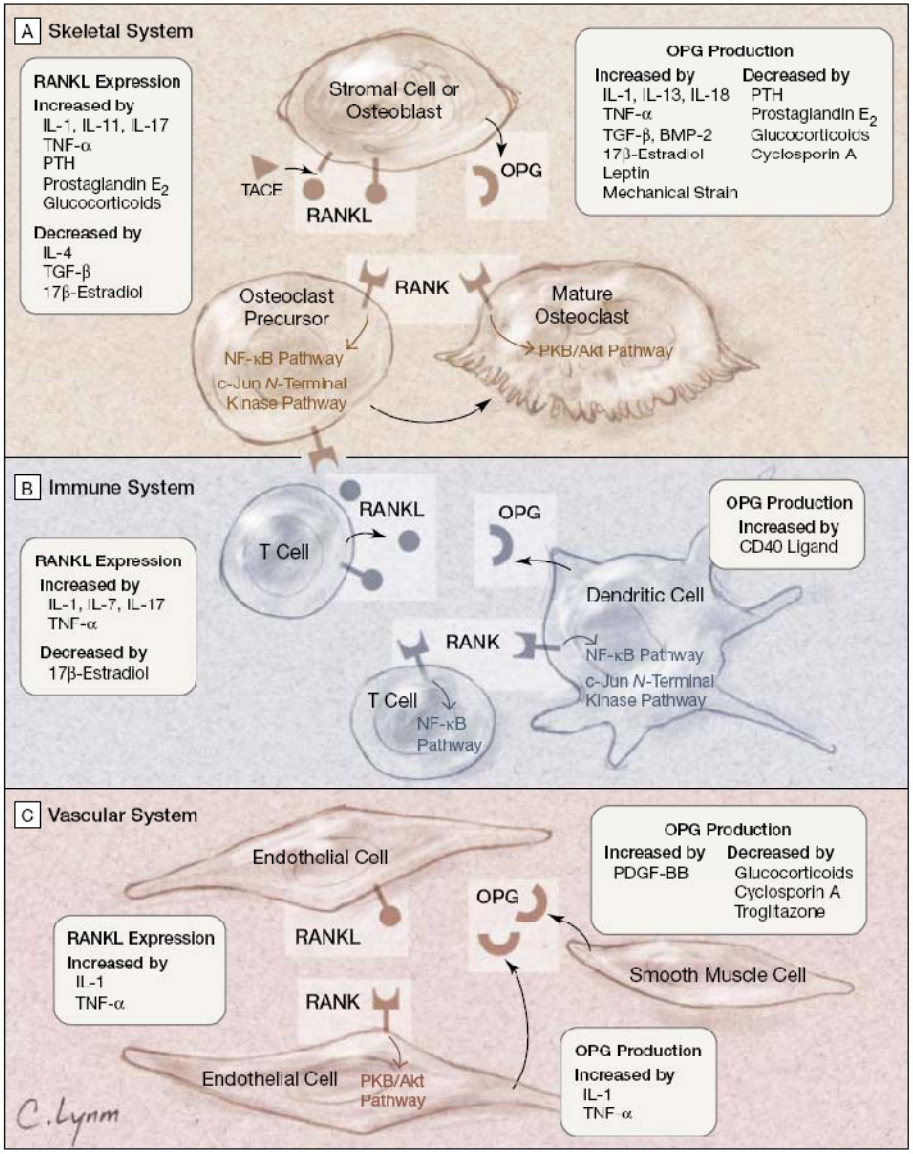
  4) autocrine EC survival factor

- ➢ OPG-TG / RANKL-KO / RANKL-KO mice: osteopetrosis
- ➢ OPG –KO mice: osteoporosis, arterial calcification

→ OPG: protective factor for bone and vascular system: mechanism ??

- ➢ Elevated serum OPG: high cardiovascular mortality, high osteoporosis

RANK/RANKL/Osteoprotegerin



Hofbauer LC *et al . JAMA.* 2004

Molecular determinants and inducers - summary

1) Inflammatory signal: pro-calcific environment

2) BMP-2: strong inducer for bone formation

3) Msx2: BMP-2-inducible transcription factor in medial calcification

4) Runx2/Cbfa: osteoblast-specific transcription factor

5) Osteocalcin: osteoblast-specific marker

6) Alkaline phosphatase (ALP): functional osteoblast phenotypic marker

7) RANK/RANKL/Osteoprotegerin (OPG): balancing factors in osteoblast and osteoclast & osteoporosis and arterial calcification

8) Osteopontin (OPN): pro-inflammatory and pro-atherogenic, but anti-calcific and decalcific factor ?

Osteoporosis and vascular calcification

- Osteoporosis is frequently associated with vascular calcification, and there is a positive association between the severity of aortic calcification and bone loss

- Denosumab (RANKL monoclonal Ab) – reduction of osteoporosis and vascular calcification

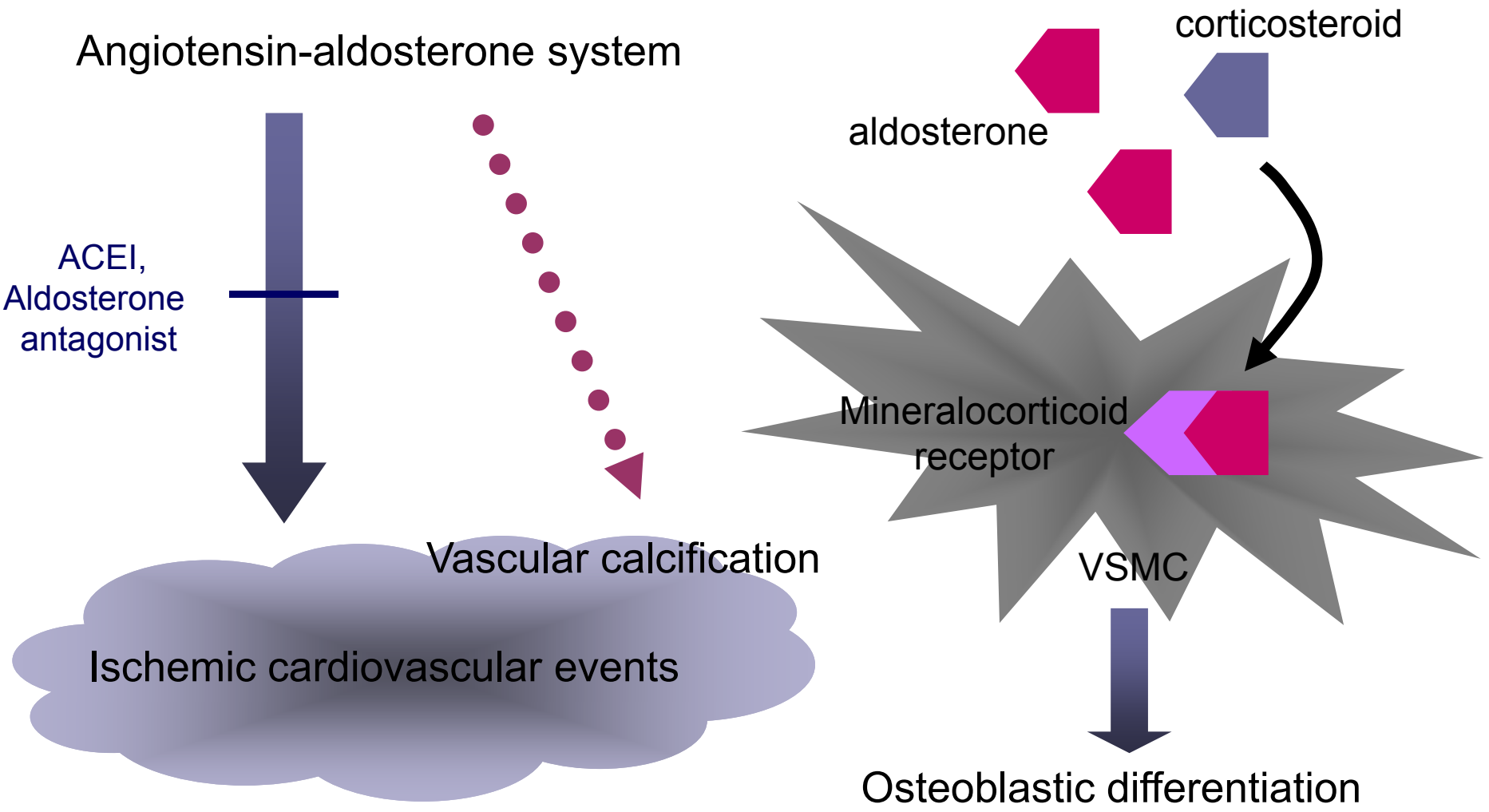
- **Mechanism is a conundrum !**

Hypothesis

- **vascular calcification promotes bone loss**
- **bone loss promotes vascular calcification**
- **a common etiology**

(estrogen deficiency?, LDL? OPG/RANKL)

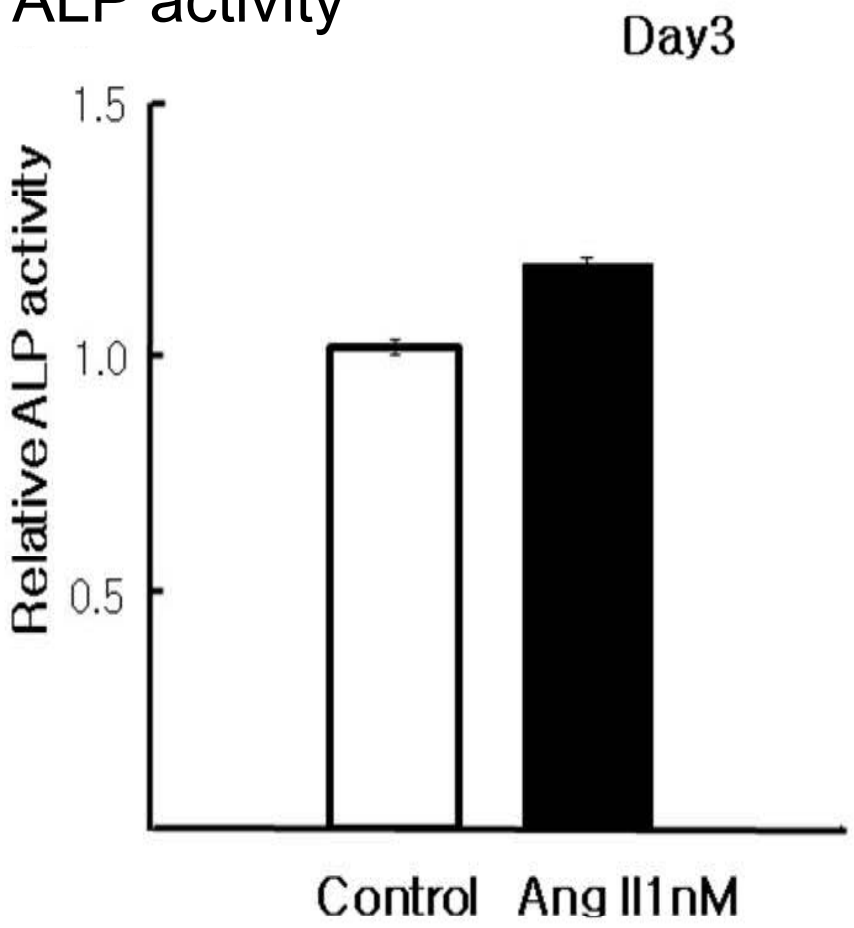
Angiotensin II and vascular calcification



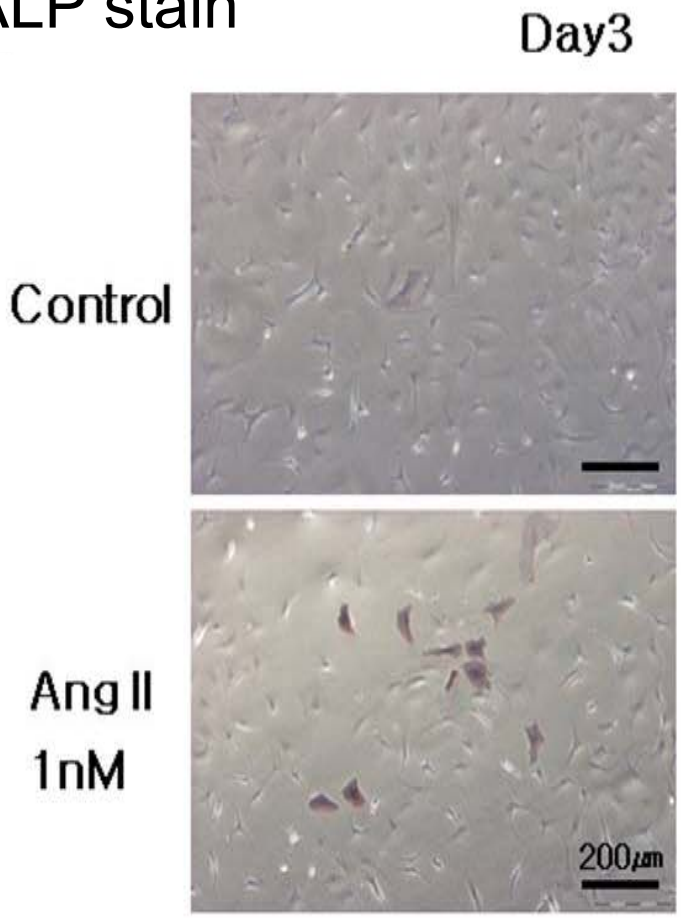
Jaffe, Demer *et al. Arterioscler Thromb Vasc Biol.* 2007;27:799

Angiotensin II stimulates VSMCs calcification

ALP activity



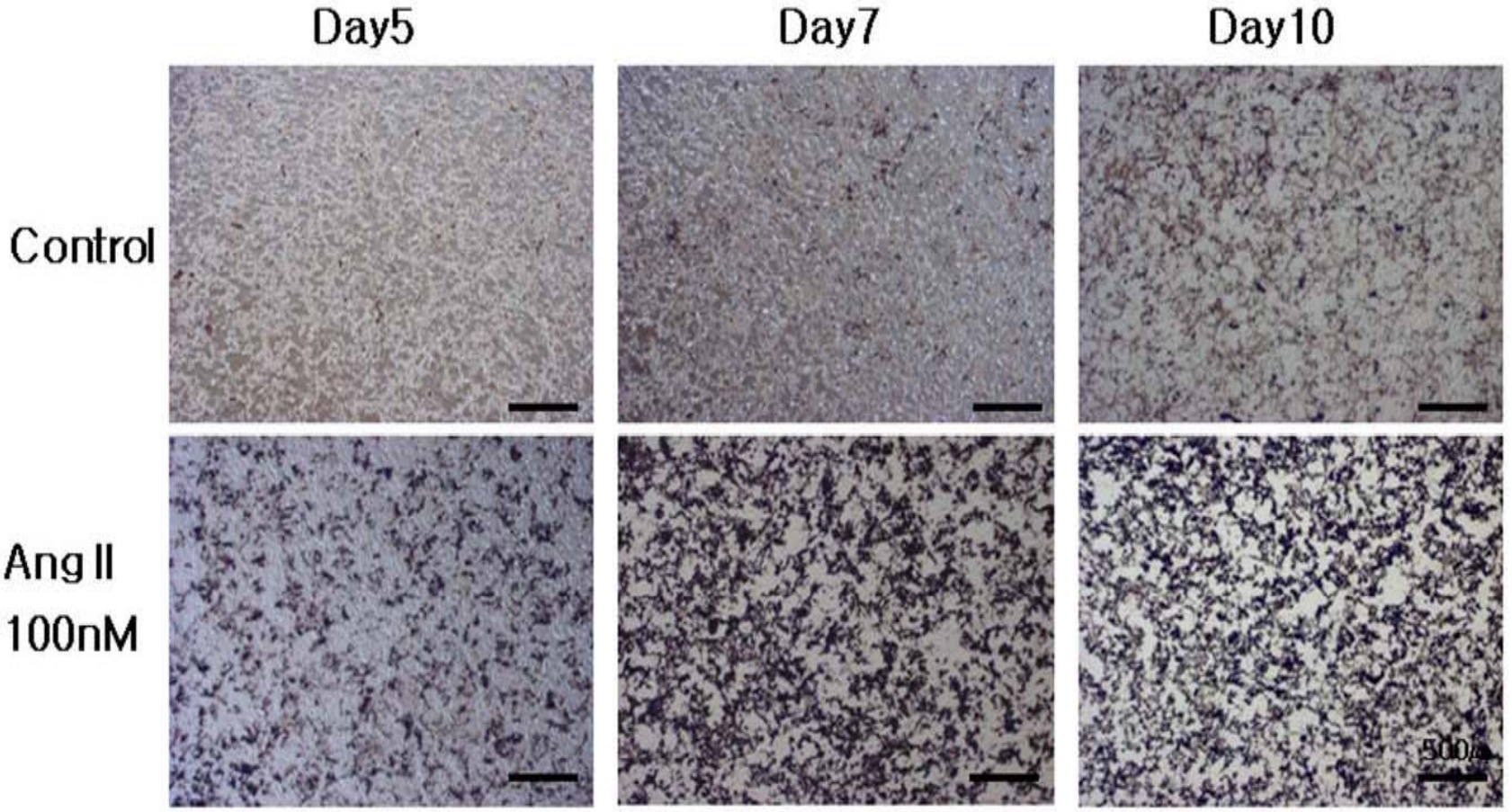
ALP stain



ALP: Alkaline phosphatase

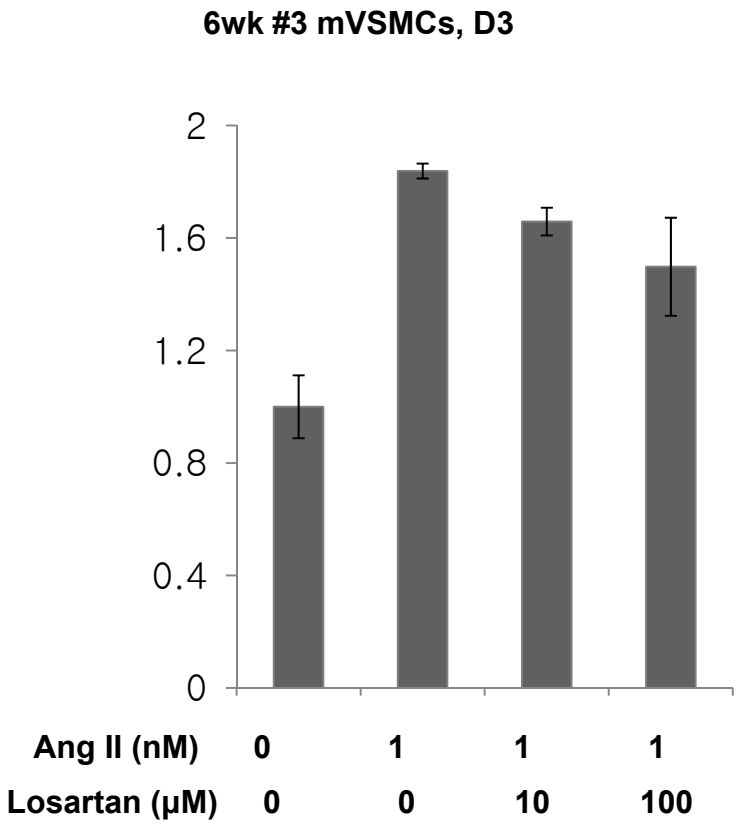
Angiotensin II stimulates VSMCs calcification

Von Kossa Stain



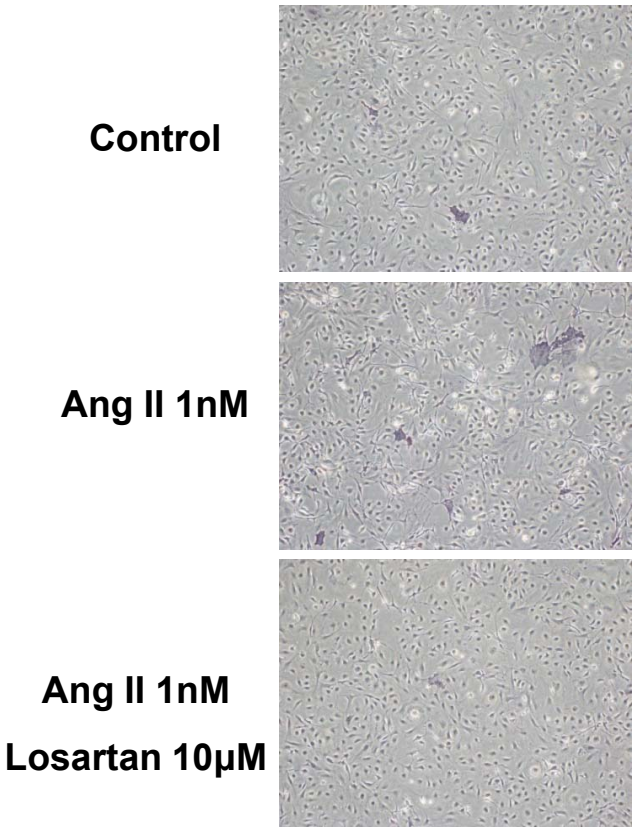
Angiotensin II stimulates VSMCs calcification through AT1 receptor signaling

ALP Activity



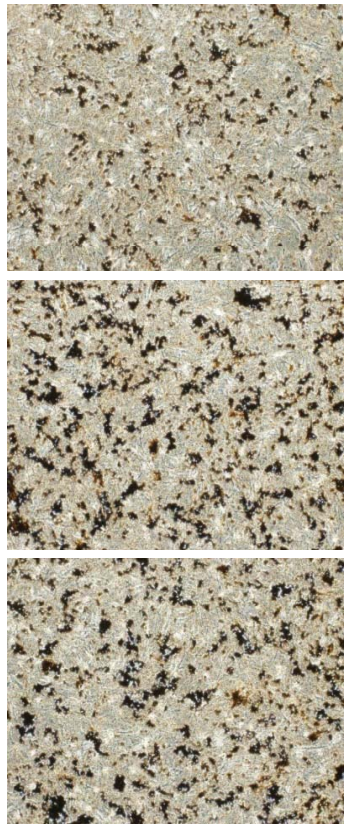
ALP Staining

6wk #6 mVSMCs, D5

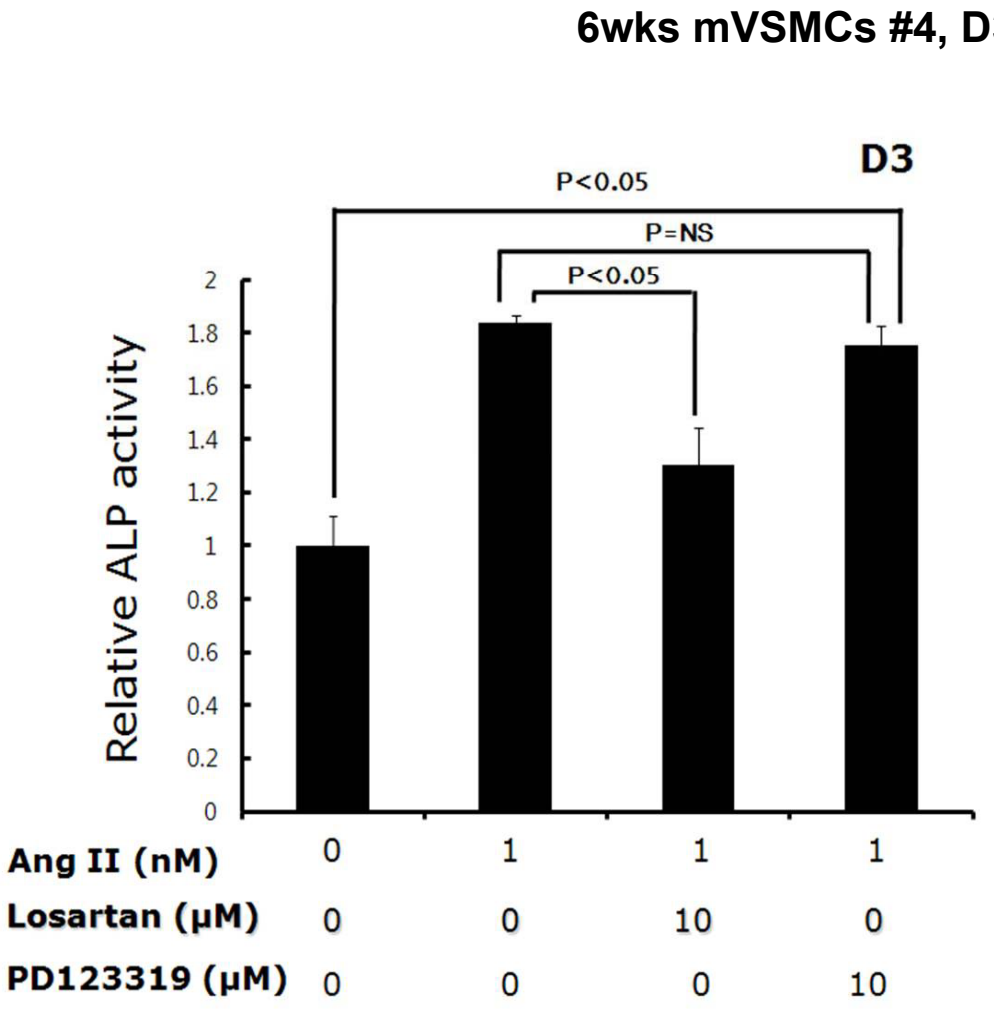


Von Kossa

20wk #6 mVSMCs, D10



Angiotensin II stimulates VSMCs calcification through AT1 receptor signaling



ALP: Alkaline phosphatase

Treatment of vascular calcification

	Potential target		Therapeutic effects	Current or prospective treatment[a]	Complication
	Process	Compounds/factors			
1.	Mineral balance	Hyperphosphatemia hypercalcemia Ca × P FGF-23/Klotho Vitamin D Parathyroid hormone	1. Maintains Ca and P serum levels 2. Inhibition of initialization and growth of the calcium apatite crystal 3. Prevention of VSMC osteo/chondrogenic transition 4. Prevention of VSMC apoptosis	**Bisphosphonates** **Sevelamer** **Calcimimetics** **Thyroidectomy** Pit-1 ↓ FGF-23/Klotho ? 1alpha-hydroxylase ↓	Crosstalk with bone metabolism; dialysis protocols
2.	Inflammation	TNF-alpha IL-6 LDL Glucocorticoid receptor	1. To prevent inflammation 2. Prevention of oste/chondrogenic transition 3. Increase VSMC survival and viability	**Statins** Inflammatory cytokines ↓ PPAR-gamma agonists ↑ HDL ↑	Crosstalk with immune system and tissue remodeling
3.	Regulatory proteins/enzymes	ALK NPP1 ANK MGP Fetuin-A OPG OPN BMP-7 Transglutaminase2	1. Maintenance of PPi level 2. Maintains CaP salts in circulation and local level in a soluble state 3. Increase VSMC survival and viability	ALK ↓ NPP1, ANK ↑ MGP ↑ Fetuin-A ↑ OPG ↑ OPN ? BMP-7 ? Transglutaminase2 ?	Multiple effects and crosstalk with bone metabolism

[a] Possible drugs/approaches in bold type have been proven in clinical trials and are currently used; suggestions in small type are hypothetical. NPP/ANK, key regulators of pyrophosphate metabolism; NPP1, nucleotide pyrophosphatase phosphodiesterase 1 (generates PPi from nucleoside triphosphates); ANK, ankylosis protein (multiple-pass transmembrane protein that mediates intracellular to extracellular channeling of PPi); OPN, osteopontin; ↑, increasing levels maybe effective; ↓, decreasing levels maybe effective; ?, effect unknown.

Kapustin A, Shanahan CM. *Curr Opin Pharmacol.* 2009

Treatment of vascular calcification

	Potential target		Therapeutic effects	Current or prospective treatment[a]	Complication
	Process	Compounds/factors			
4.	Apoptosis and vesicle release	Gas6-Axl signaling Akt Apoptotic bodies Matrix vesicles	1. Increase VSMC survival and viability 2. Modifying vesicle release and VSMC adaptation to prevent mineralization?	**Statins** FGF-23/Klotho ↑ Matrix vesicles ? Ca Channel blockers	Crosstalk with immune system, tissue remodeling; carcinogenesis; unknown biological role of matrix vesicles
5.	Osteo/chondrogenic differentiation	Cbfa1/Runx2 Osterix Msx2 Wnt signaling	1. To prevent osteo/chondrogenic conversion of VSMC	Cbfa1/Runx2 ↓ Osterix ↓ Msx ↓ Wnt signaling ↓	Crosstalk with bone metabolism
6.	Mineral resorption	'Osteoclastic' Macrophages/monocytes Carbonic anhydrase II	Induction of calcium deposit resorption	Pi ↓ Carbonic anhydrase II ↑	Crosstalk with bone metabolism

[a] Possible drugs/approaches in bold type have been proven in clinical trials and are currently used; suggestions in small type are hypothetical. NPP/ANK, key regulators of pyrophosphate metabolism; NPP1, nucleotide pyrophosphatase phosphodiesterase 1 (generates PPi from nucleoside triphosphates); ANK, ankylosis protein (multiple-pass transmembrane protein that mediates intracellular to extracellular channeling of PPi); OPN, osteopontin; ↑, increasing levels maybe effective; ↓, decreasing levels maybe effective; ?, effect unknown.

Kapustin A, Shanahan CM. *Curr Opin Pharmacol.* 2009

Treatment of vascular calcification – clinical trials

Coronary artery calcium (CAC) and/or abdominal aortic calcium (AAC)

Effect of *Simvastatin* (80 mg) on Coronary and Abdominal Aortic Arterial Calcium (from the Coronary Artery Calcification Treatment with Zocor [CATZ] Study)

James G. Terry, MS[a,*], J. Jeffrey Carr, MD, MS[b,d], Ethel O. Kouba, PhD[a], Donna H. Davis, BS[a], Lata Menon, MS, RN[a], Kathryn Bender, PharmD[c], E. Ted Chandler, MD[a], Timothy Morgan, PhD[d], and John R. Crouse III, MD[a,d]

(Am J Cardiol 2007;99:1714–1717)

Effect of Intensive Versus Standard Lipid-Lowering Treatment With Atorvastatin on the Progression of Calcified Coronary Atherosclerosis Over 12 Months

A Multicenter, Randomized, Double-Blind Trial

Axel Schmermund, MD; Stephan Achenbach, MD; Thomas Budde, MD; Yuri Buziashvili, MD; Andreas Förster, MD; Guy Friedrich, MD; Michael Henein, MD; Gert Kerkhoff, MD; Friedrich Knollmann, MD; Valery Kukharchuk, MD; Avijit Lahiri, MD; Roman Leischik, MD; Werner Moshage, MD; Michael Schartl, MD; Winfried Siffert, MD; Elisabeth Steinhagen-Thiessen, MD; Valentin Sinitsyn, MD; Anja Vogt, MD; Burkhard Wiedeking, MD; Raimund Erbel, MD

(*Circulation*. 2006;113:427-437.)

Although the target reduction in LDL was achieved, there was no treatment effect on the rate of calcified plaque progression → Statin could not attenuate CAC progression

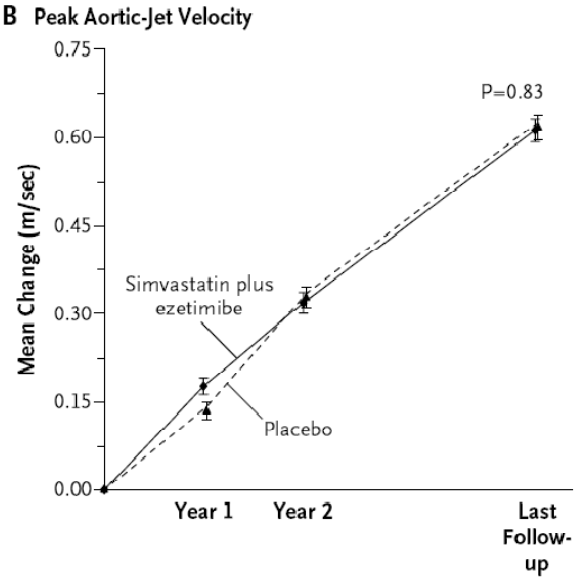
Treatment of valvular calcification – clinical trials

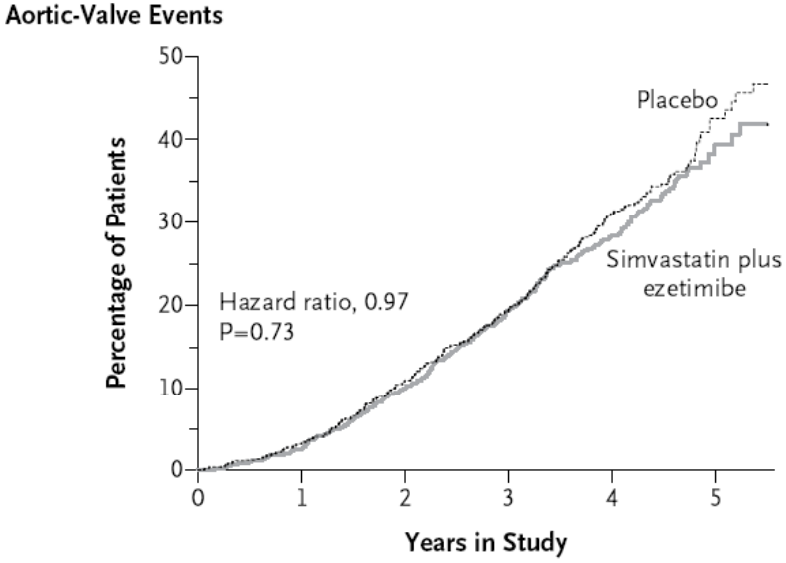
Aortic valve calcification: aortic stenosis

Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis

Anne B. Rossebø, M.D., Terje R. Pedersen, M.D., Ph.D., Kurt Boman, M.D., Ph.D., Philippe Brudi, M.D., John B. Chambers, M.D., Kenneth Egstrup, M.D., Ph.D., Eva Gerdts, M.D., Ph.D., Christa Gohlke-Bärwolf, M.D., Ingar Holme, Ph.D., Y. Antero Kesäniemi, M.D., Ph.D., William Malbecq, Ph.D., Christoph A. Nienaber, M.D., Ph.D., Simon Ray, M.D., Terje Skjærpe, M.D., Ph.D., Kristian Wachtell, M.D., Ph.D., and Ronnie Willenheimer, M.D., Ph.D., for the SEAS Investigators*

N Engl J Med 2008;359:1343-56.

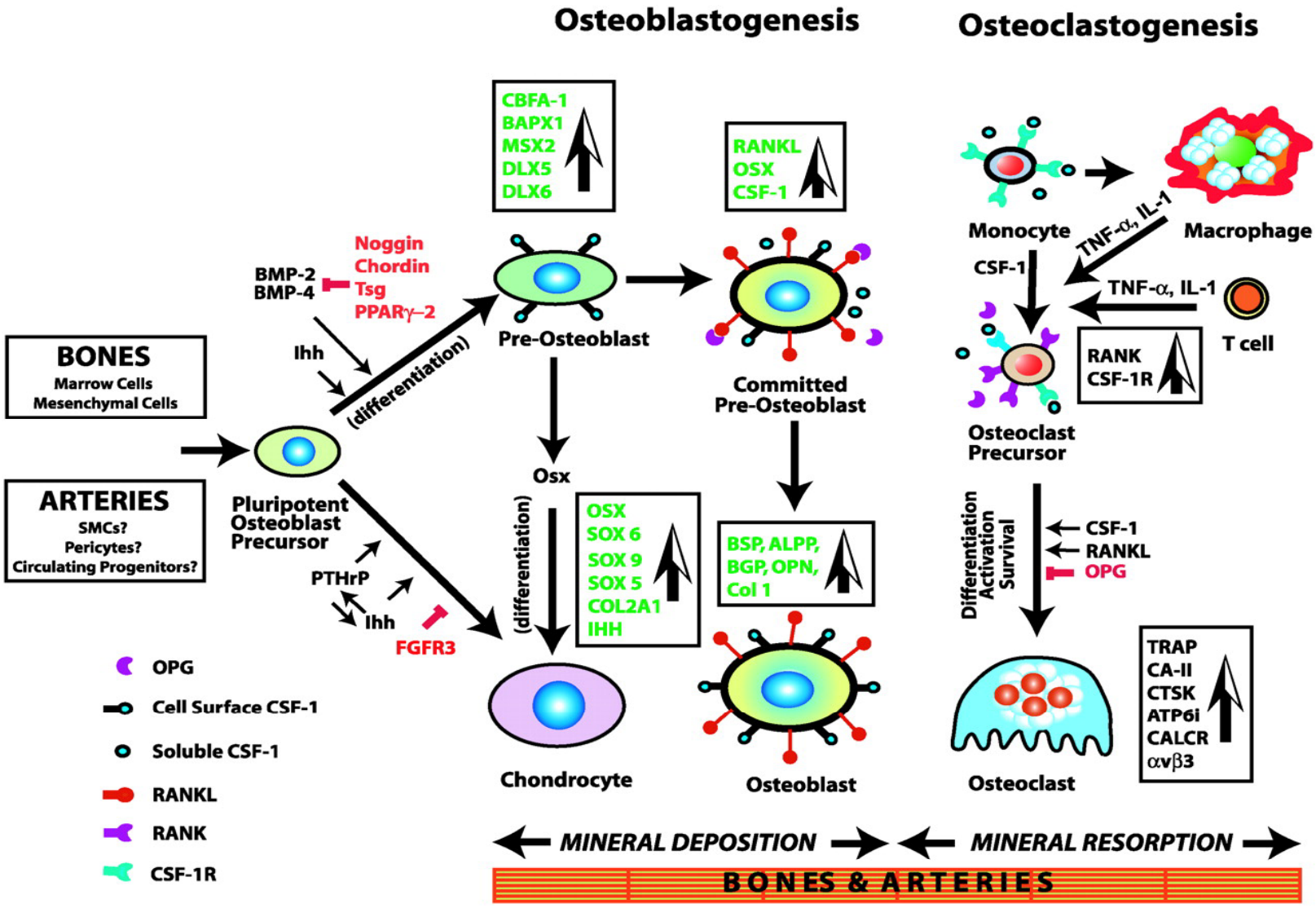




[가설] lipid-lowering treatment might prevent progression of aortic-valve stenosis and thus reduce the need for aortic-valve replacement.

[결과] Simvastatin and ezetimibe did not reduce the composite outcome of combined aortic valve events and ischemic events in patients with aortic stenosis.

Treatment of vascular calcification – new target



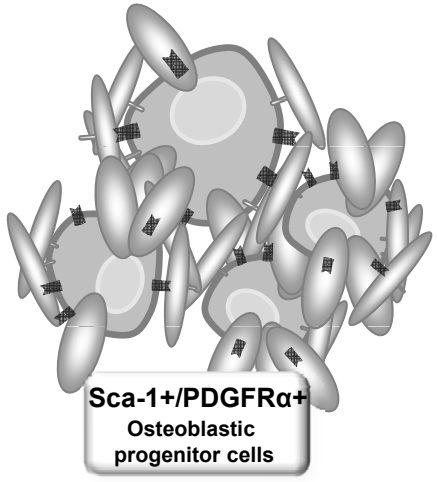
Doherty TM et al. PNAS. 2003

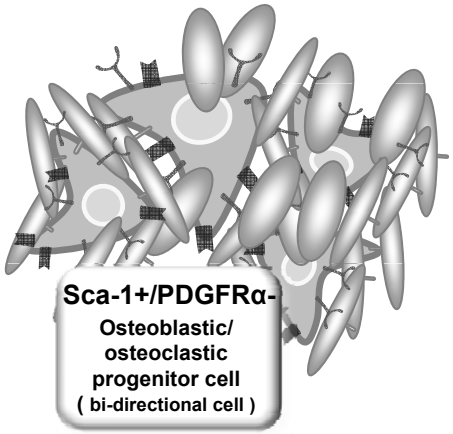
Cellular determinants – origin of calcifying cells

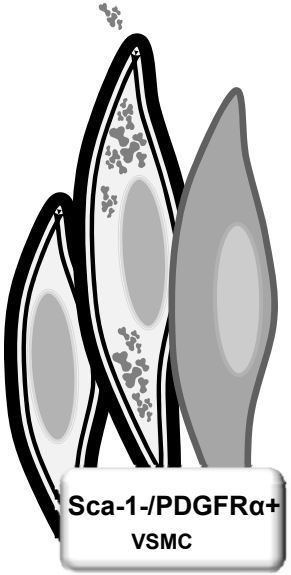
Origin of osteoblastic or osteogenic cells in vasculature is a great topic of debate.

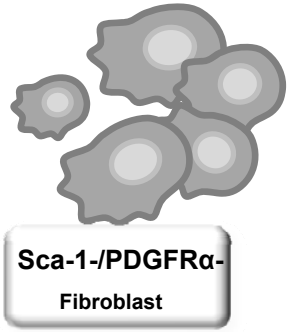
- vascular smooth muscle cell (VSMC): contractile → proliferative, synthetic form
- calcifying vascular cell (CVC): may be a certain clone or subpopulation of VSMC
- pericyte: a kind of mesenchymal progenitors
- mesenchymal stem cell (circulating or resident), adventitial myofibroblast (resident ?) and fibrocyte (circulating)
- calcifying vascular progenitor cells (circulating, resident)

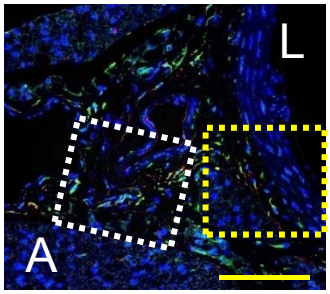
Treatment of vascular calcification – new cellular target



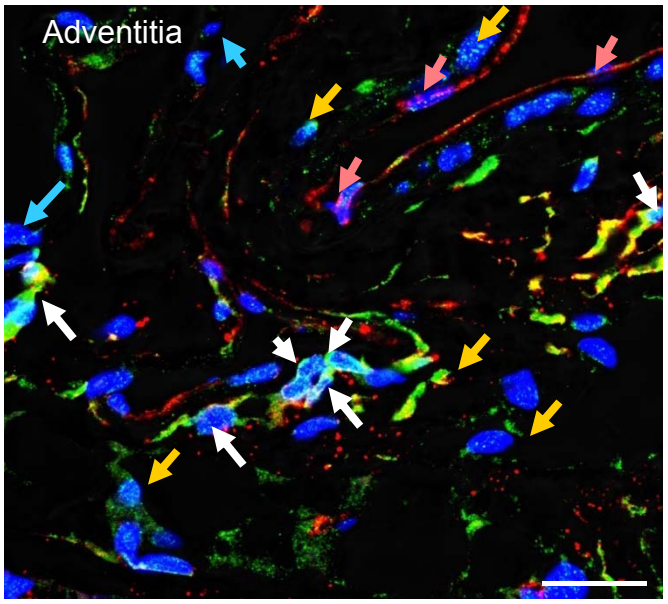


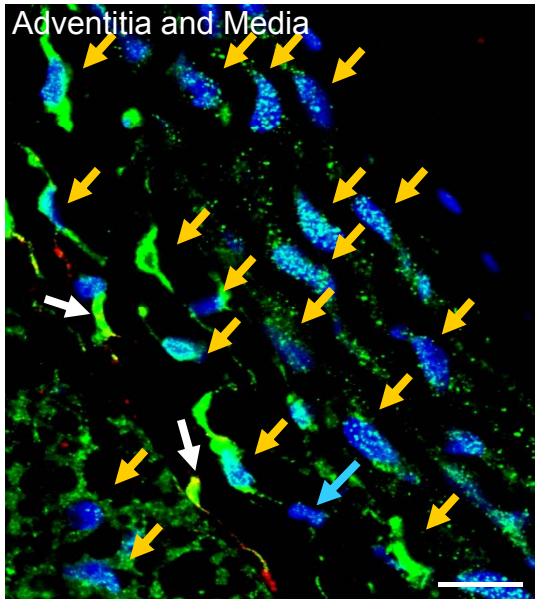




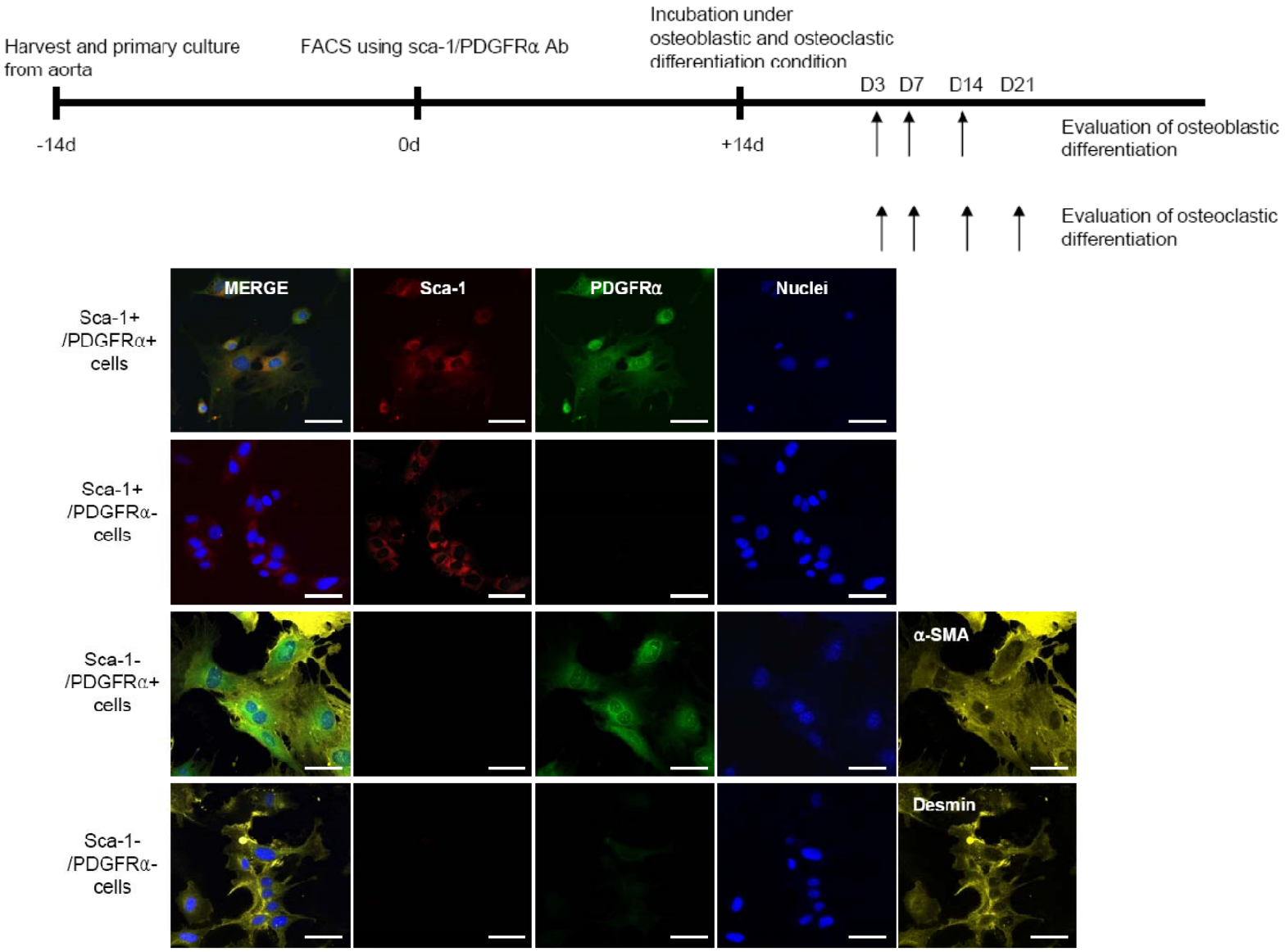


Sca-1+PDGFRα +
Sca-1+PDGFRα -
Sca-1-PDGFRα +
Sca-1-PDGFRα -

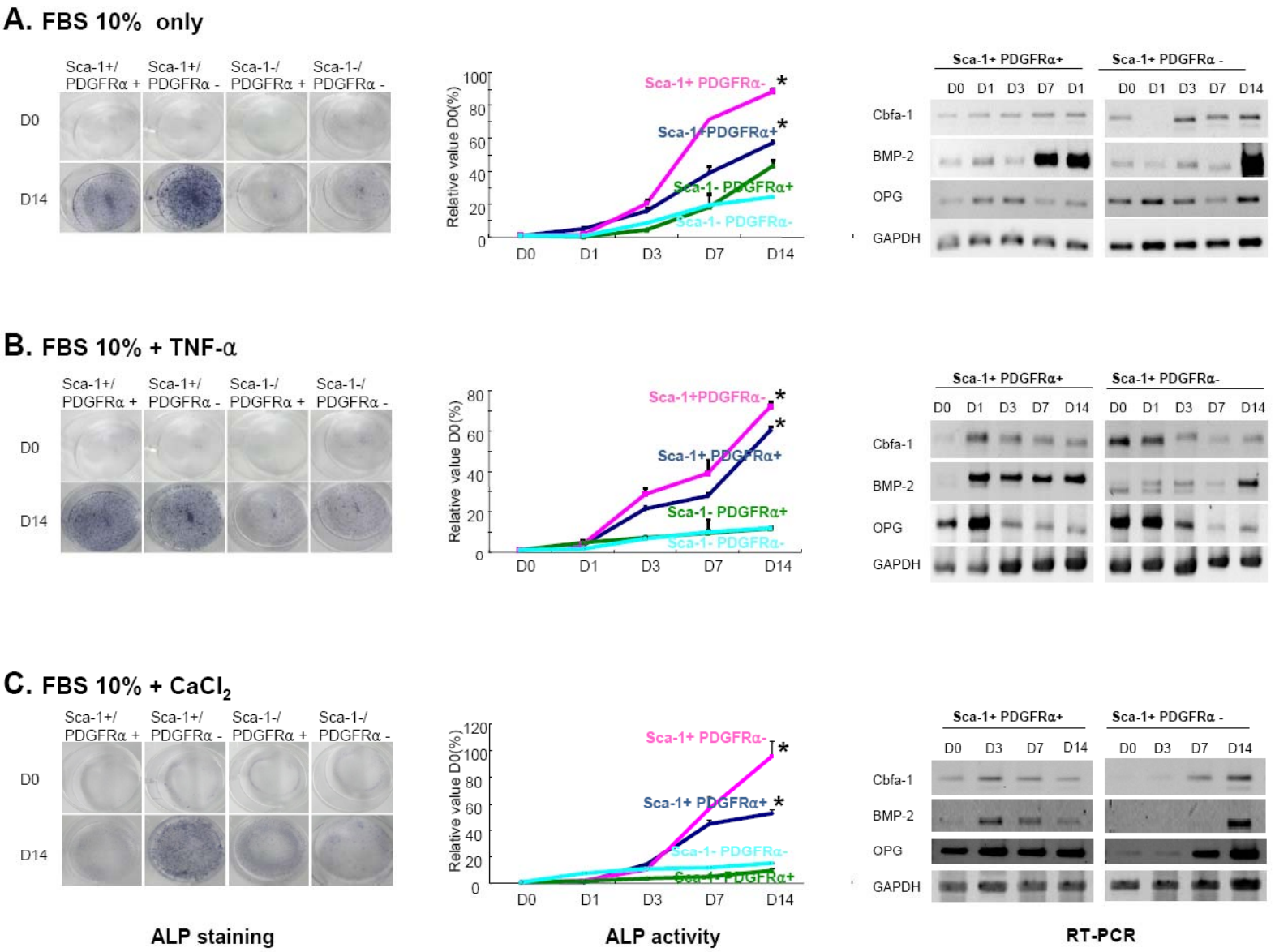




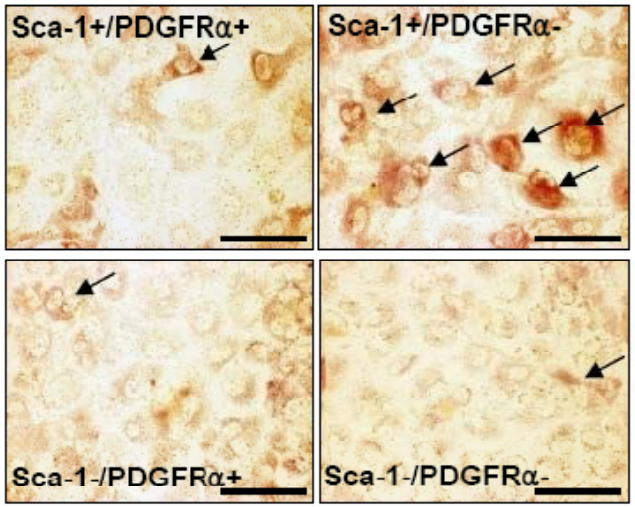
Vascular calcifying progenitor cells



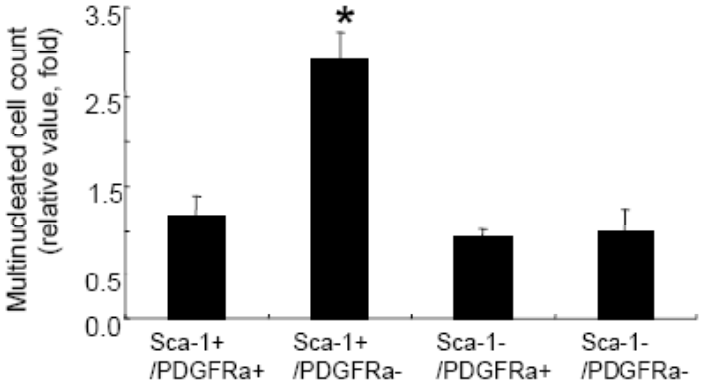
Osteoblastic differentiation of calcifying progenitor cells

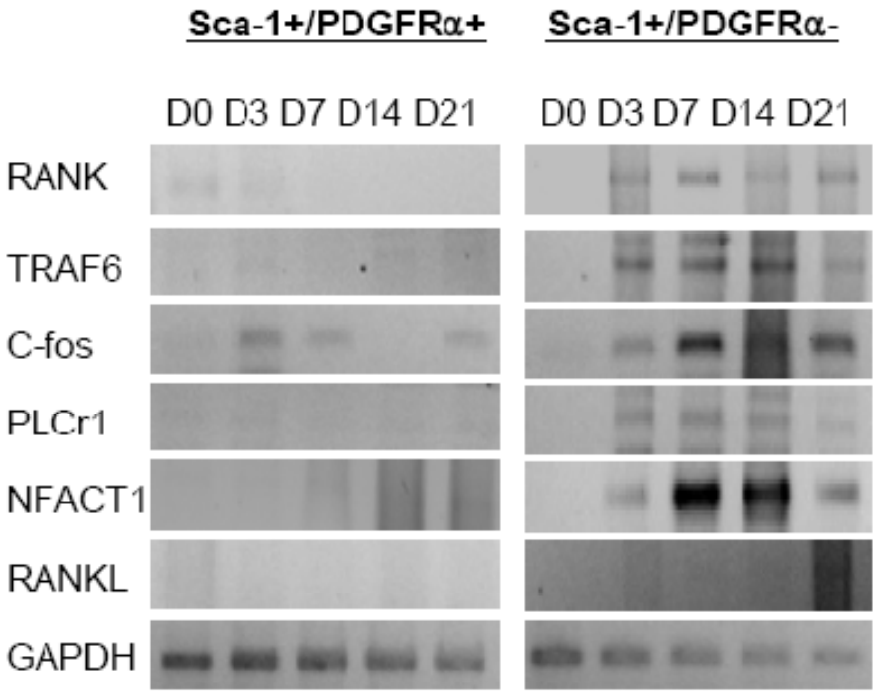


Osteoclastic differentiation of calcifying progenitor cells

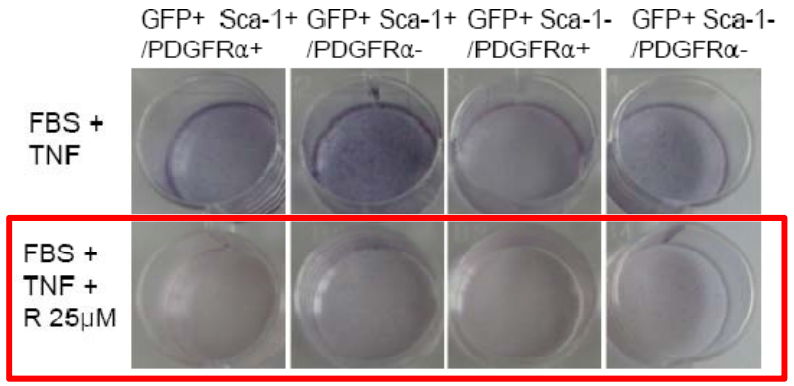


TRAP staining (FBS + RANKL + M-CSF)

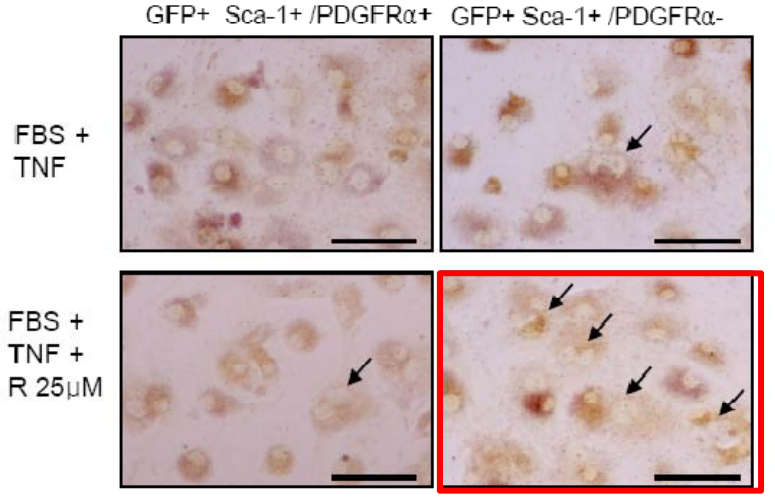




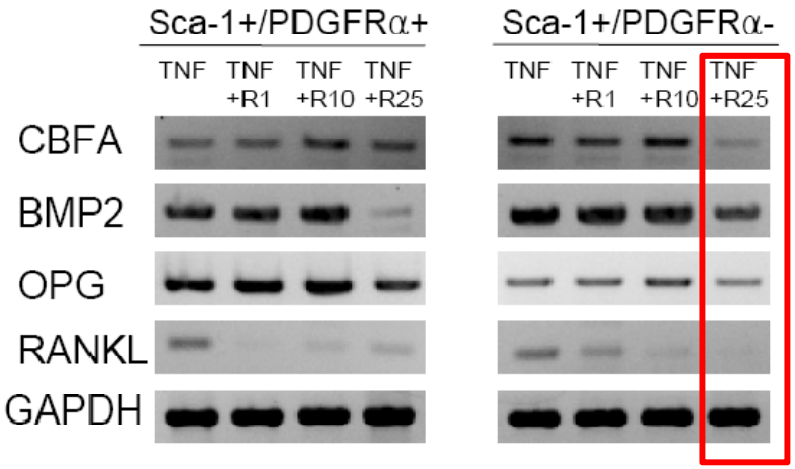
PPARγ activation suppresses osteoblastic differentiation and promotes osteoclastic differentiation



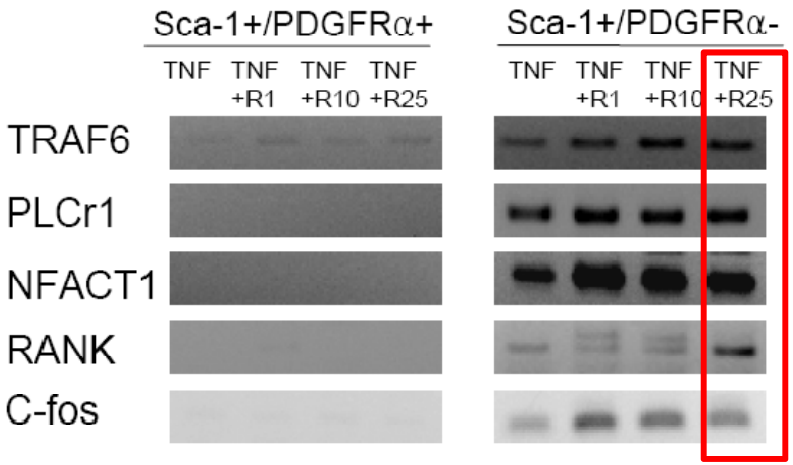
ALP staining



TRAP staining

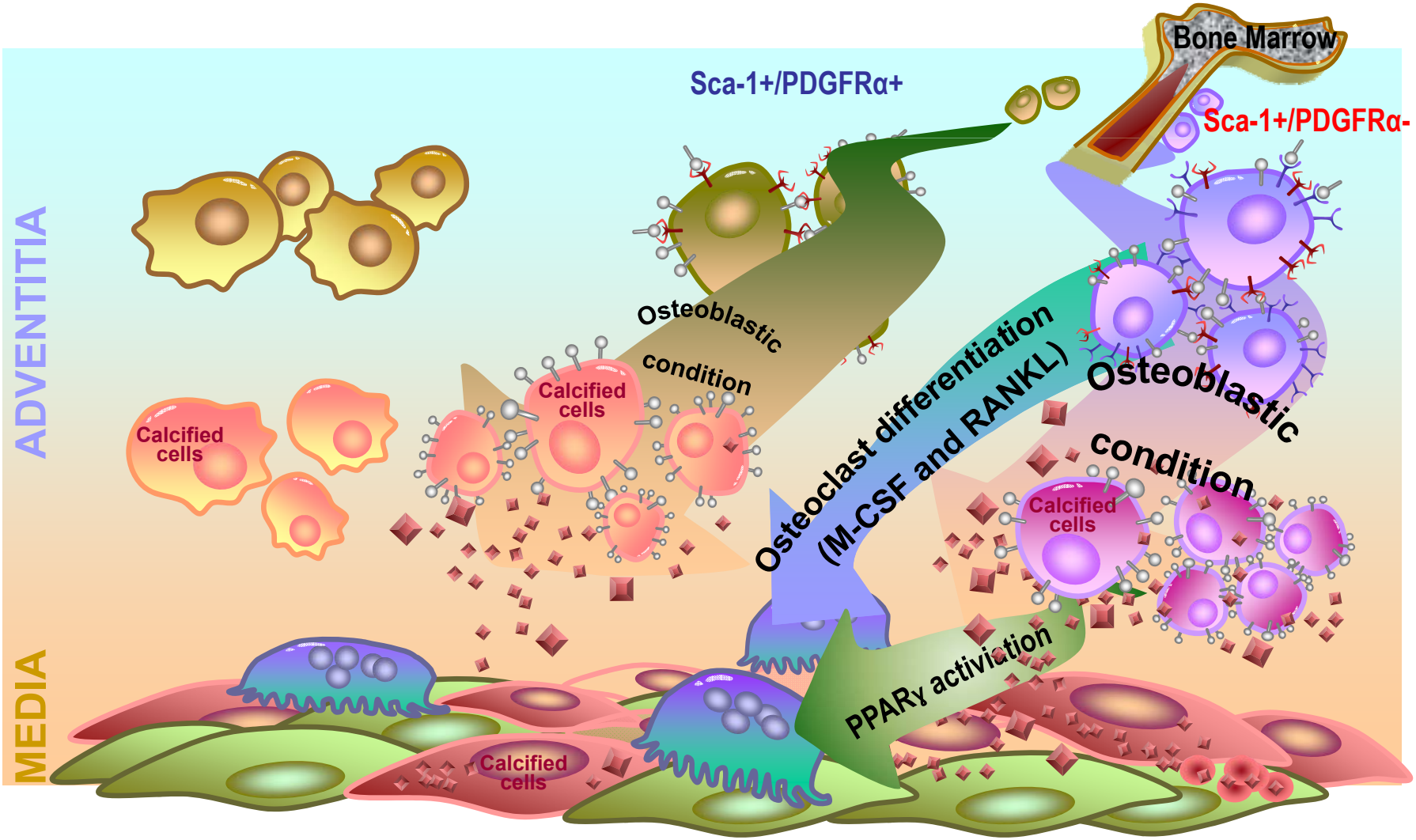


Osteoblast-related genes



Osteoclast-related genes

R=Rosiglitazone (PPARγ agonist)



Osteoblastic progenitor cells
Sca-1+/PDGFRα+

Osteoblastic/ osteoclastic progenitor cell (bi-direction cell)
Sca-1+/PDGFRα-

VSMC
Sca-1-/PDGFRα+

Fibroblast
Sca-1-/PDGFRα-

Osteoclast

RANKL
RANK
OPG
BMP-2

CONTENTS

Conclusions

1. Vascular calcification is an important feature of progressive atherosclerosis, a poor prognostic factor of future adverse cardiovascular events.

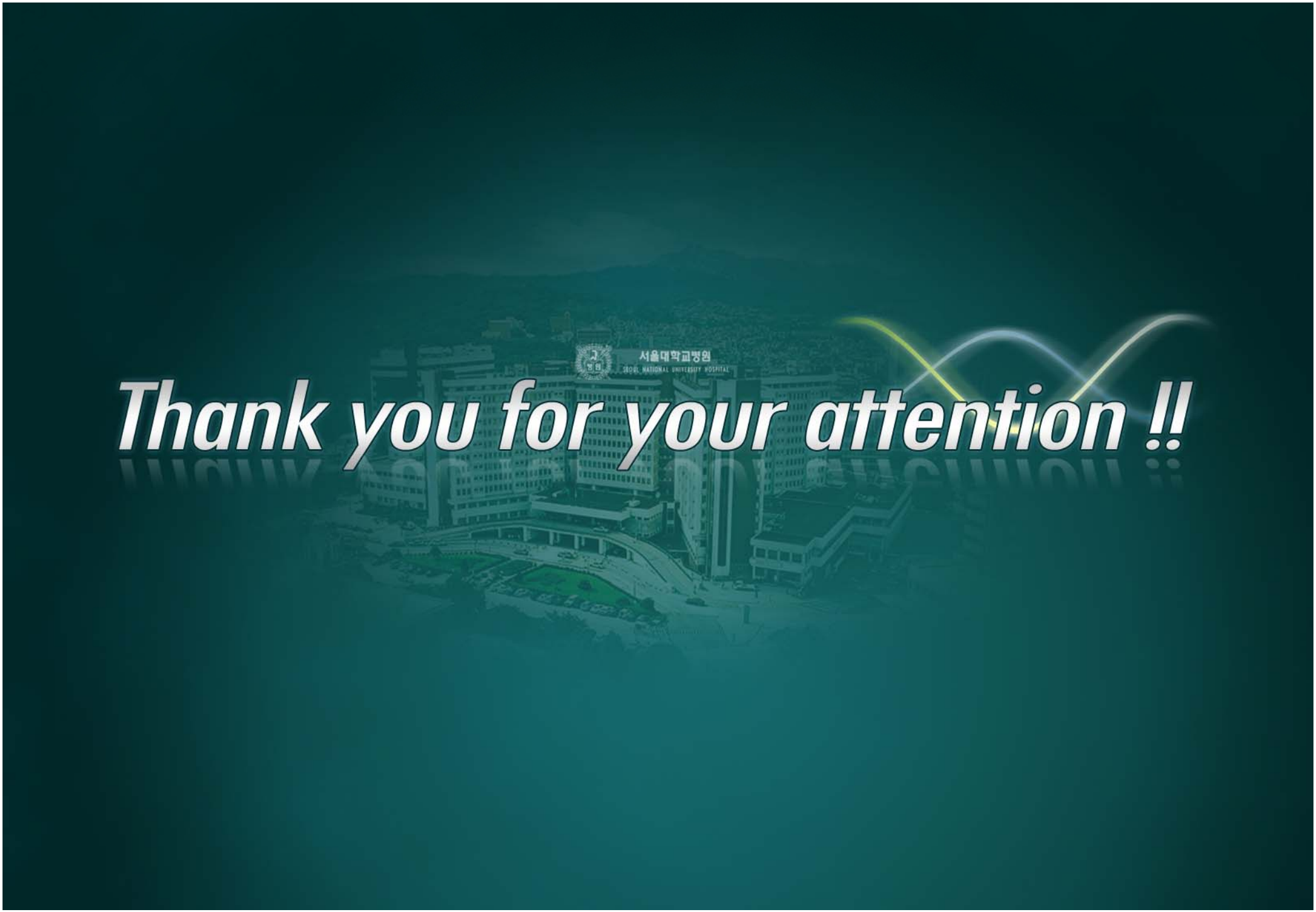
2. Recent studies have shown that the pathophysiologic process of vascular calcification is tightly-regulated, active process .

3. Recent advances in understanding molecular and cellular mechanisms of vascular calcification may lead to the development of new therapeutic strategies for cardiovascular diseases.

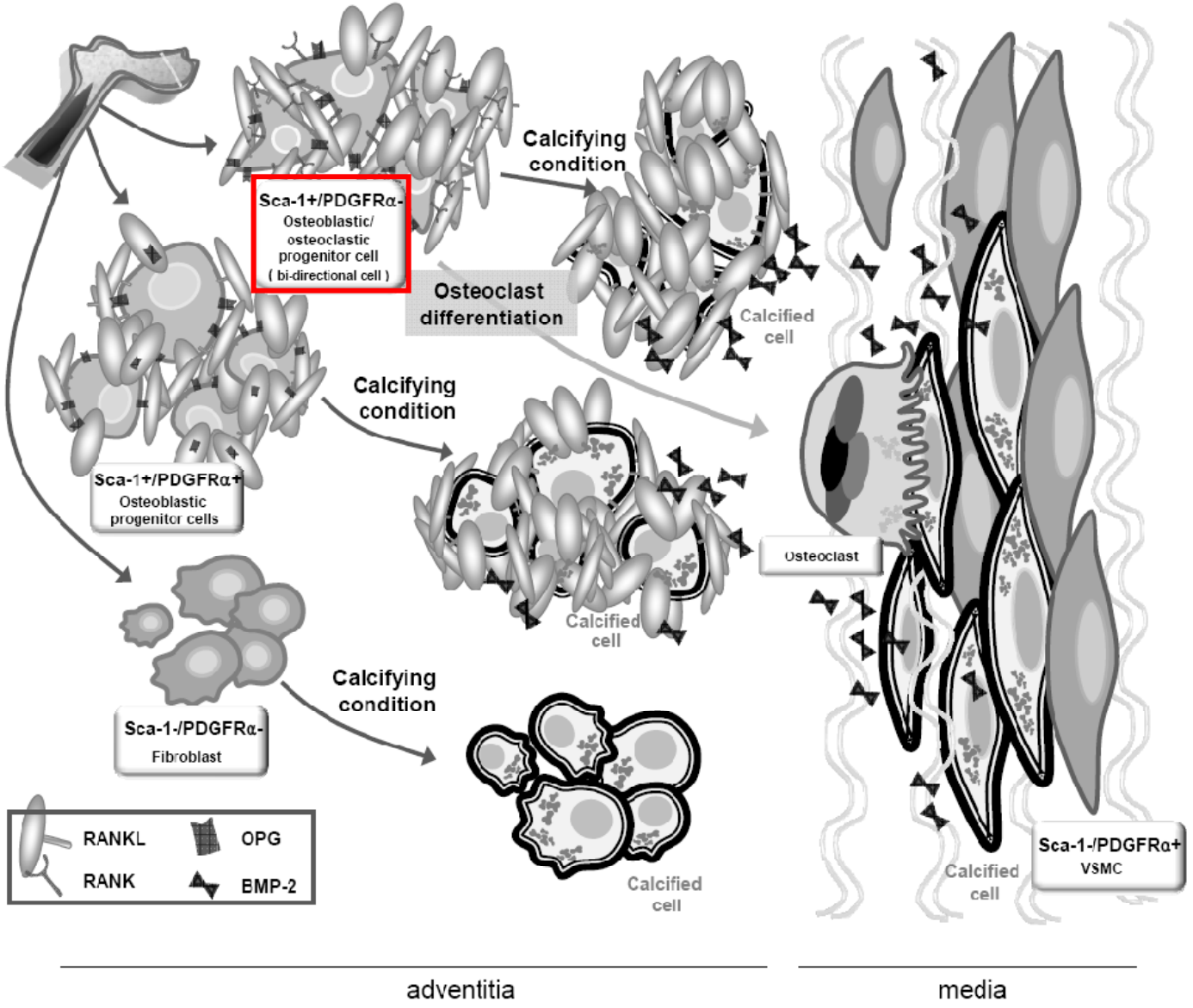
4. Eventually, we hope that vascular calcification, once considered irreversible process, would be regressed and reserved through decalcifying therapy in the future.

Acknowledgements

Seoul National University Cardiovascular Research Lab

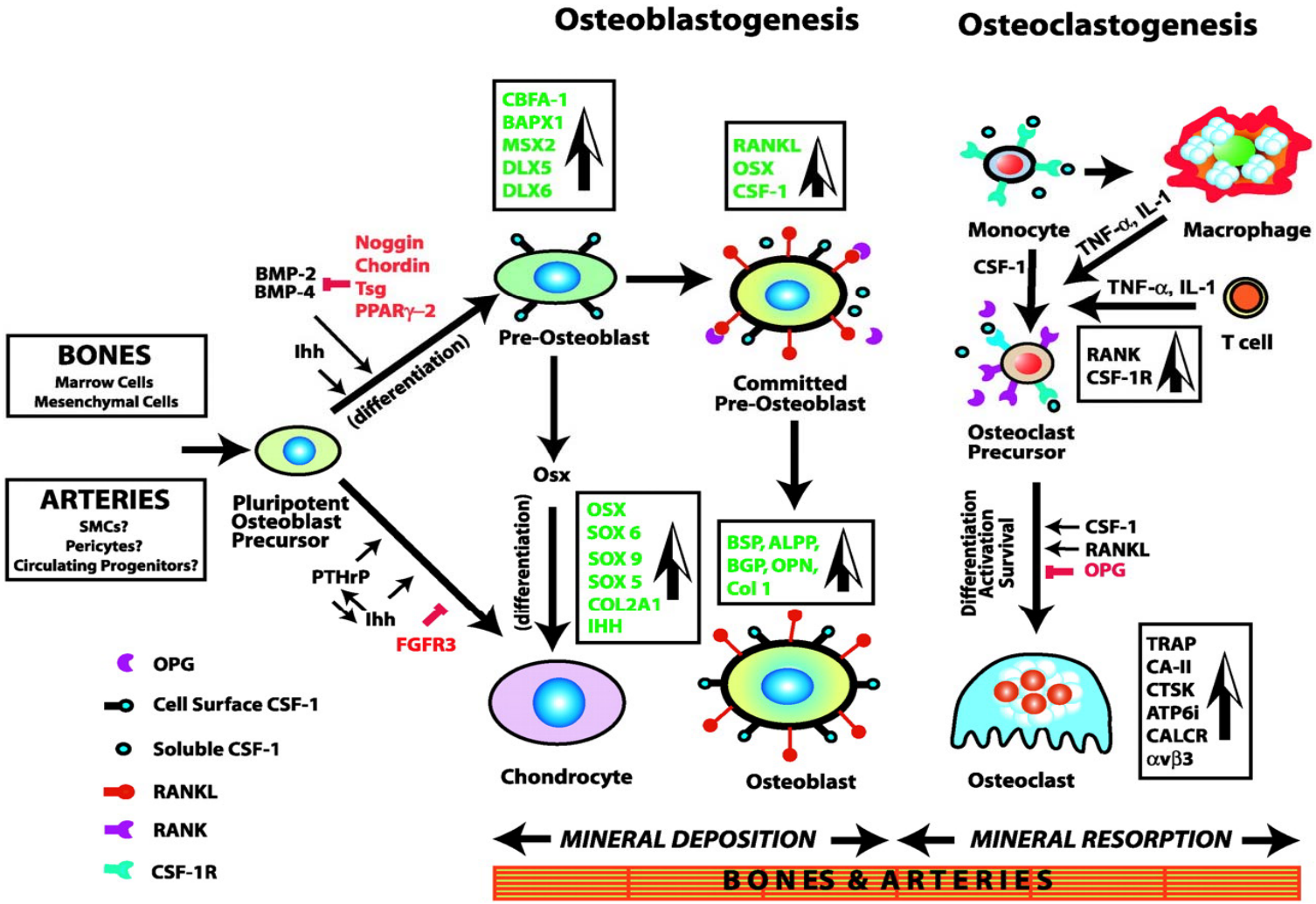

서울대학교병원
SEOUL NATIONAL UNIVERSITY HOSPITAL
Thank you for your attention !!

Treatment of vascular calcification - hypothesis



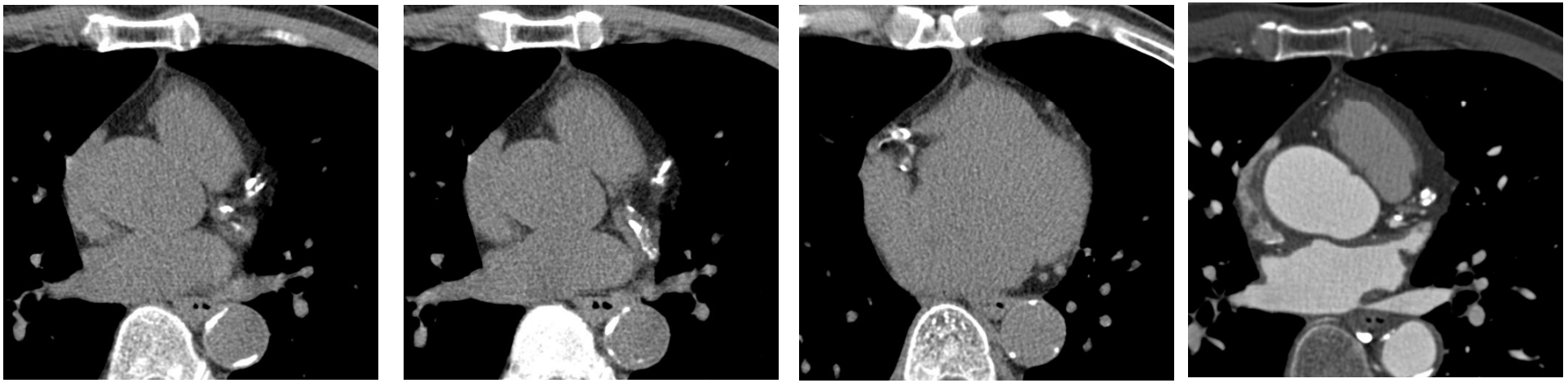
Cho HJ *et al . Unpublished data*

Mechanism of osteogenesis, showing the major genes, growth factors, and signaling pathways culminating in fully mature chondrocytes, osteoblasts, and osteoclasts



Doherty T M et al. PNAS 2003;100:11201-11206

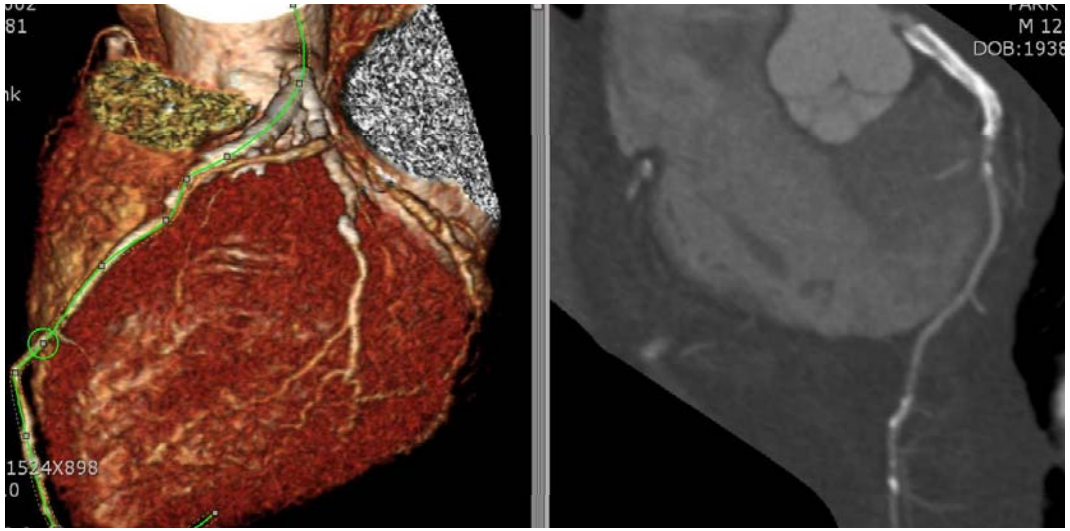
Vascular calcification : clinical significance ?

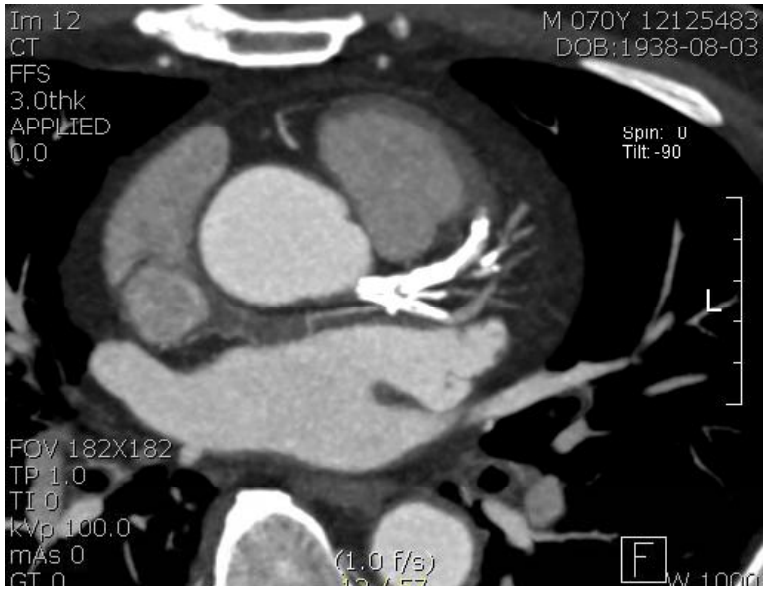


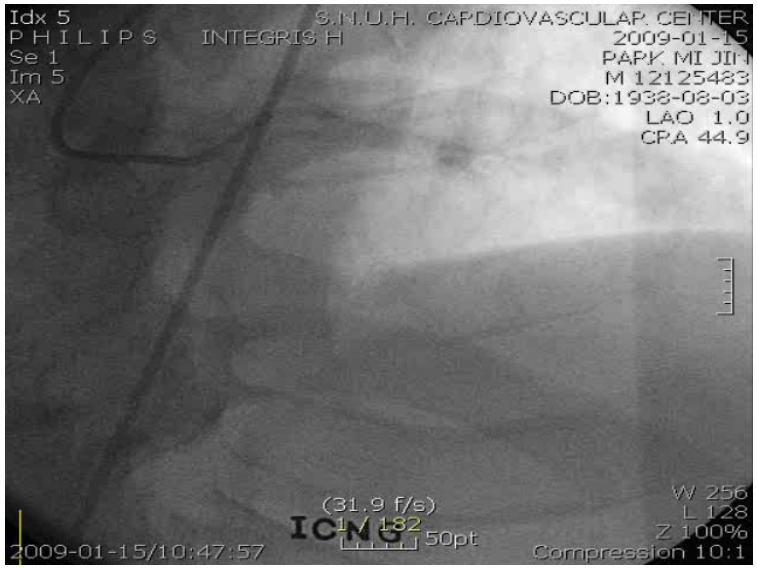
정기 검진상 우연히 발견된 High calcium score (CAC score = 3609)

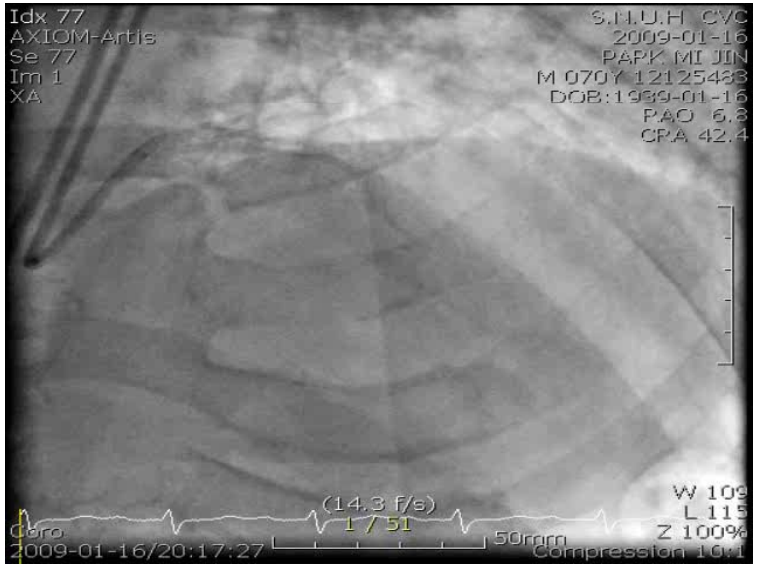
CAC score: coronary artery calcium score

Vascular calcification : clinical hurdles

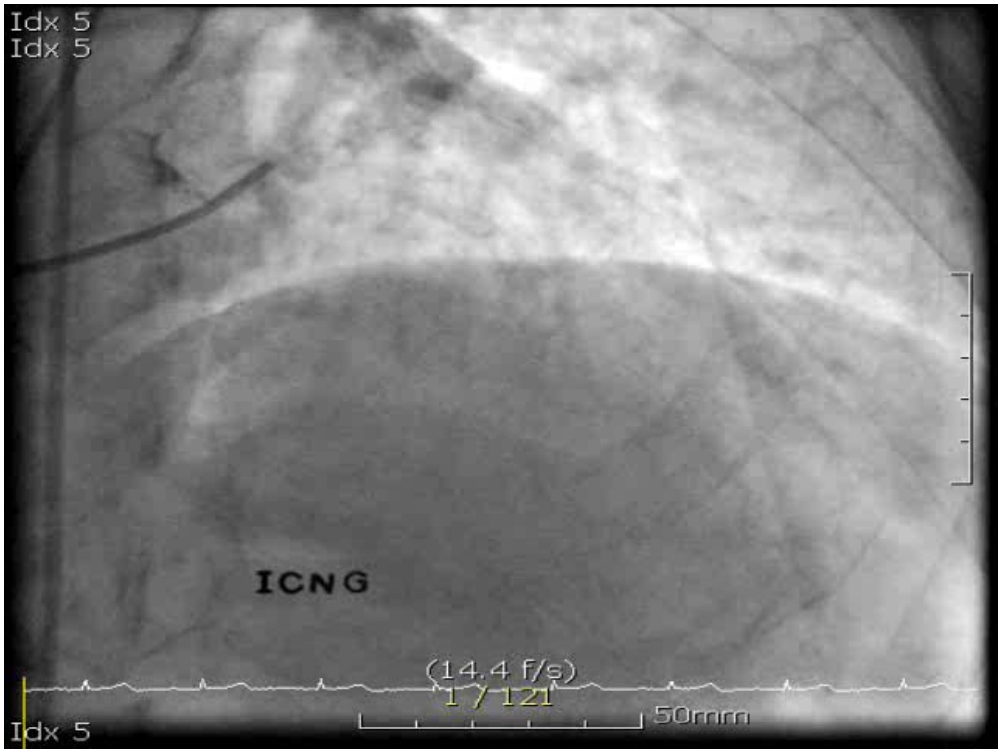


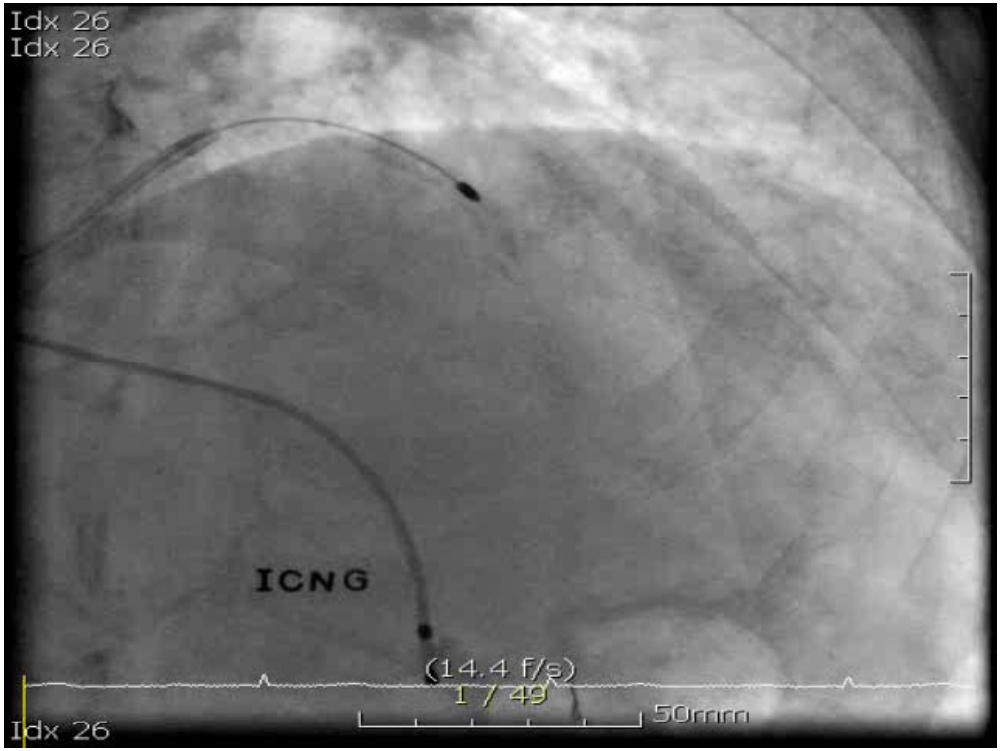




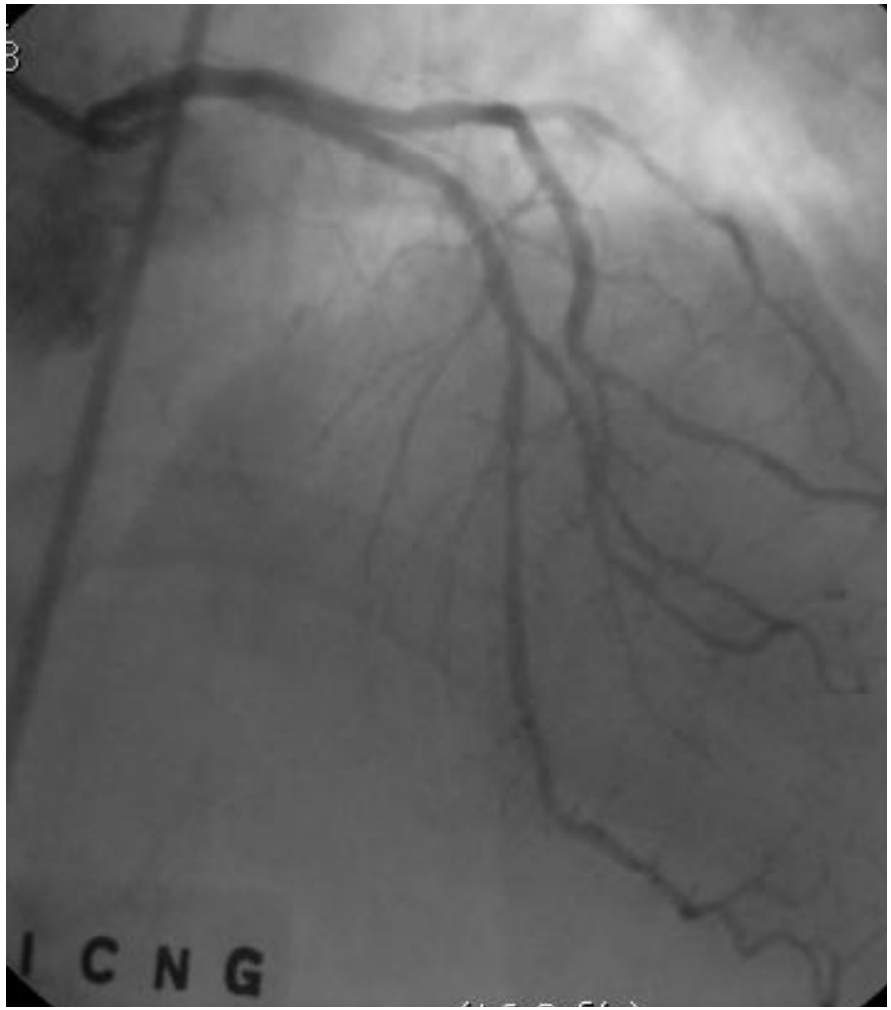


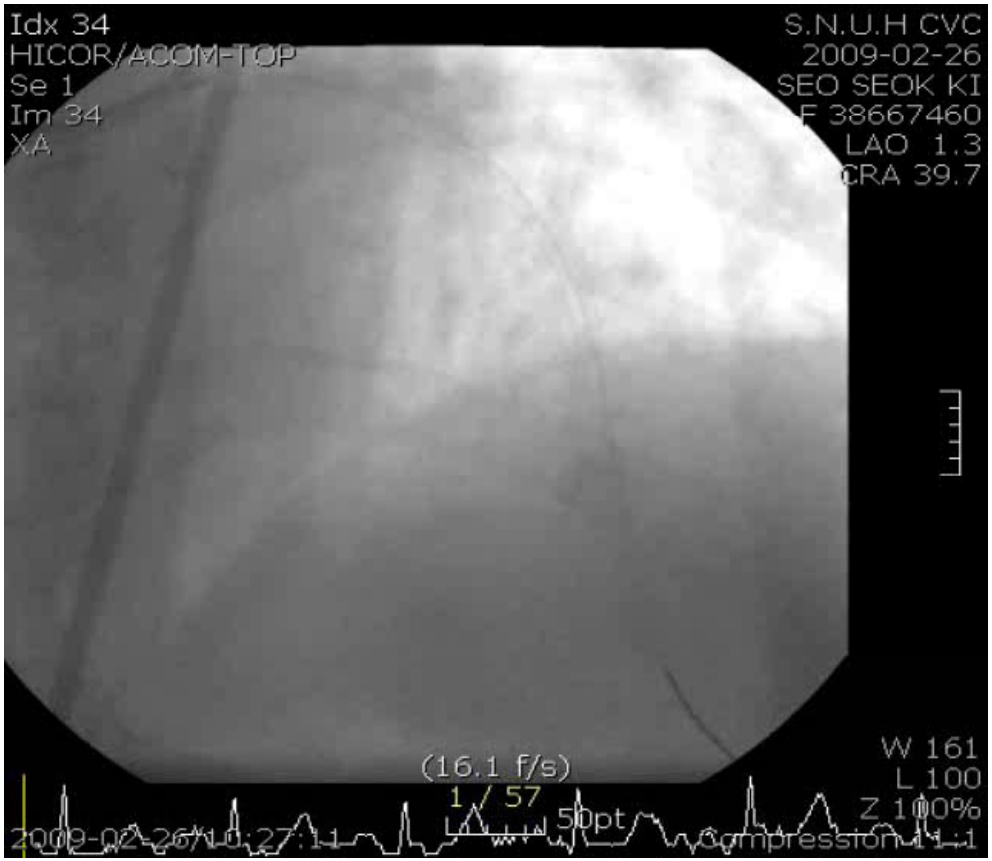
Vascular calcification : clinical hurdles





Vascular calcification : clinical hurdles





Heavy calcification

→ not just a marker of severe disease, but an enemy of treatment

Molecular determinants and inducers - summary

1) Inflammatory signal: pro-calcific environment
2) BMP-2: inducer for bone formation
3) Msx2: BMP-2-inducible transcription factor in medial calcification
4) Runx2/Cbfa: osteoblast-specific transcription factor
5) Osteoclacin: osteoblast-specific marker
6) Alkaline phosphatase (ALP): functional osteoblast phenotypic marker
7) RANK/RANKL/Osteoprotegerin (OPG): balancing factors in osteoporosis, arterial calcification
8) Osteopontin (OPN): pro-inflammatory and pro-atherogenic, but anti-calcific and decalcific factor ?

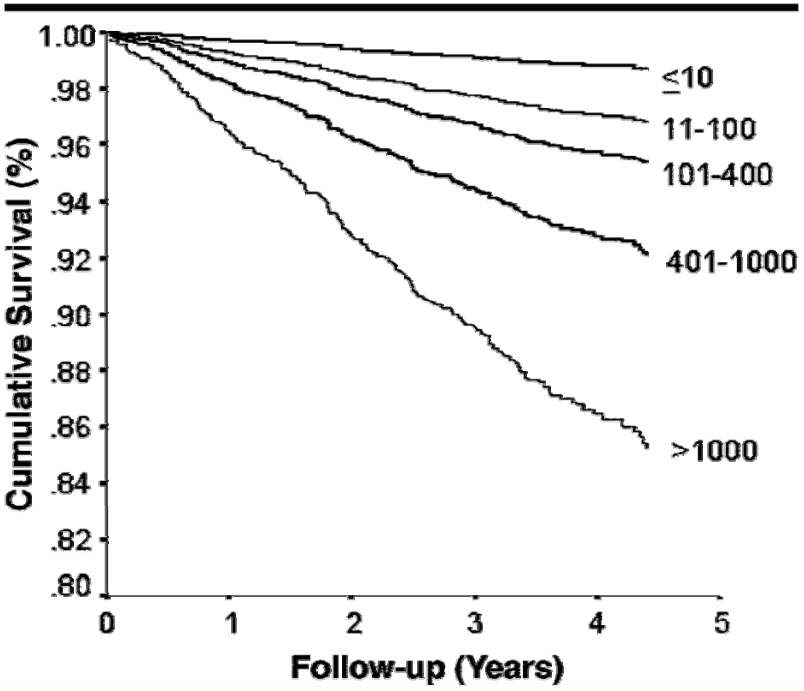
Vascular calcification : clinical significance

Table 2 Frequency of Revascularization Procedures and Cardiac Events During Follow-Up Results in the CAC Cohort

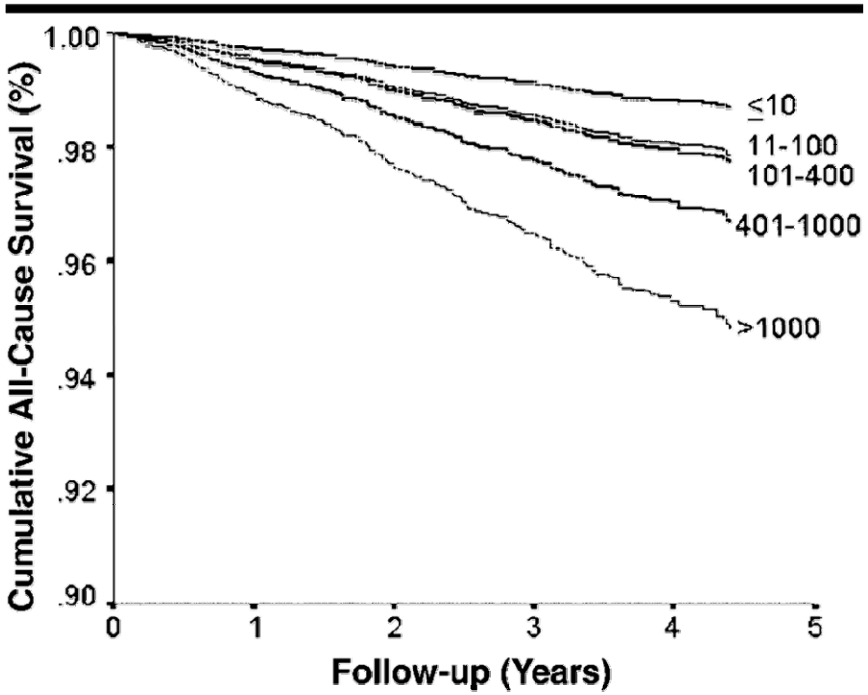
	CAC Grouping						
Groups	0	1–9	10–99	100–399	400–999	≥1000	p Value*
All CAC patients (n = 1,153)							
Patients (n)	252	52	205	274	230	140	
% ischemia	1.2%	1.9%	1.5%	4.0%	7.8%	20.0%	<0.0001
Ischemic (n = 64)							
Ischemic patients (n)	3	1	3	11	18	28	
Early cath (<60 days)	1 (33%)	1 (100%)	1 (33%)	6 (55%)	11 (61%)	13 (46%)	0.99
Revascularized <60 days	0 (0%)	0 (0%)	0 (0%)	5 (46%)	9 (50%)	13 (46%)	0.06
Revascularized ≥60 days	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	1 (5.6%)	3 (10.7%)	0.41
CD/MI*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7.1%)	0.23
Annualized CD/MI rate	0%/yr	0%/yr	0%/yr	0%/yr	0%/yr	2.7%/yr	0.88
Nonischemic (n = 1,089)							
Nonischemic patients (n)	249	51	202	263	212	112	
Early cath (<60 days)	2 (0.8%)	1 (2.0%)	0 (0%)	5 (1.9%)	3 (1.4%)	6 (5.4%)	0.01
Revascularized <60 days	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2.7%)	0.007
Revascularized ≥60 days	2 (0.8%)	0 (0%)	6 (3.0%)	6 (2.3%)	11 (5.2%)	7 (6.3%)	0.001
CD/MI*	1 (0.4%)	0 (0%)	0 (0%)	2 (0.8%)	6 (2.8%)	2 (1.8%)	0.02
Annualized CD/MI rate	0.2%/yr	0%/yr	0%/yr	0.3%/yr	1.0%/yr	0.6%/yr	0.10

CAC score as a prognostic marker

Unadjusted for Risk



Adjusted for Risk

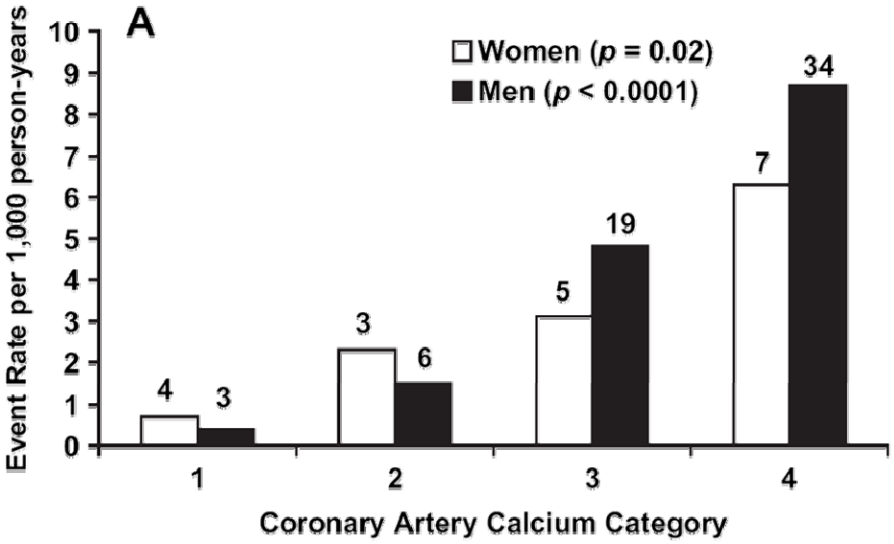


Survival according to baseline CAC score

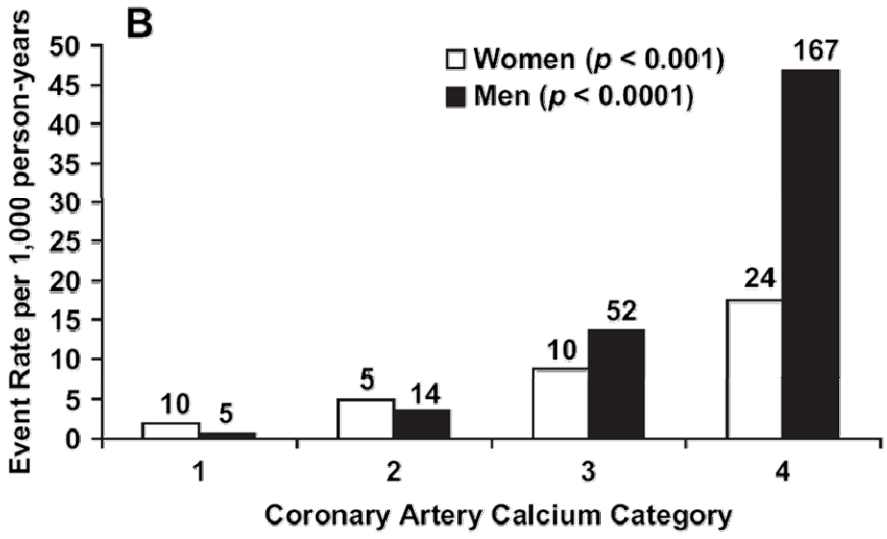
Shaw et al, Radiology, 2003

CAC score and CV event

Death + MI



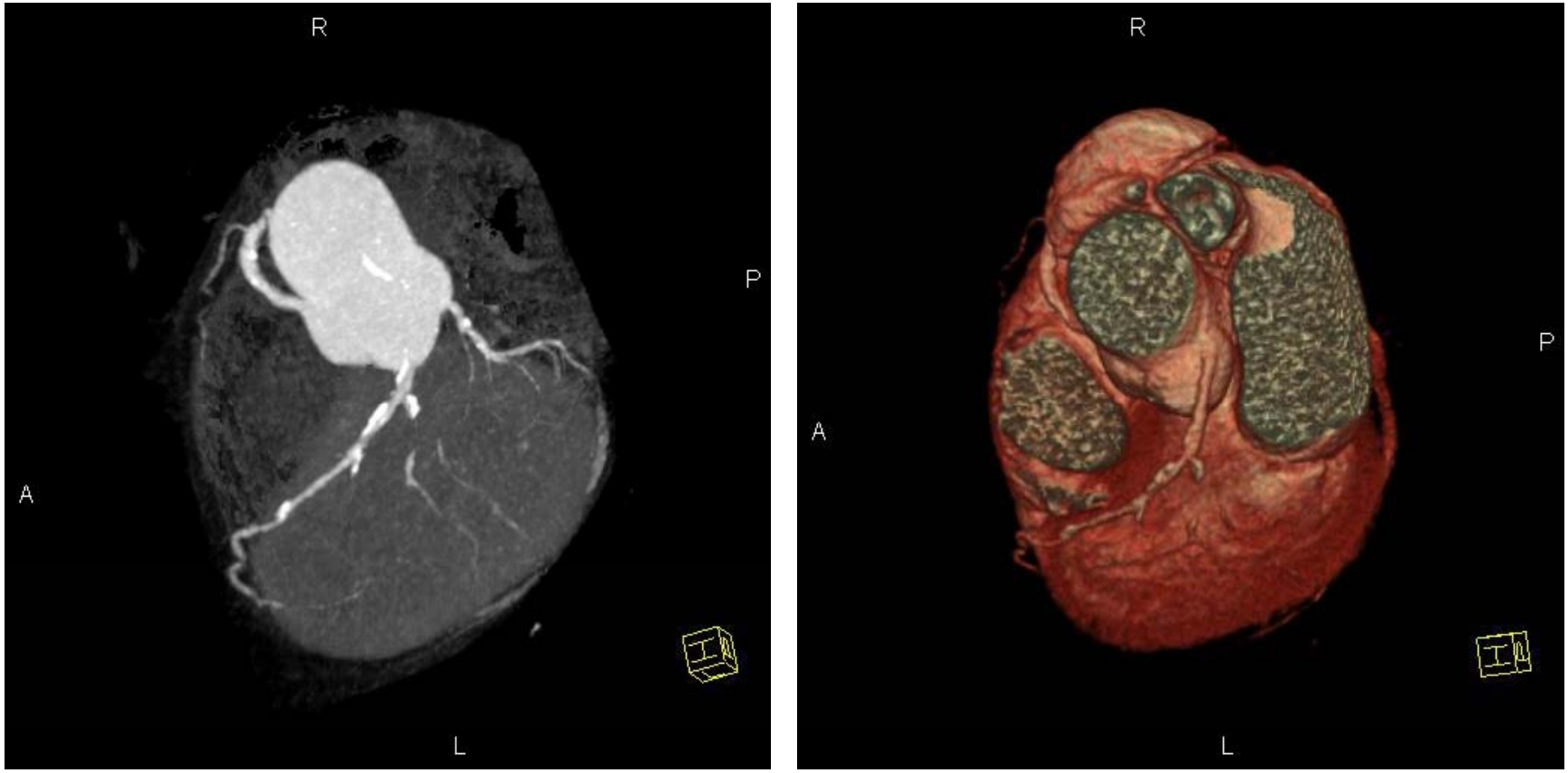
Death + MI + Revasc



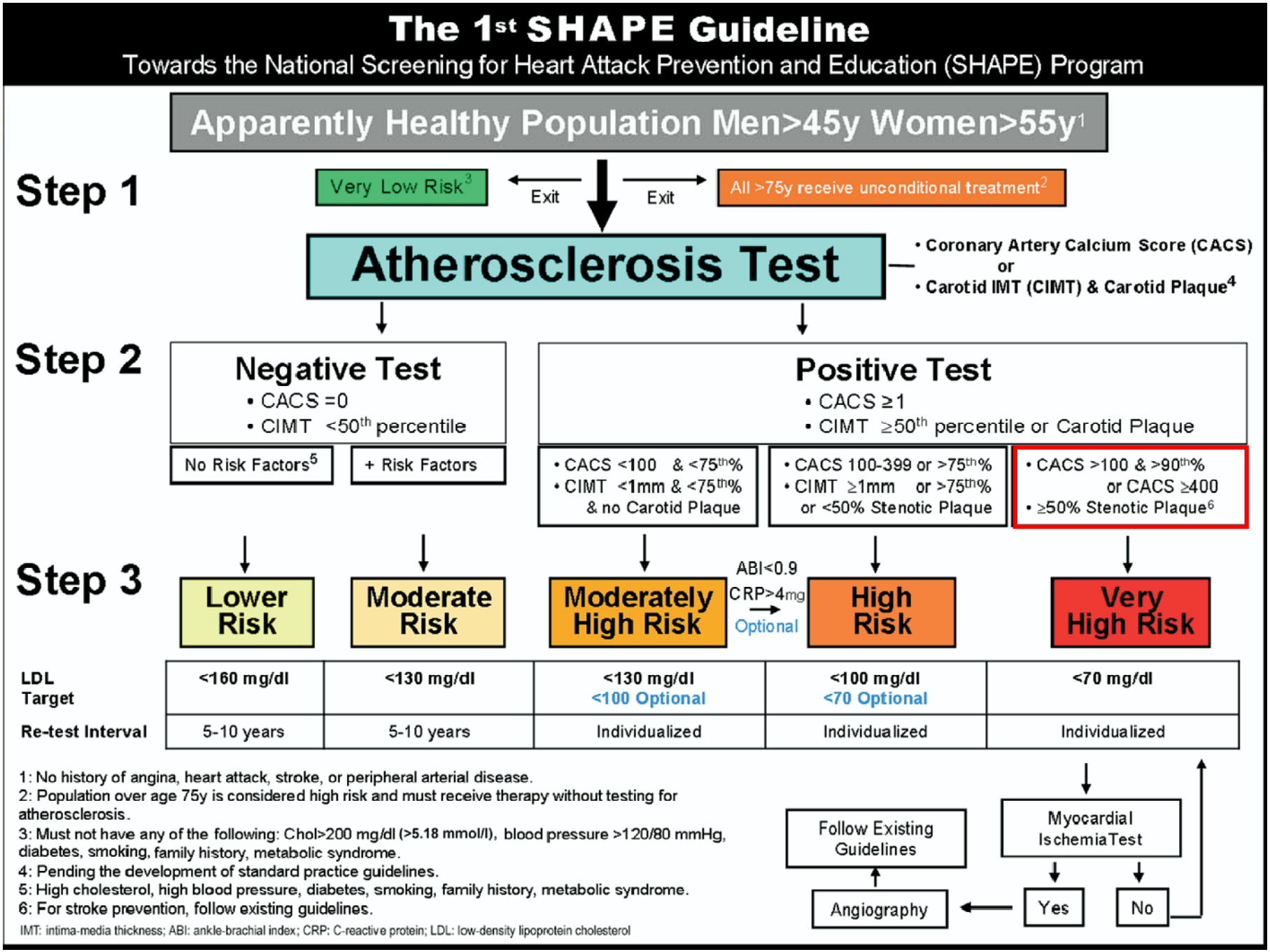
Cooper Clinic Study

LaMonte et al, Am J Epidemiol 2005

Vascular calcification : clinical significance



mLAD 70%, dLCx 50%, mRCA 50% with calcification



Naghavi et al, Am J Cardiolgoy, 2006