

Projects progress report

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The Fisher Laboratory

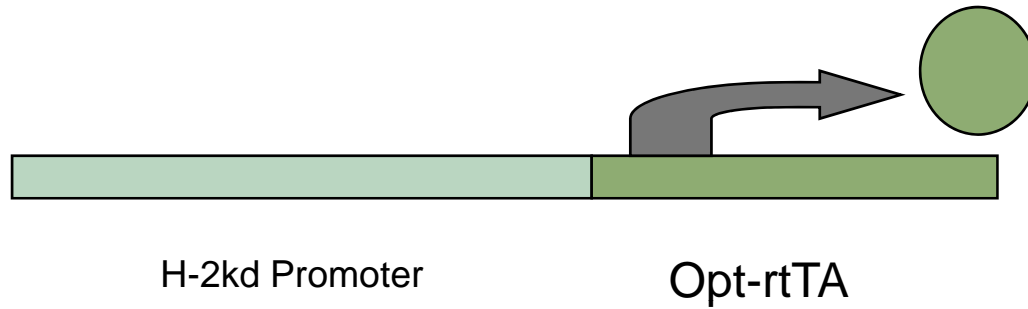
NYU School of Medicine

- Current transgenic model: hAR is up-regulated before hyperglycemic and/or hyperlipidemic conditions.
- **We want a model where hAR can be conditionally up regulated to determine effects after plaque formation begins.**

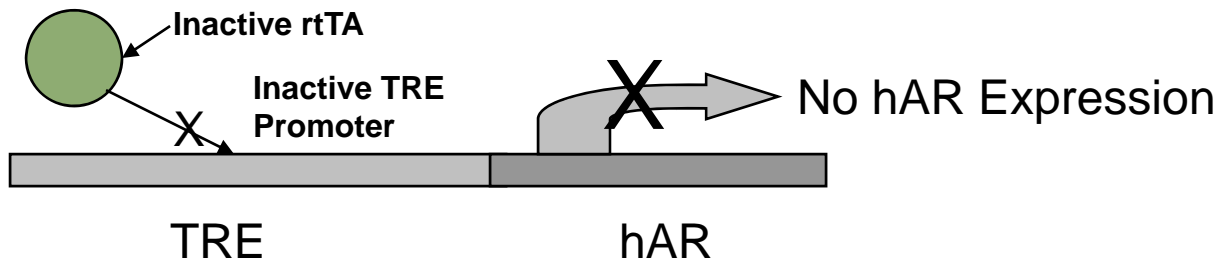
Tetracycline-on (Tet-on) System

- Variation of the Tet-off system
- It is a binary transgenic system in which the expression of a trans gene is dependent on the activation of an inducible transcriptional activator.
- It works by the activation of rtTA via doxycycline binding. Active rtTA binds to TRE to activate it.

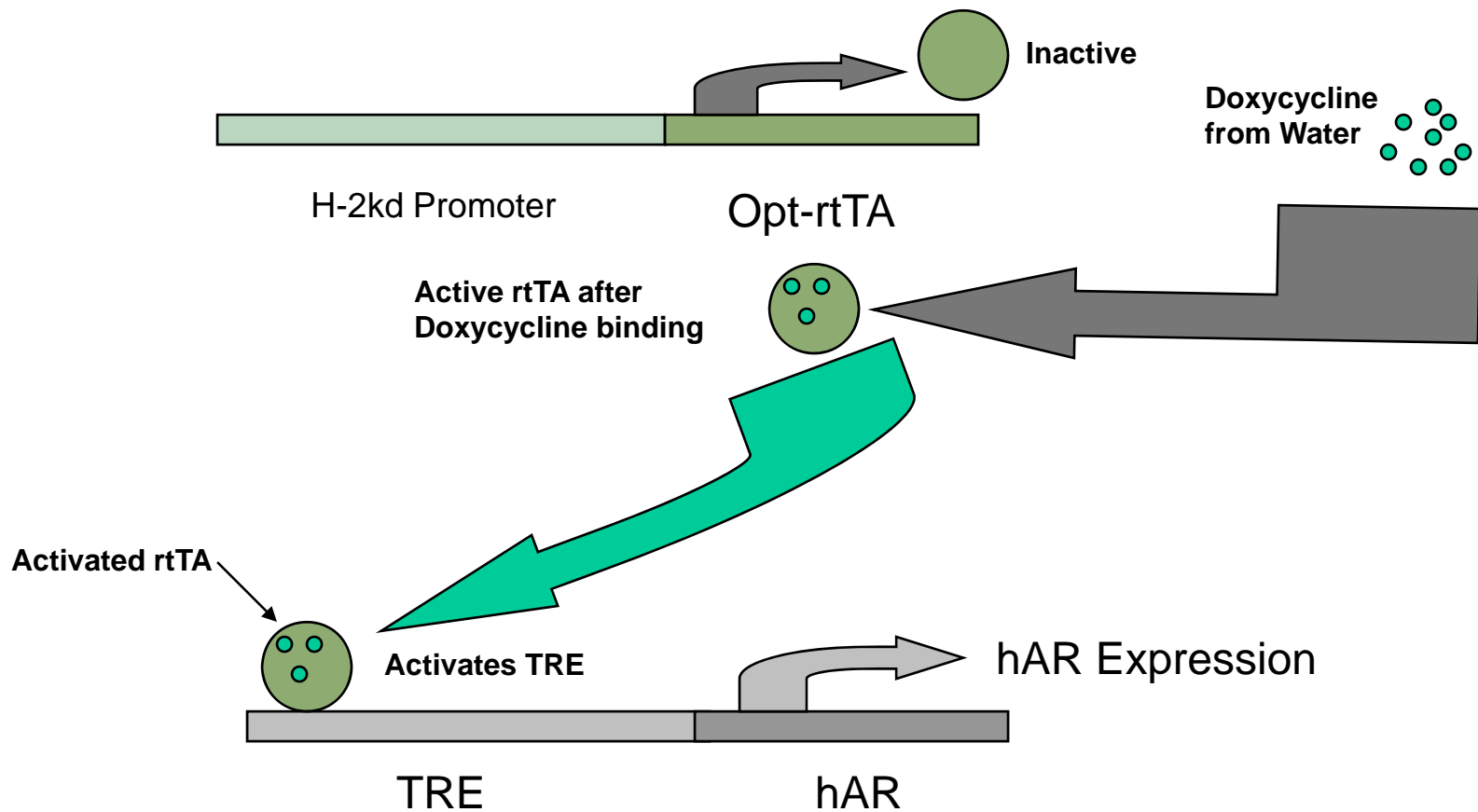
Tet-on system has two constructs



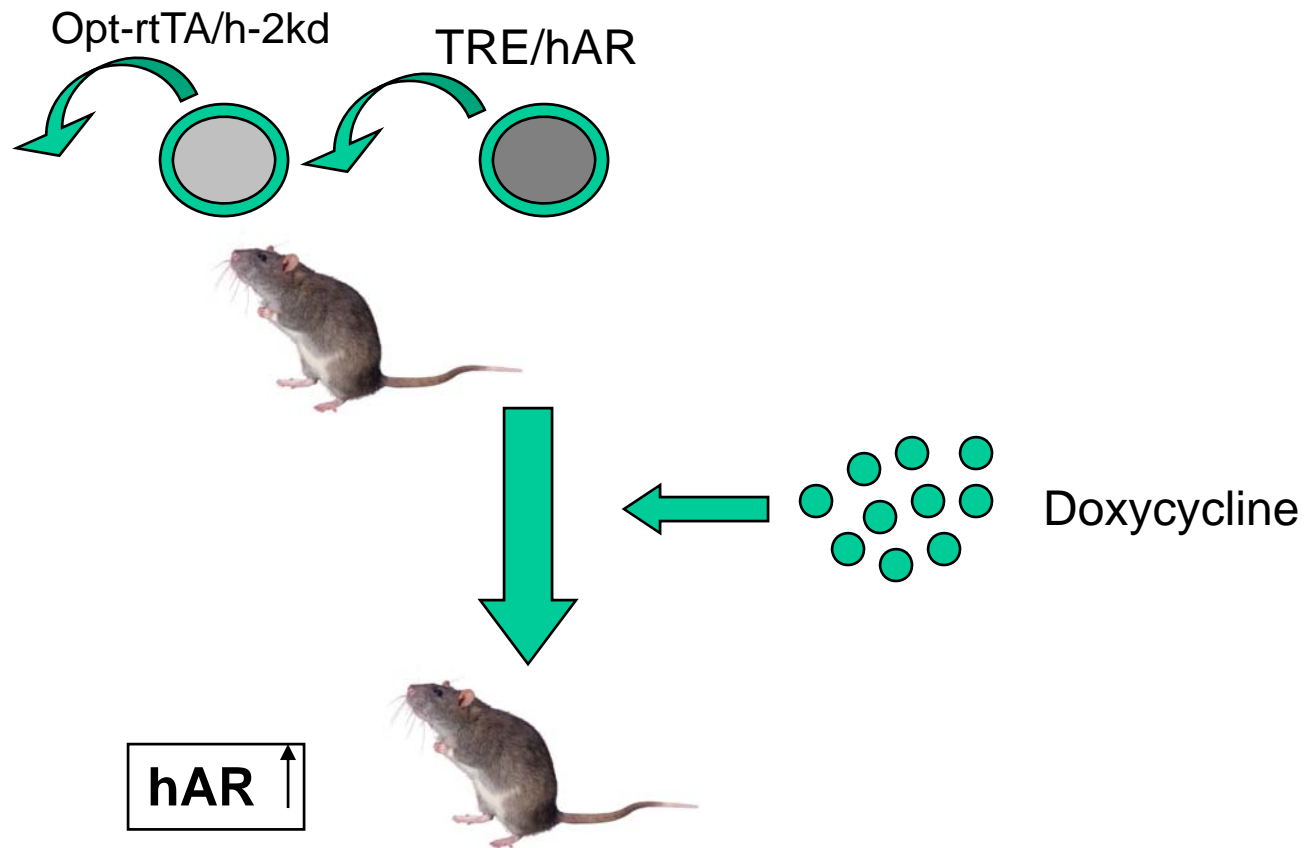
No Doxycycline
Present



Mechanism for activation of the Tet-on system



Discussions with JAX: put both plasmids into the LDLR^{-/-} mouse



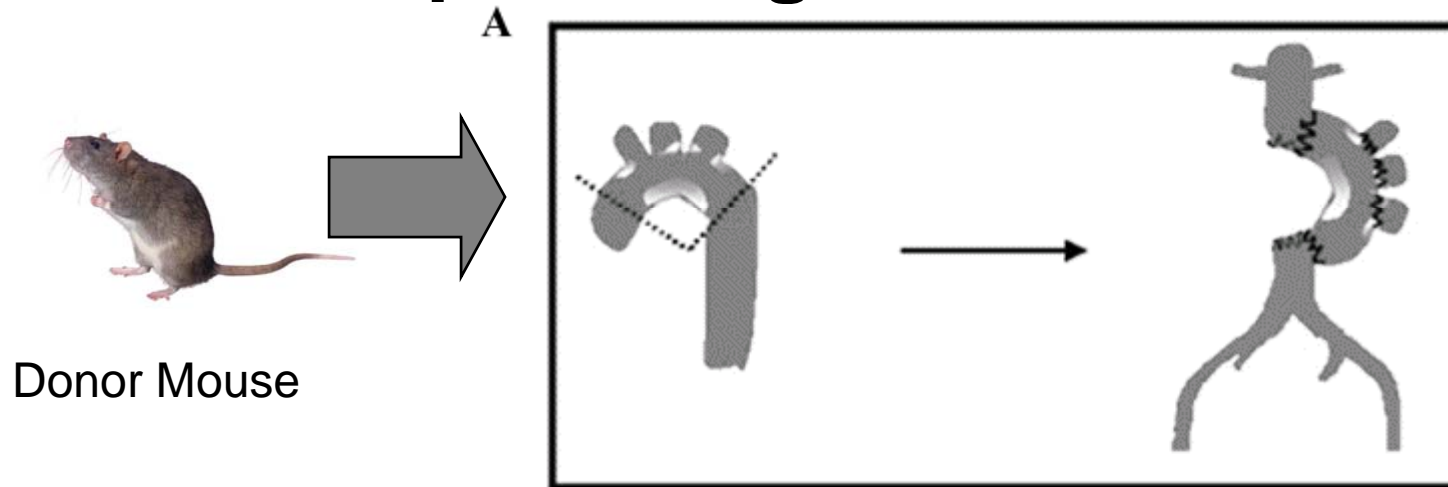
Current status of the project

- The two constructs for making Tet-on controlled transgenic mice, namely rtTA and TRE/hAR, have been made and sequenced
- After confirming their bioactivity in transient transfection study, those constructs will be sent to Jackson laboratory for generating transgenic mice

Atherosclerosis regression study in hAR transgenic mice

- Fisher lab and collaborators have introduced mouse models in which dyslipidemia is rapidly reversed in either a transplantation procedure or by conditional gene inactivation
- Regression of plaques has been observed and molecular pathways identified
- Question: does diabetes impair regression effects of “aggressive lipid management”?

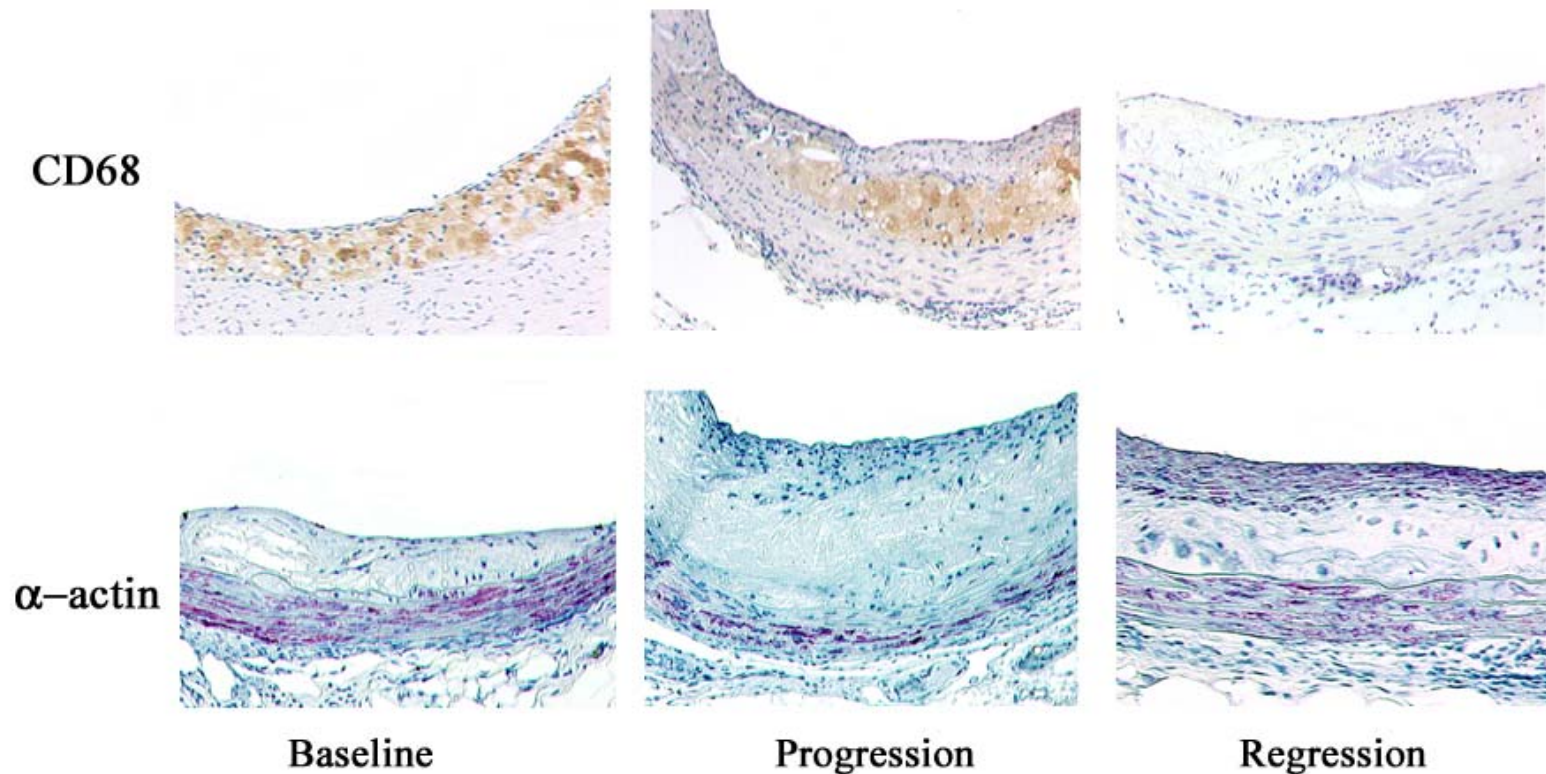
Transplant Regression Model



B



Absence of foam cells and the formation of a fibrous cap 4 weeks after placing lesions into the Wild-type environment



Trogan et al, ATVB, 2004

Experimental design for transplant atherosclerosis regression study

Donor



LDLR^{-/-}



Akita^{+/-}



Akita^{+/-}-hAR



WT



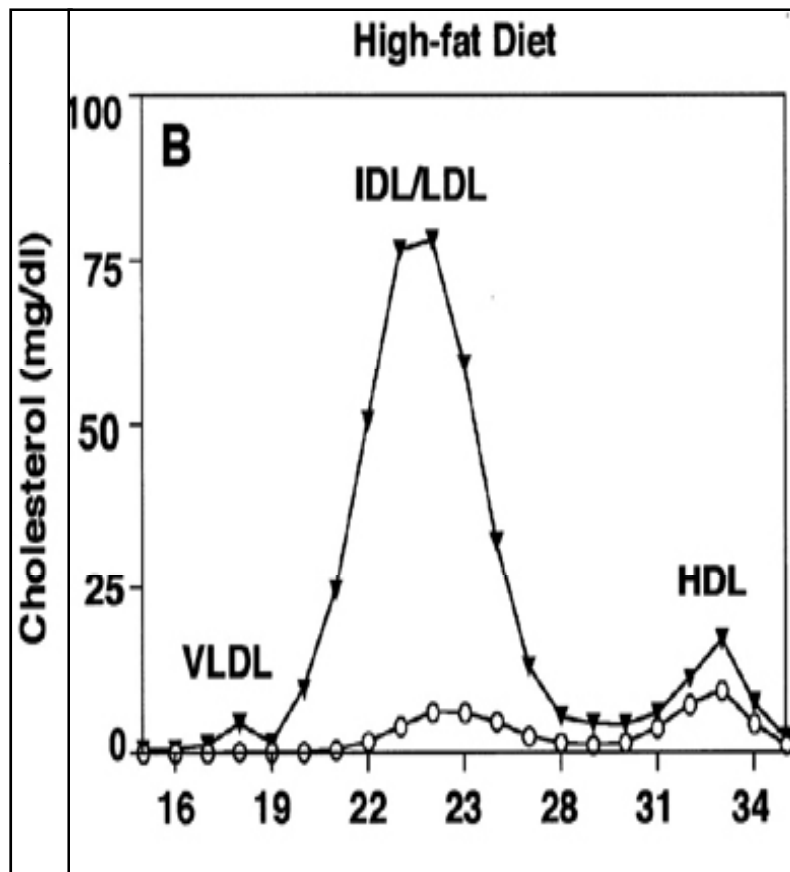
WT/hAR



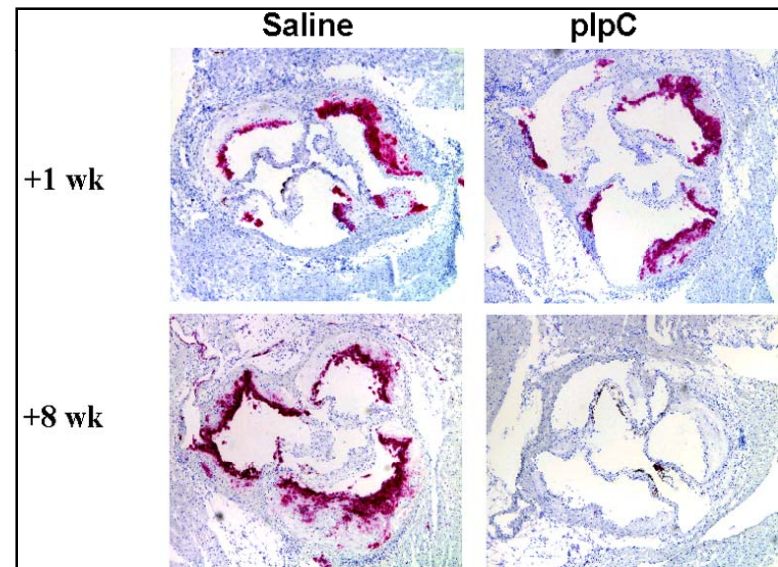
Baseline LDLR^{-/-}

Non-surgical Models of Regression

Changes in plasma lipoprotein profile and Atherosclerosis in REVERSA mice after MTP gene ablation



Lieu et al., Circulation 2003



Rong et al., unpublished

Experimental design for non-surgical regression study



Reversa mice

Western Diet (16 wks)

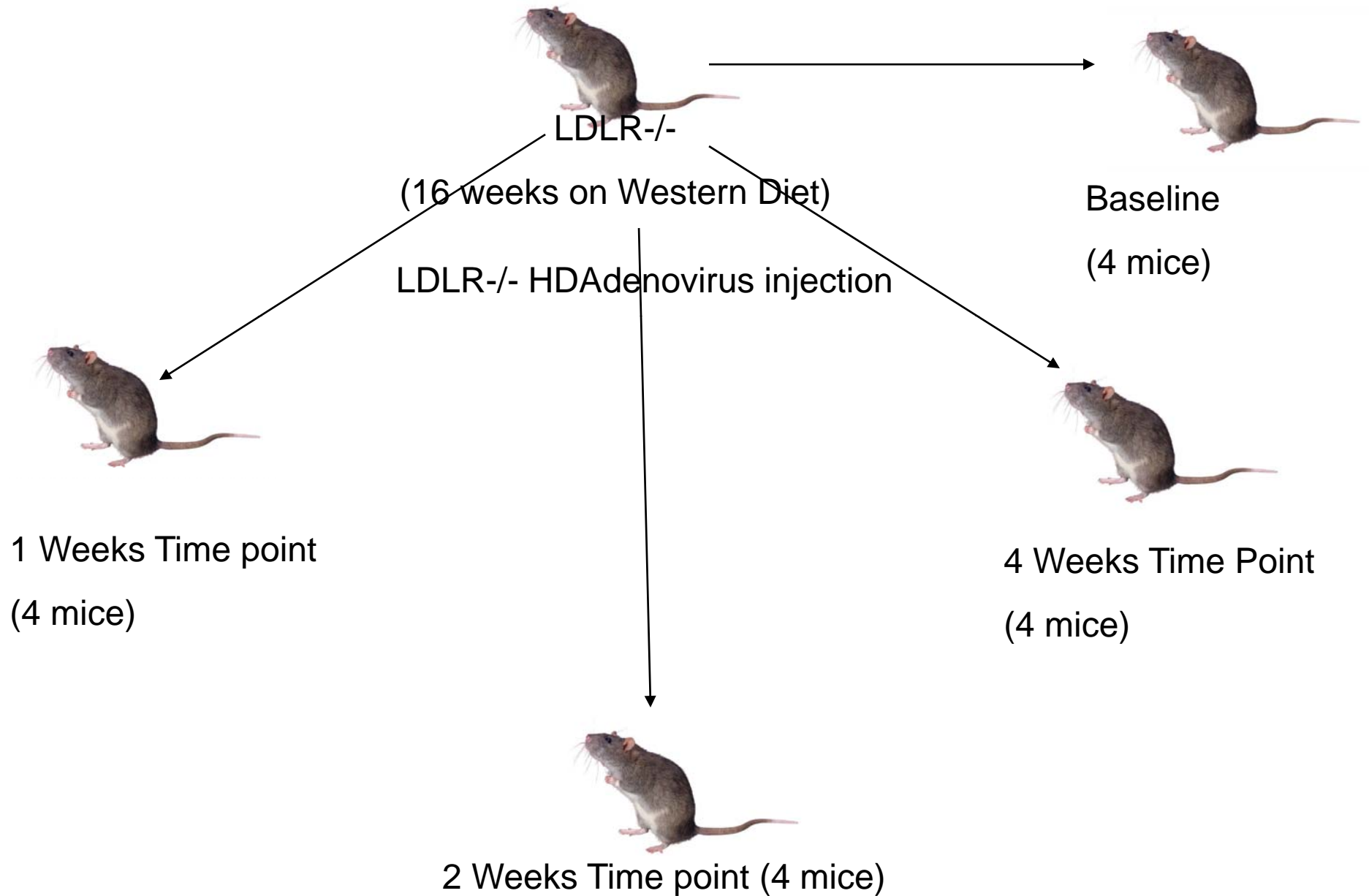


↓
PIPC injections +/- STZ

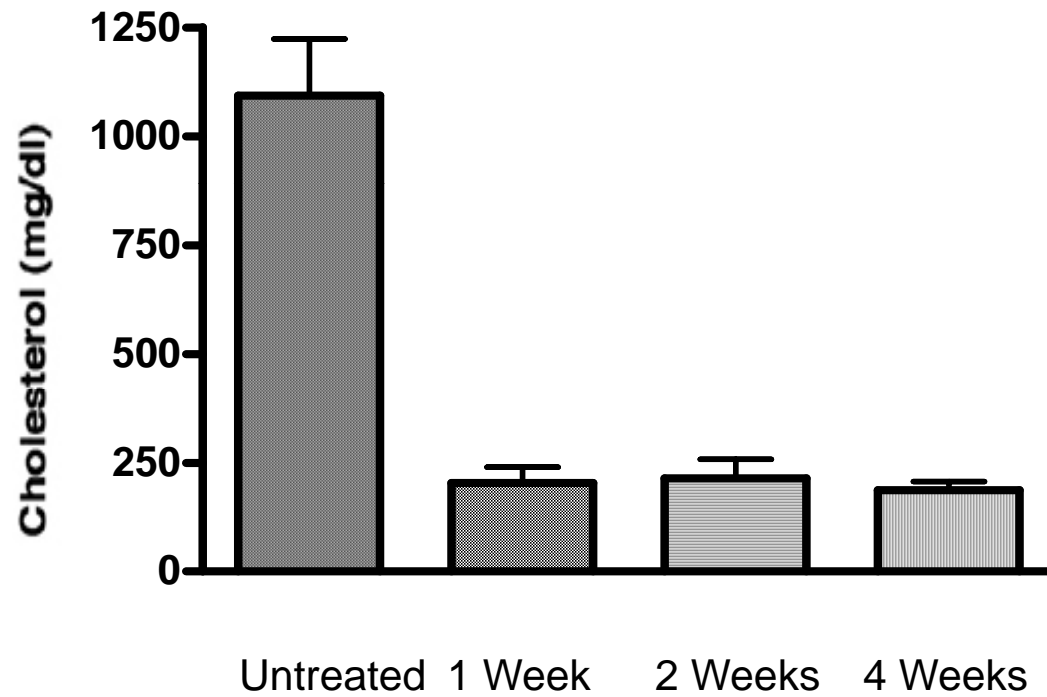


30 Days: analyses

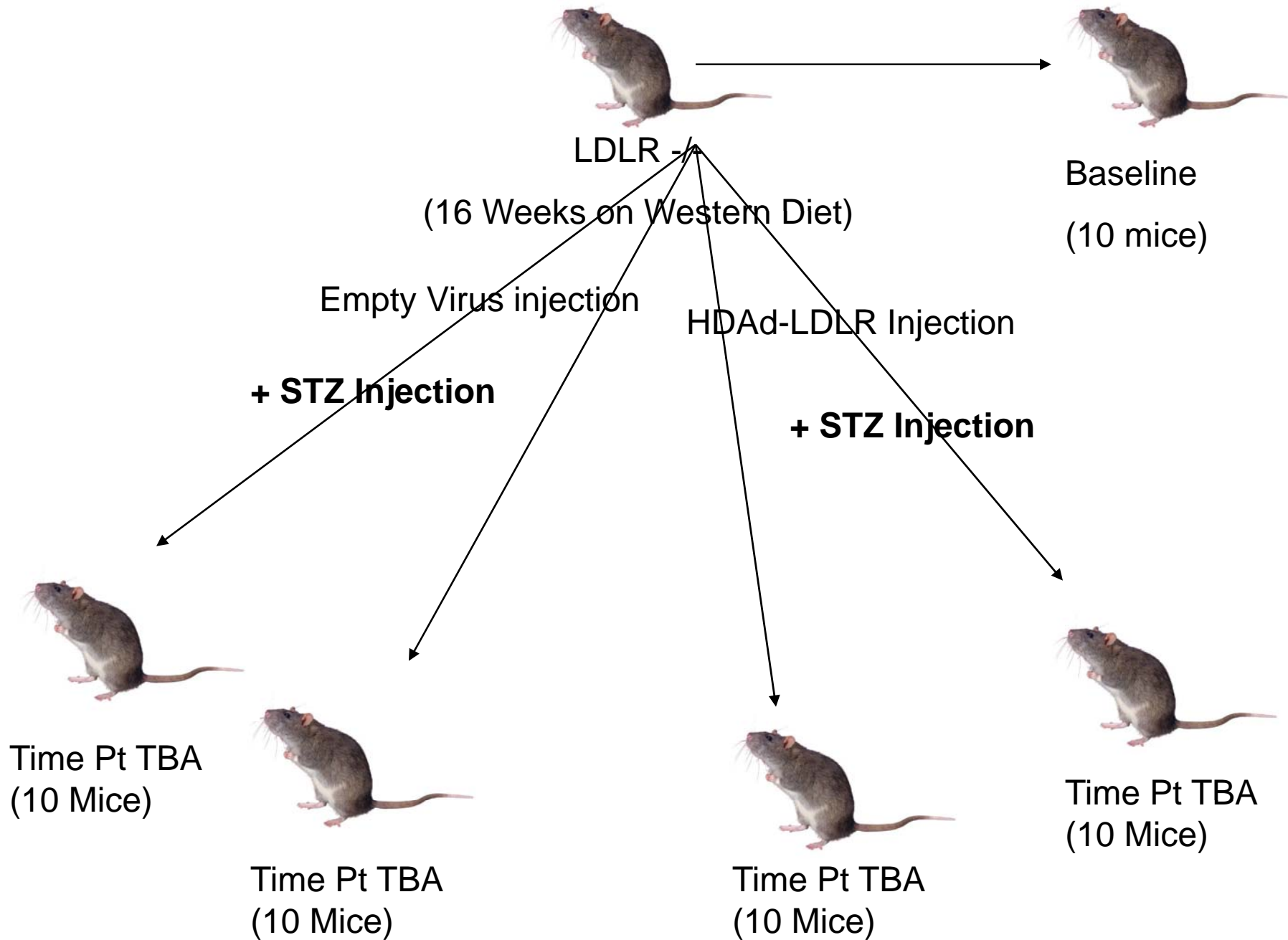
Non-surgical Model II: Adenoviral Approach



Total plasma cholesterol level in baseline LDLR^{-/-} mice, 1 week, 2 weeks or 4 weeks after HD-Ad-LDLR injection

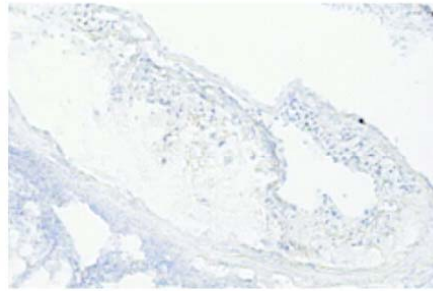


Experimental design of diabetic study with HD-Ad

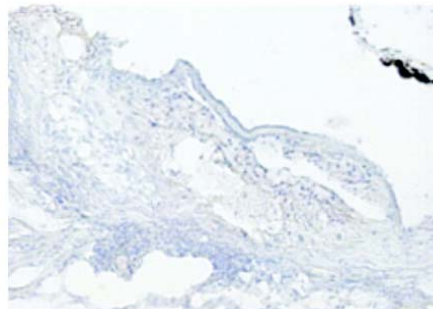


Role of aldose reductase (AR) in hypoxic injury

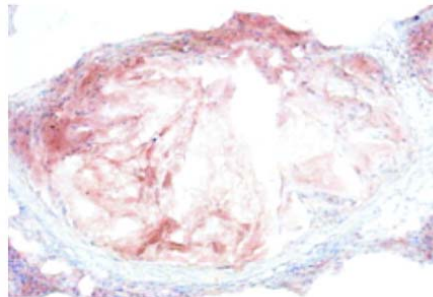
hAR-expressing mice have more AR protein expression in the atherosclerotic lesion area compared with nontransgenic mice



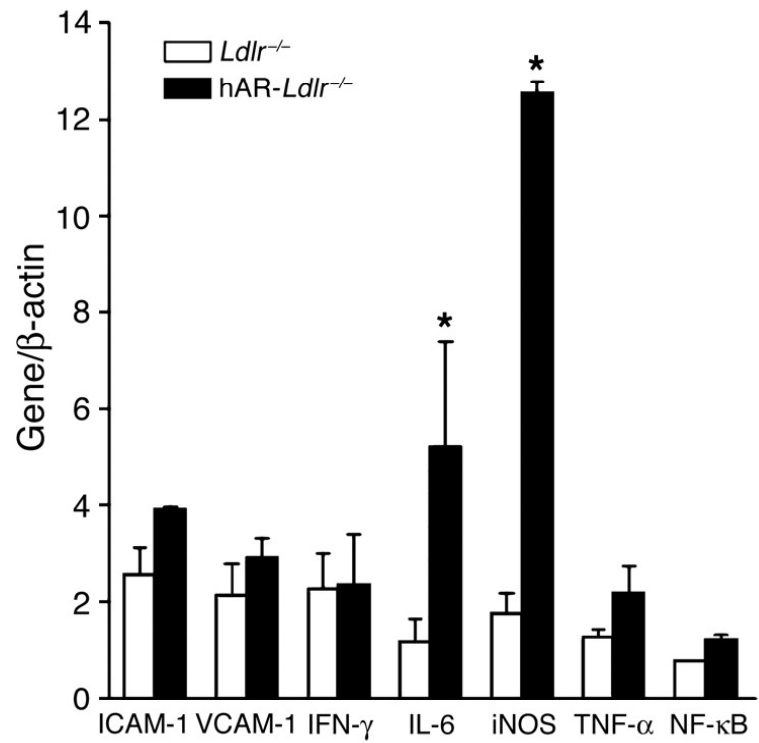
Negative



Ldlr^{-/-}

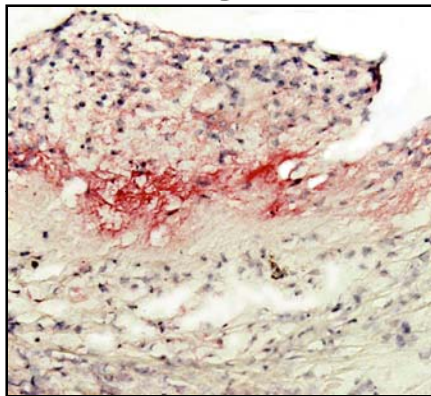


hAR-*Ldlr*^{-/-}

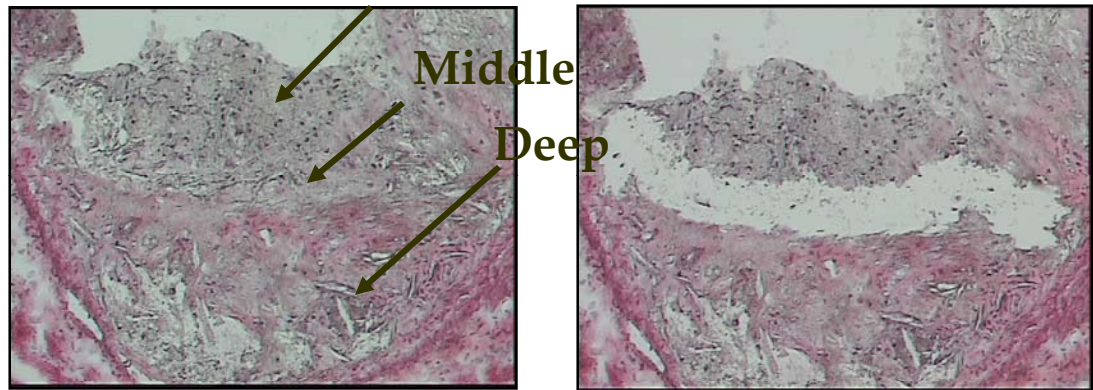


Mouse Plaques Exhibit Hypoxic Changes at Histological and Molecular Levels

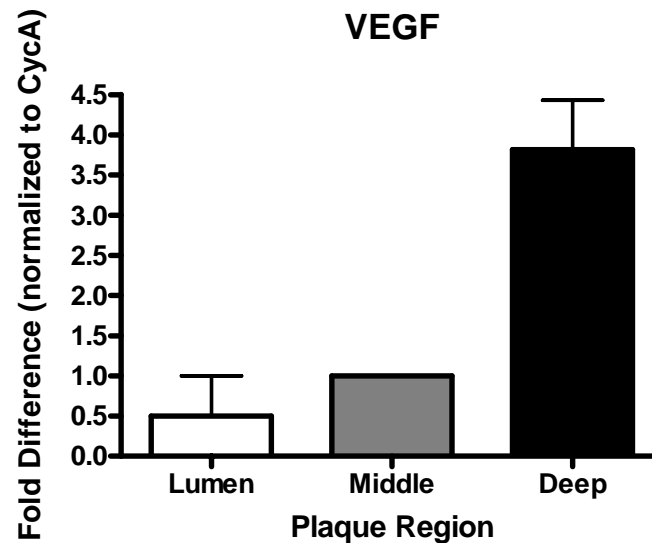
VEGF Staining



LCM Images



VEGF



- Make observations from the mouse models on pathways implicated in oxidant stress to determine separate contributions of hypoxia, hyperglycemia, and hAR
- If warranted, macrophages will be transfected with AR and stable transfection cell lines established
- Macrophages will be cultured in high and low glucose in a chamber that mimics an hypoxia environment to investigate underlying mechanisms

Acknowledgments



- David Habiell
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