

초청 강연 III

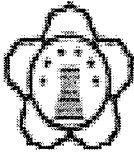
Discovery of Atherosclerosis-specific Targeting
Peptides using Phase Display

- 좌장 | 삼성서울병원 김영욱

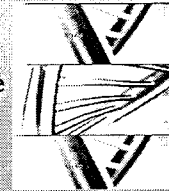
- 경북의대 이병현

Discovery of Atherosclerosis-Specific Targeting Peptides Using Phage Display

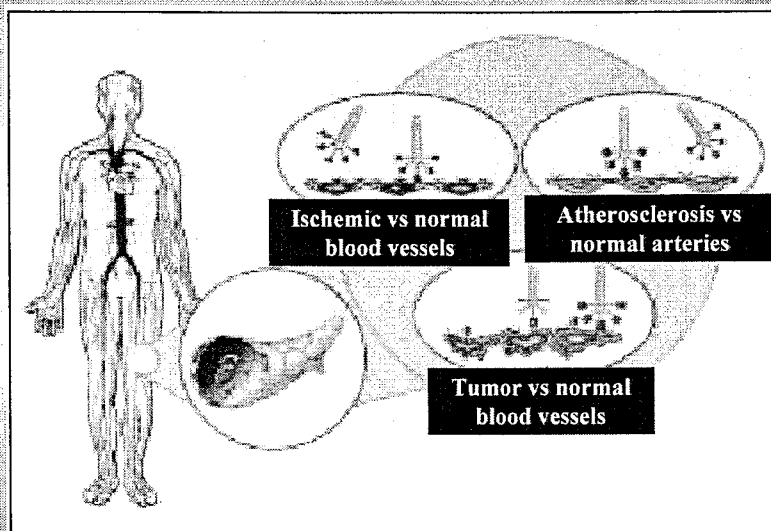
Byung-Heon Lee, M.D., Ph.D.

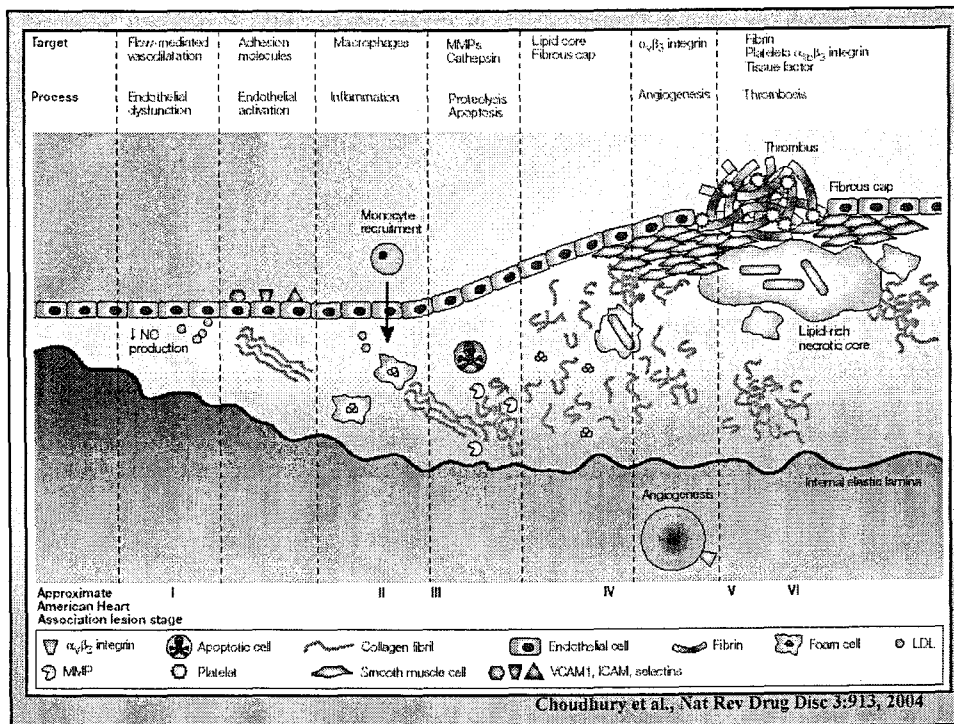


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Molecular diversity of vasculatures

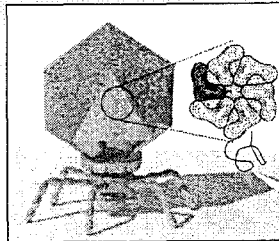
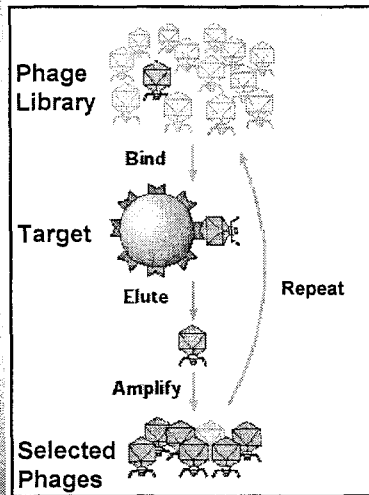




Current imaging targets and ligands for atherosclerosis

Target	Ligand	Agent
Vasa vasorum		
• $\alpha_v\beta_3$ integrin	• RGD peptide	• LEFPC
• $\alpha_v\beta_3$ integrin	• ED-B fibronectin Ab	• ^{125}I -radioisotope
Macrophage		
• CCR-2	• MCP-1	• ^{125}I
• Phagocytosis	• USPIO	• USPIO
• MMP	• MMP polyinhibitor	• ^{111}In
• Scavenger receptors	• Antibody	
• Apoptosis	• Annexin V	• Tc-99m
Endothelial cell		
• GRP78	• CAPGPSKSC	

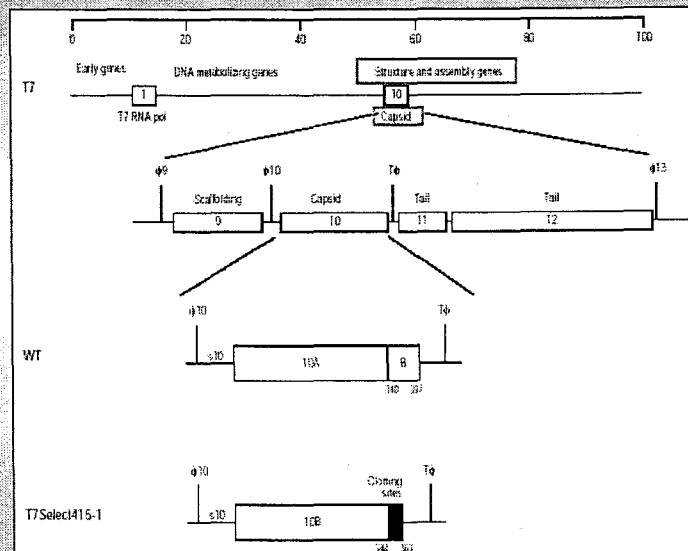
Biopanning using phage display



Displayed peptide

Double-stranded DNA bacteriophage
 Icosahedral particle (diameter 55nm)
 Head, 1 conical tail, 6 tail fibers
 415 copies of T7 capsid protein –
 10A (344 aa) and 10B (397 aa)

Genetic map of T7 phage



Constructing recombinant T7 phage

(aa 351)

EcoRI

HindIII

GAATTCCTGCNNKNNKNNKNNKNNKNNKNNKNNKNNKTTGCTAAGCTT

CTTAAGGACGNNMNNMNNMNNMNNMNNMNNMNNMNNMACGATTCGAA

N S C X X X X X X X C * A

(N = A or C or G or T)

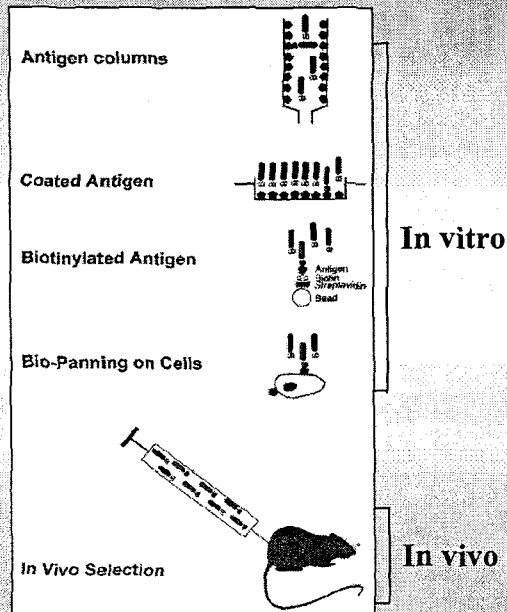
(K = G or T)

(M = A or C)

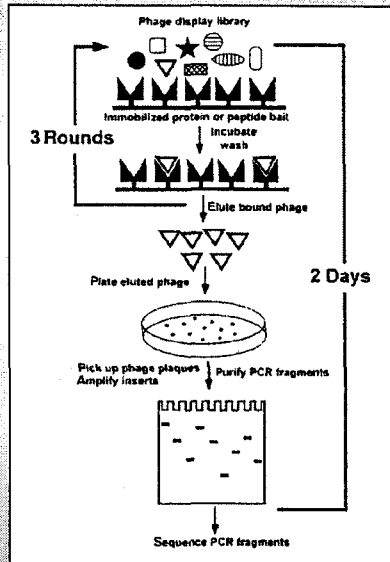
EcoRI-HindIII digestion
Ligation & Packaging

T7 Phage-displayed peptide libraries
(CX₇C variety: $20^7 = 1.28 \times 10^9$)

Approaches for phage display

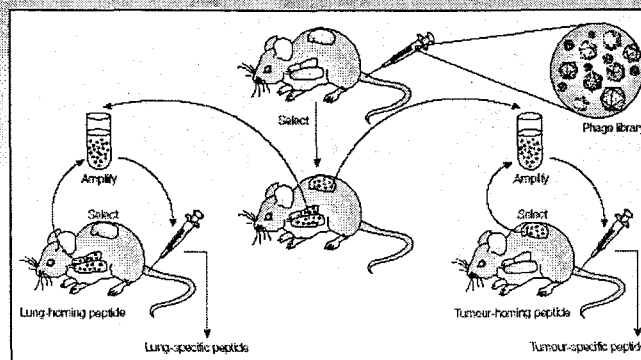


In vitro phage display



- Map protein-peptide interactions
- Identify peptides with special function
 - Receptor antagonists or agonists
 - Mimics of epitopes (peptidomimetics)
 - Enzyme inhibitors
- Identify cell-specific markers

In vivo phage display



- Tissue or vascular targeting ligand mimetics
 - Specific organs (e.g., lung)
 - Tumor blood vessels (angiogenesis)
 - Atherosclerotic plaques
 - Vascular injury & restenosis

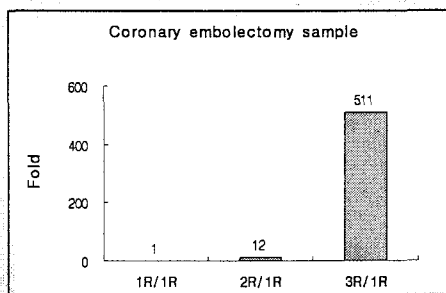
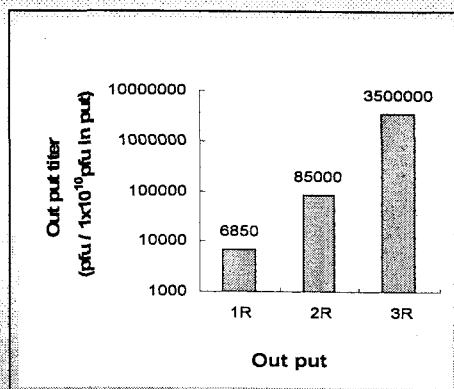
Ruoslahti, Nat Rev Cancer, 2002

Experimental approach to discover atherosclerosis-specific peptide

1. Coronary or femoral artery atherectomy specimens
 - 경북대병원 심장내과/혈관외과
 - Cell homogenates
 - In vitro / Ex vivo phage display
2. LDL receptor knockout mouse
 - 이화여대
 - Atherosclerosis mouse model
 - In vivo phage display

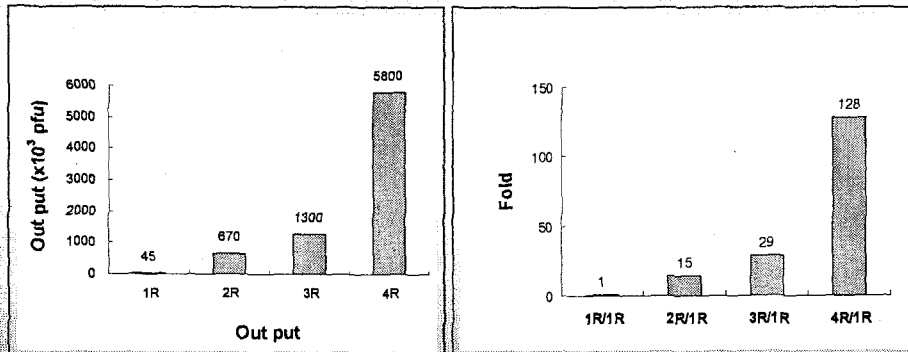
Biopanning of phages selective for human atherosclerotic plaques

Coronary atheroma



Biopanning of phages selective for human atherosclerotic plaques

Femoral atheroma



Peptides selected for human atherosclerotic plaques

Coronary arteries

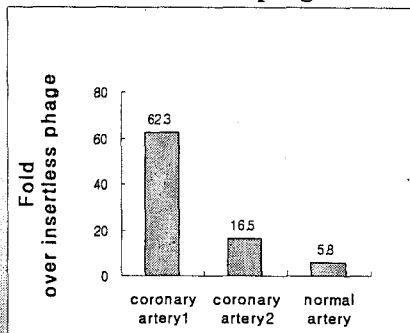
Clones	Frequency
Atheroma-1	(8/27)
Atheroma-2	(4/27)

Femoral arteries

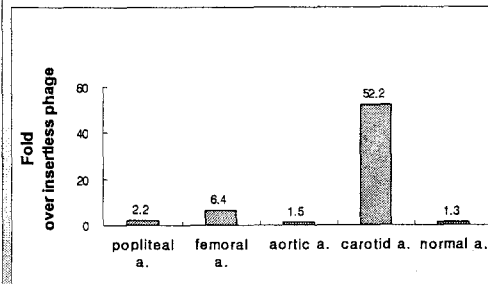
Clones	Frequency
Atheroma-3	(3/27)

Ex vivo binding of phages to human atherosclerotic plaques

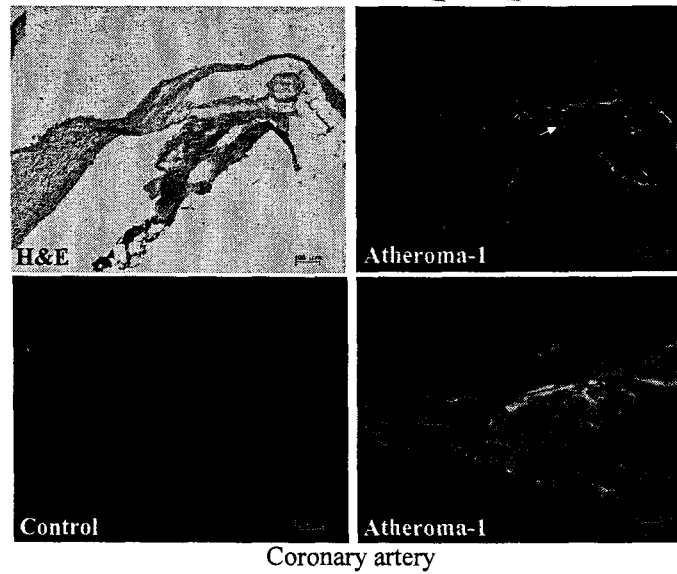
Atheroma-1 phage



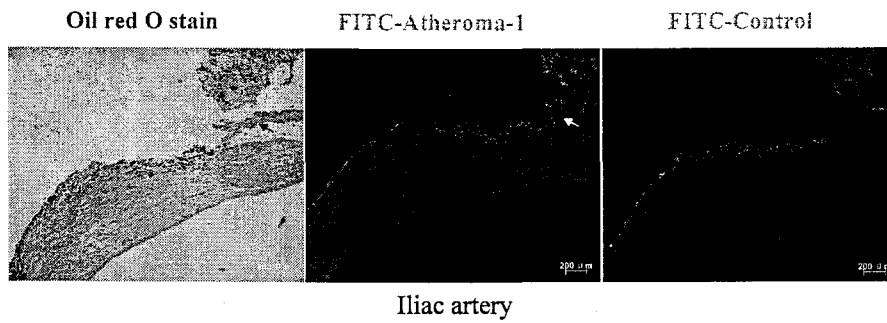
Atheroma-3 phage



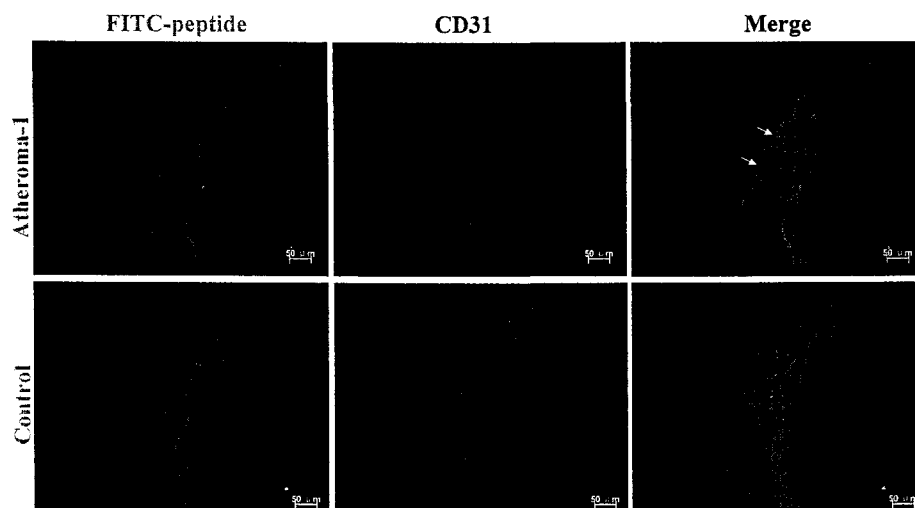
Atheroma-1 peptide overlay on human atherosclerotic plaques



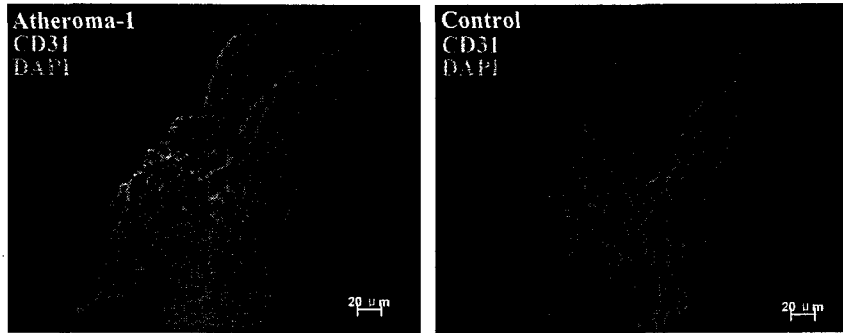
Atheroma-1 peptide overlay on human atherosclerotic plaques



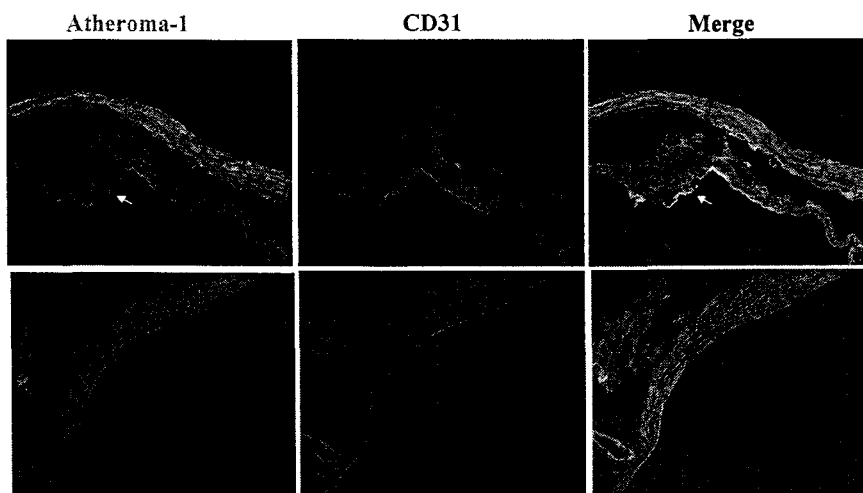
Atheroma-1 peptide overlay on atherosclerotic aorta in LDLr -/- mice



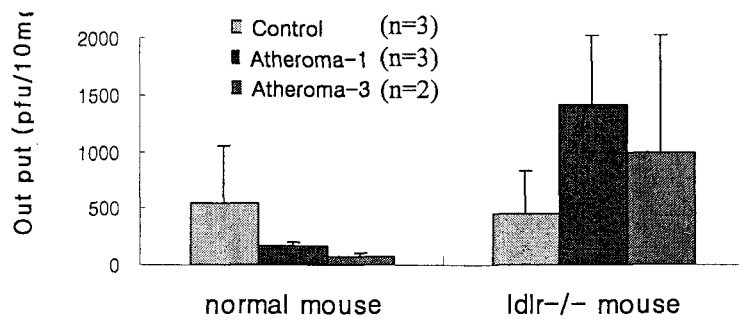
**Atheroma-1 peptide overlay on
atherosclerotic aorta in LDLr^{-/-} mice**



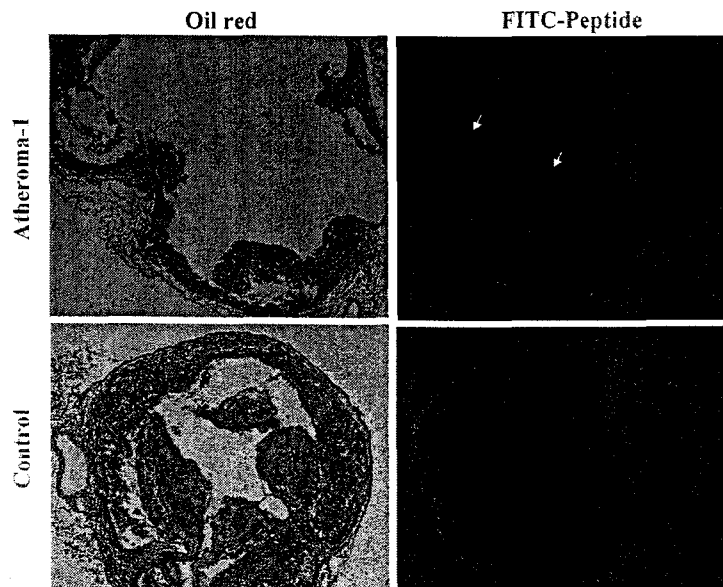
**Atheroma-1 peptide overlay on
atherosclerotic aorta in LDLr^{-/-} mice**



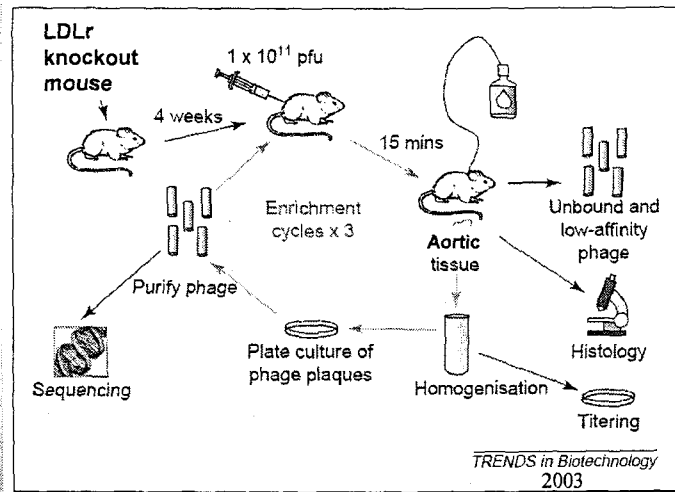
In vivo homing of phages to atherosclerotic aorta in LDLr -/- mice



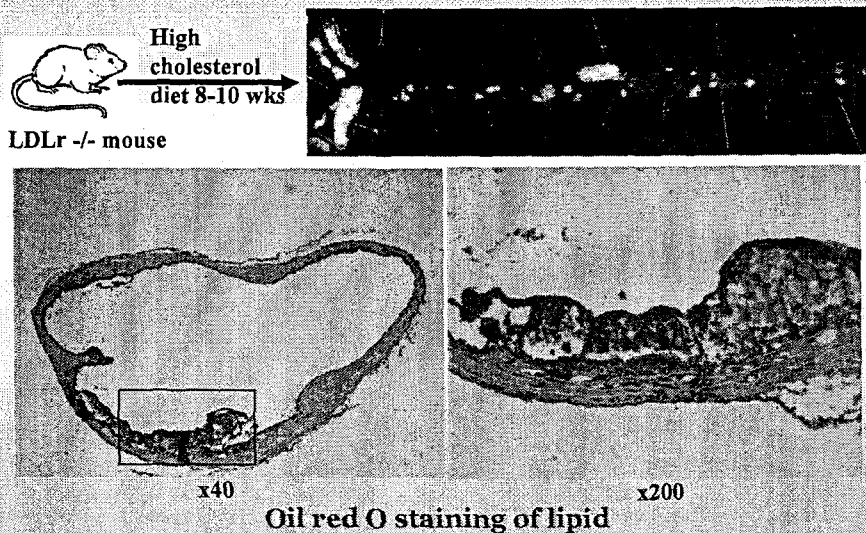
In vivo homing of Atheroma-1 peptide to atherosclerotic plaques in LDLr -/- mice



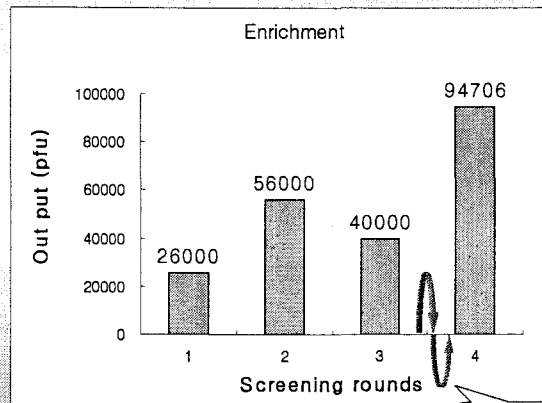
In vivo phage display with LDLr ^{-/-} mouse



Atherosclerotic plaques in LDL receptor knockout mice



In vivo biopanning of phages homing to atherosclerotic plaques in LDLr ^{-/-} mice



Negative selection

Peptides selected by in vivo phage display

Clones	Frequency
LDL-1	7/30
LDL-2	3/30
LDL-3	2/30

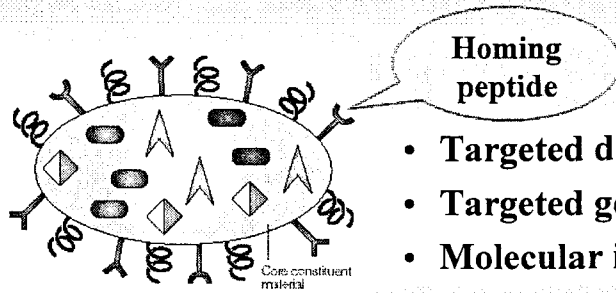
Phage display as a scaffold for ligand mimetics

Peptide	Proteins containing homologous sequences	Accession number
Atheroma-1	IL-4 precursor	P46652
	Monocyte differentiation antigen CD14 precursor	P10810
LDL-1	Kininogen precursor; contains bradykinin	O08677
	LDL receptor-related protein 4 (ANP-converting enzyme)	Q9Z319

Future directions

- Construction of nanovectors coated with the homing peptides
- Targeted delivery of the nanovector to atherosclerotic plaques and molecular imaging in animal models

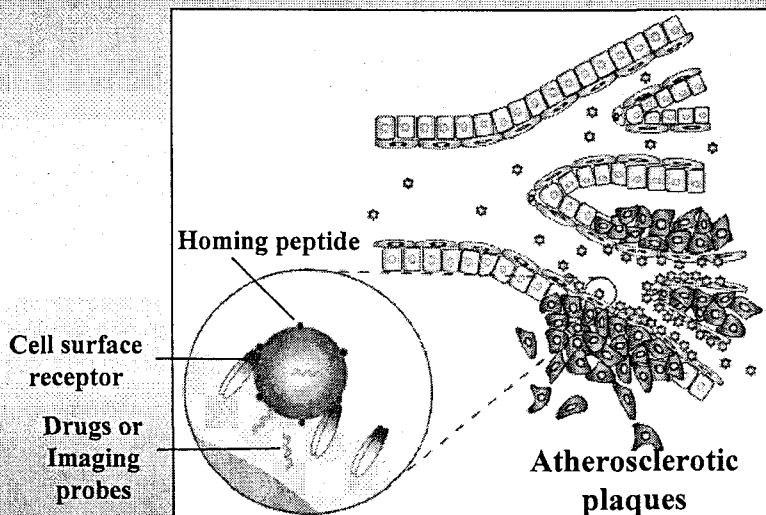
Multifunctional nanovectors or "smart probe"



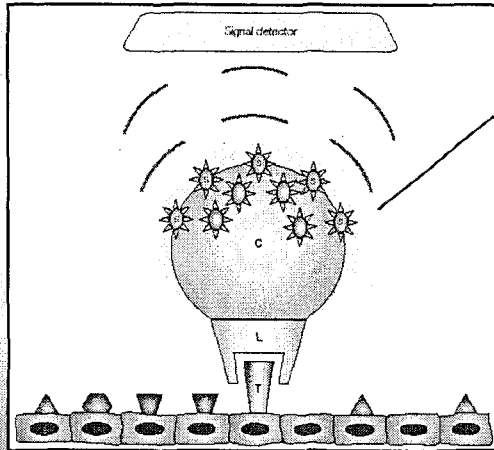
Therapeutic or imaging payload	Biological surface modifier
Drug A	PEG
Drug B	Targeting moieties
Contrast enhancer	
Permeation enhancer	

- Targeted drug delivery
- Targeted gene therapy
- Molecular imaging
- Higher efficacy
- Less side effects
- Earlier diagnosis

Model for targeting atherosclerosis by nanovectors coated with homing peptides



Model for targeted molecular imaging of atherosclerosis

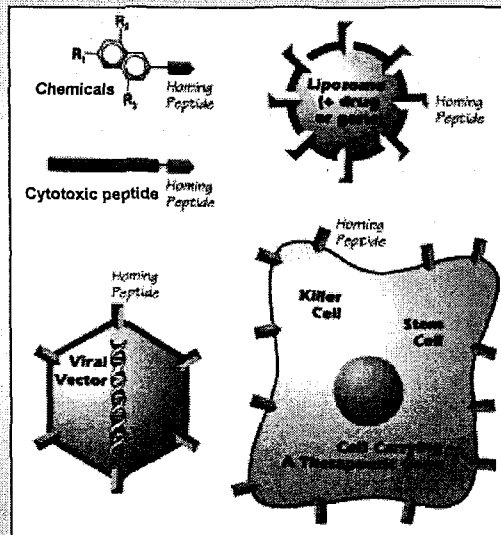


Signals:

- Radioisotopes (PET)
- Fluorescence dyes (Optical imaging)
- Luminescence dyes (Optical imaging)

Nat Rev Drug Disc, 2004

Potential therapeutic compounds and devices coated with homing peptides



Seminars Cancer Biol, 2000