Radiolabeled Adenoviral Sub-unit Proteins for Molecular Imaging and Therapeutic Applications in Oncology

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Overall Objective

To develop and optimize new ligand systems, based on adenoviral vectors (intact adenovirus, adeno-viral fiber protein, and the knob protein), for delivering suitable radionuclides into tumor cells for molecular imaging and combined gene/radionuclide therapy of cancer.

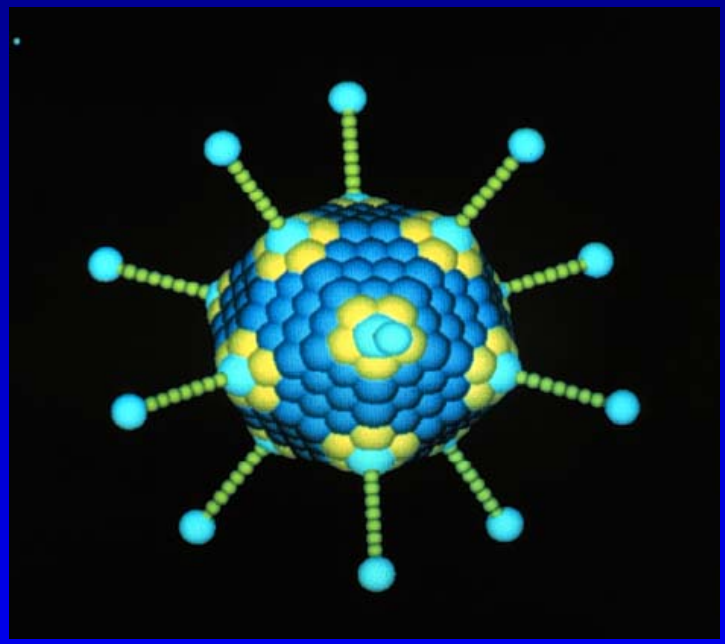
Issues Involved in Combined Gene/Radioimmunotherapy

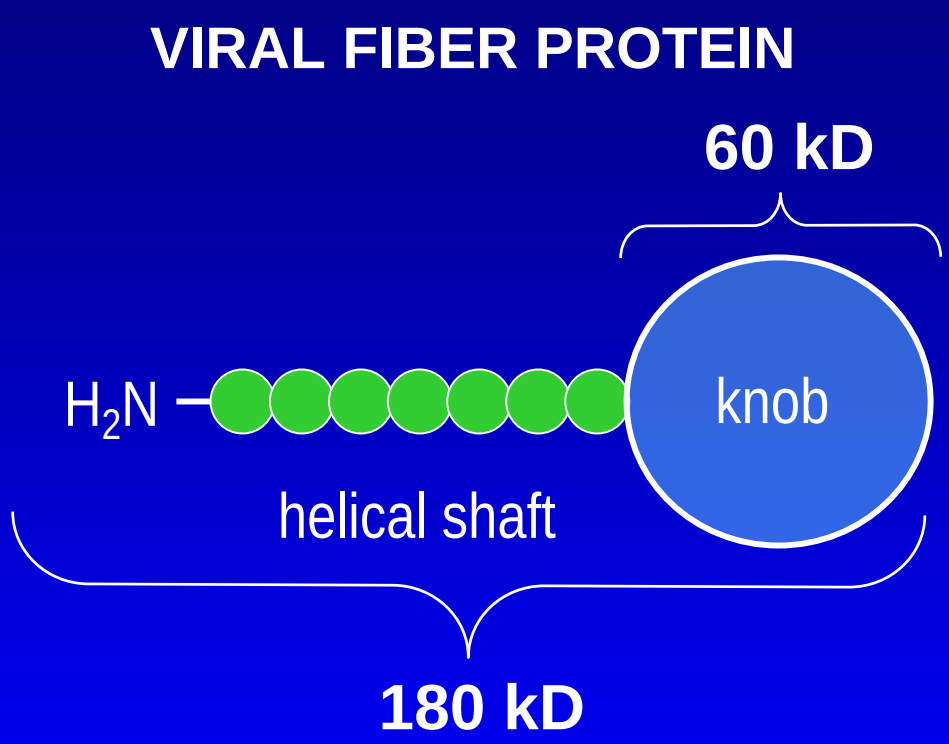
- Current clinical trials of gene therapy using adenoviral vectors involve non-systemic delivery of therapeutic genes
- Distribution and delivery of viral subunit proteins following i.v. administration not understood and must be studied and optimized
- Retention of the selective binding and internalization into tumor cells of radiolabeled viral vectors is an unmet challenge

Radionuclides Suitable for Gene/ Radioisotopic Imaging and Therapy

Imaging		Therapy		
PET	SPECT	ß⁻	Auger	α's
F-18	Tc-99m	Sc-47	Ga-67	At-211
Co-55	In-111	Cu-67	I-125	Bi-212
Ga-68	I-123	Sn-117m	Hg-195m	Bi-213
I-124	Pb-203	I-131	Tl-201	Ac-225

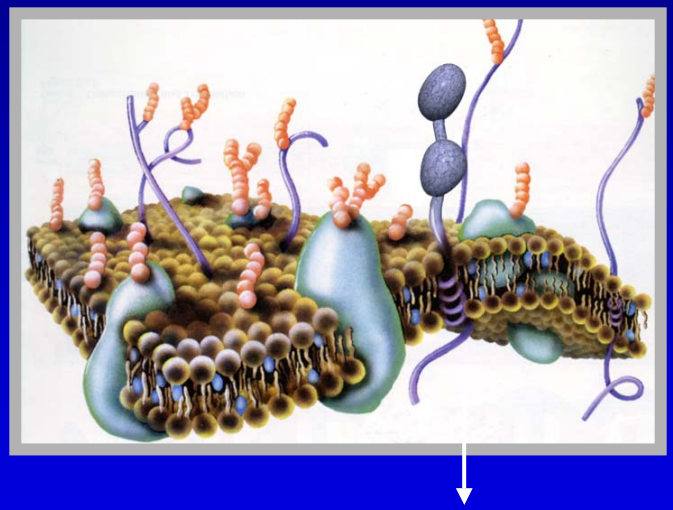
Adenovirus Binding to CAR



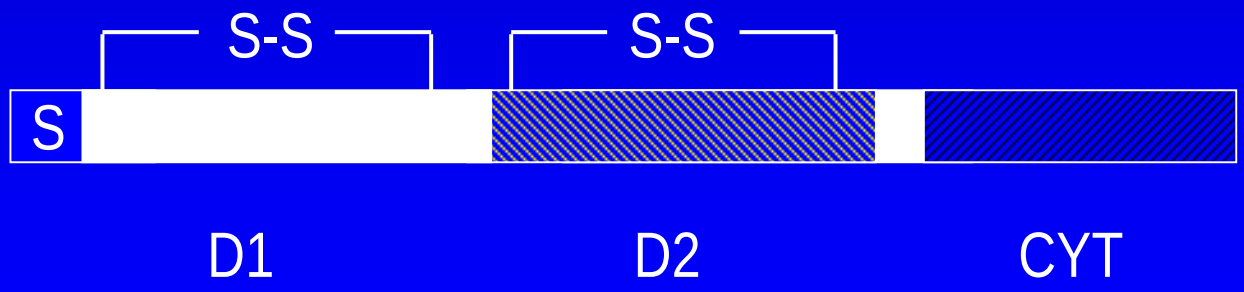


- purified fiber binds to CAR and blocks infection
- knob domain carries CAR-binding activity

CAR: Coxsackievirus and Adenovirus Receptor



- 46 kD Ig superfamily member
- 2 extracellular Ig-like domains: D1 and D2



In-vivo Studies

Viral components used

- Intact adenovirus (Ad, ~80 nm diam.)
- Native adenoviral fiber protein (AdFP, 180 kD trimer); purified from infected human cultured cells
- Fiber knob protein (AdFKP, 60 kD); recombinant, synthesized in E. Coli
- AdFKP-DTPA conjugates

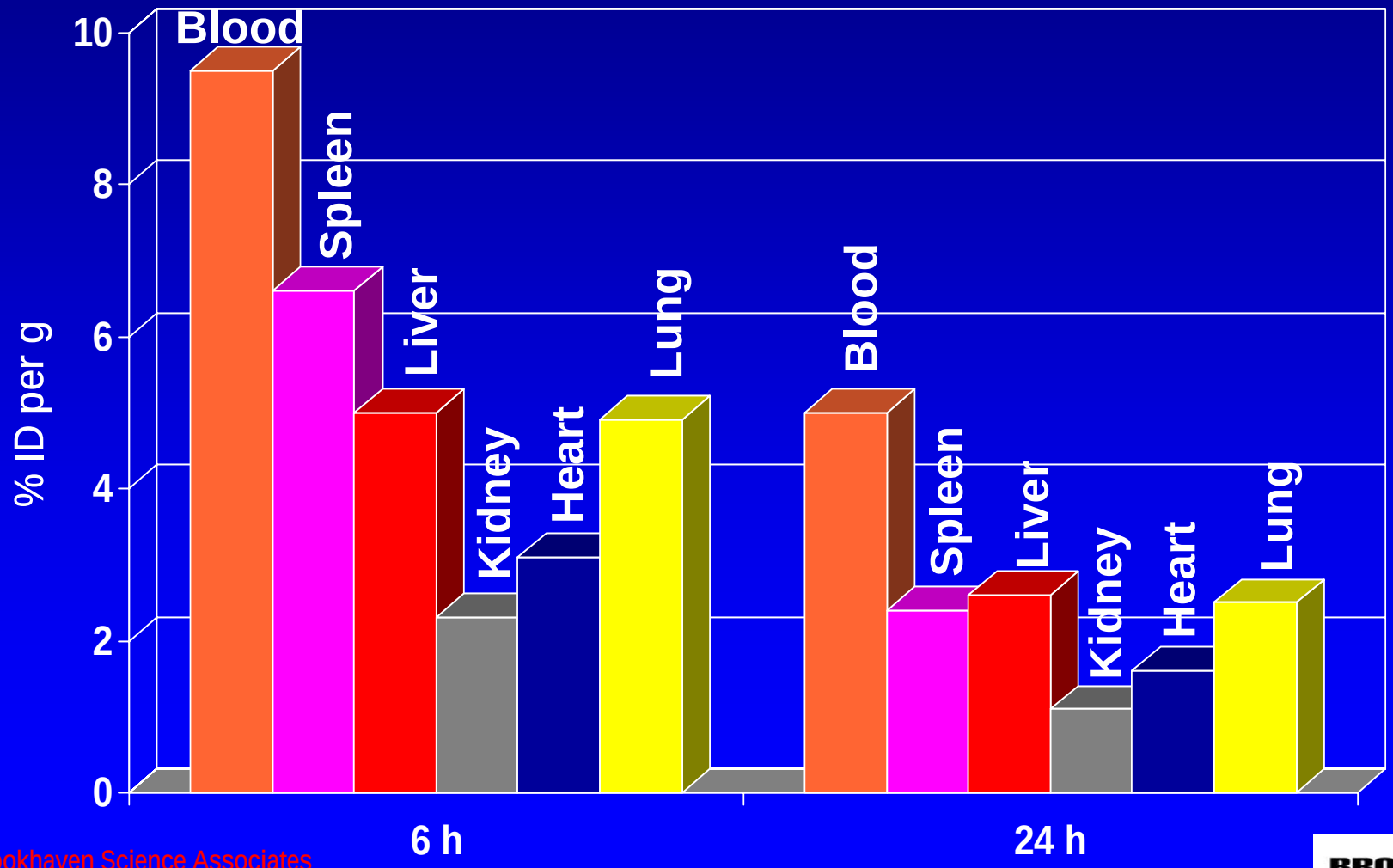
In-Vivo Studies, Cont'd

- Viral components radiolabeled with I-131 and In-111
  - Binding activity determined using reaction with biotinylated CAR followed by chemiluminiscent detection
- In-vivo distribution studied in mice and rats (i.v. injection)
  - Whole body autoradiography
  - Tissue sample counting

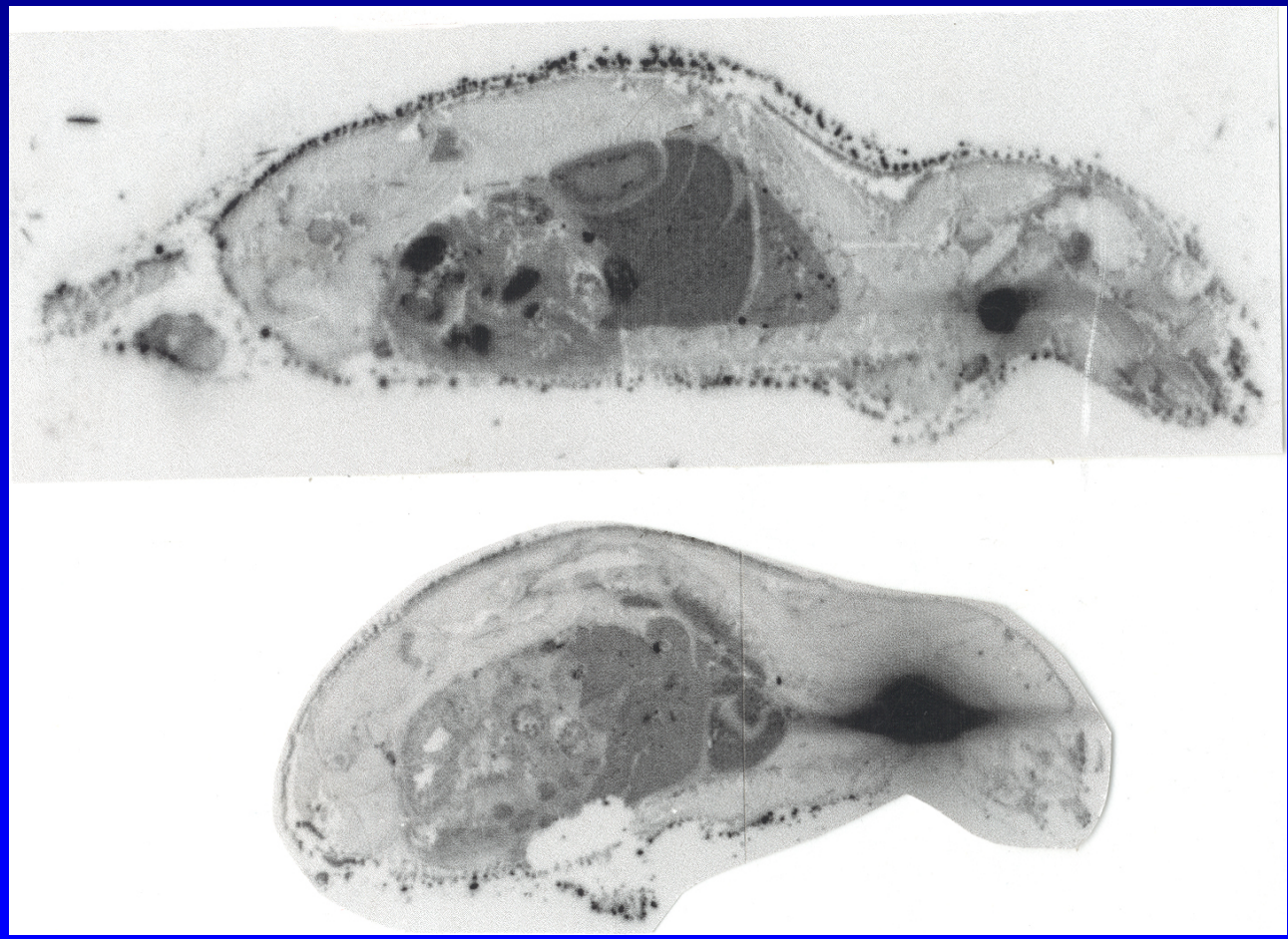
Preliminary Results

- Ad vectors localized (i.v.) preferentially in CAR-expressing organs (e.g., lung, liver) .
- CAR binding of radioiodinated AdFP and AdFKP is maintained.
- In-111-6-His-AdFKP-DTPA retains binding specificity to CAR . Histidine appears to have a protective effect.
- CAR binding of Ad-2 wild serotype is better (~8x stronger) than Ad-12, in particular following radiolabeling.

Biodistribution of I-131-AdFP in Mice



Whole body autoradiography in rats 6h (top) and 24h (bottom) following the injection of ^{131}I-labeled adenoviral fiber protein (AdFP)



Conclusions

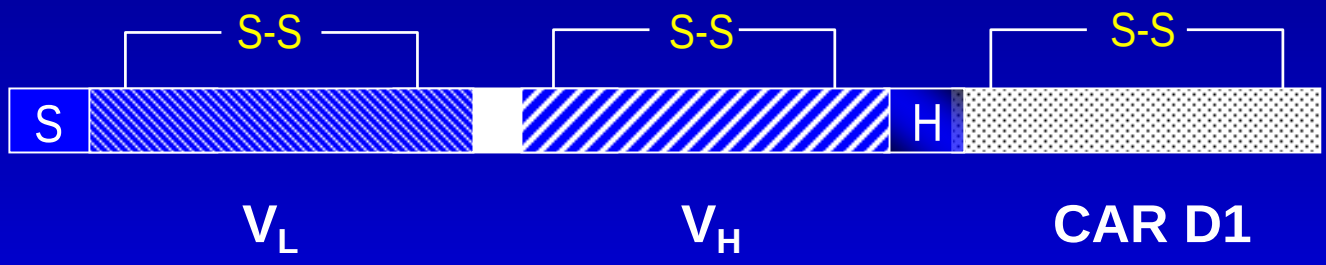
- Whole virus or its sub-unit proteins bind to the natural receptor CAR
- IV administration produces preferential uptake into CAR expressing organs (e.g., lungs, liver)
- For targeting tumors with therapeutic genes/radioisotopes, viral tropism for CAR must be altered/modified

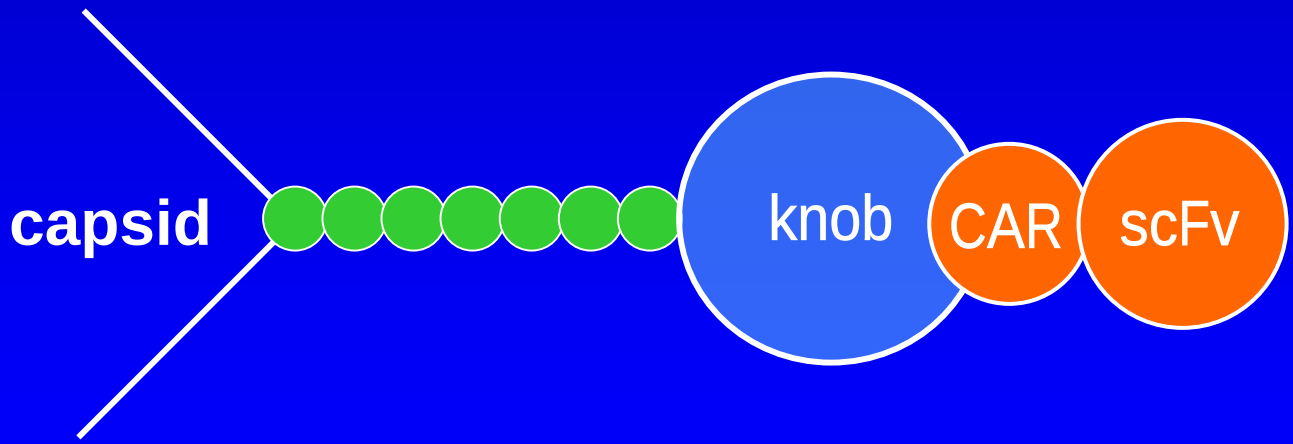
Possible Approaches to Modify Viral Tropism

- Bio-engineer the production of bifunctional fusion proteins
  - Tumor-specific scFv constructs + viral sub-unit proteins
  - scFv-viral constructs using the knob-CAR-binding region
- Bind tumor-specific MAbs/peptides/scFv constructs onto the virus capsid

Adenovirus Retargeting

“Minibody” approach: swap CAR D1 for CH3 domain





Acknowledgements

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