Center for Development of Therapeutics, Broad Institute

CDOT

- First hire Oct 2011 (now ~30 scientists)
- Collaborations are key to program success
- Outsourcing of some essential activities

Approach

- Establish tools to take on undruggable therapeutic targets
- Commitment to comprehensive approach

Goals: Make next generation impactful therapies.

Pursue deep understanding of target biology and disease systems.

Apply learnings in an integrated, open and collaborative drug discovery environment.

BROAD INSTITUTE

Medullary Cystic Kidney Disease 1

Biology of the MUC1 Disease Mutation
&
Therapeutics Discovery and Development

INSTITUTO Carlos Slim DE LA SALUD

BROAD INSTITUTE

Slim Initiative in Genomic Medicine for the Americas

The MCKD1 Project Team

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BI Deaconess Medical Center

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Wake Forest School of Medicine

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Center for Applied Genomics
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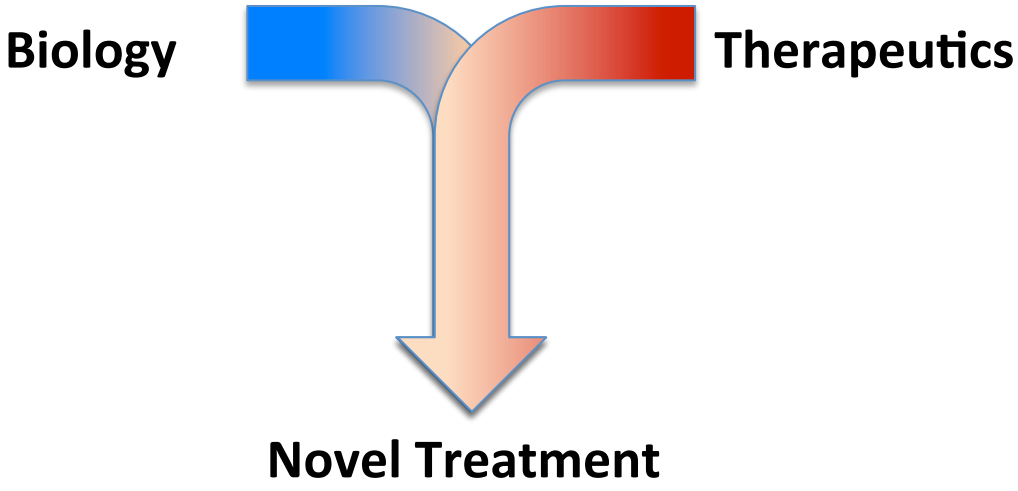
MCKD1: Biology and Therapeutics

- The biology underlying the role of mutant MUC1 in kidney disease is *unknown*.
- Our leading hypothesis is the frame shifted mutant MUC1 is a toxic protein and that *decreasing protein levels will benefit patients.*
- To rapidly drive toward new treatments, we initiated an "at-risk" therapeutics program targeted on decreasing MUC1 levels while beginning parallel research on biology of the disease.



nature genetics

LETTERS

Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in *MUC1* missed by massively parallel sequencing

Andrew Kirby[1,2], Andreas Gnirke[1], David B Jaffe[1], Veronika Barešová[3], Nathalie Pochet[1,4], Brendan Blumenstiel[1], Chun Ye[1], Daniel Aird[1], Christine Stevens[1], James T Robinson[1], Moran N Cabili[1,5], Irit Gat-Viks[1,6], Edward Kelliher[1], Riza Daza[1], Matthew DeFelice[1], Helena Hůlková[3], Jana Sovová[3], Petr Vyleťal[3], Corinne Antignac[7–9], Mitchell Guttman[1], Robert E Handsaker[1,10], Danielle Perrin[1], Scott Steelman[1], Snaevar Sigurdsson[1], Steven J Scheinman[11], Carrie Sougnez[1], Kristian Cibulskis[1], Melissa Parkin[1], Todd Green[1], Elizabeth Rossin[1], Michael C Zody[1], Ramnik J Xavier[1,12], Martin R Pollak[13,14], Seth L Alper[13,14], Kerstin Lindblad-Toh[1,15], Stacey Gabriel[1], P Suzanne Hart[16], Aviv Regev[1], Chad Nusbaum[1], Stanislav Kmoch[3], Anthony J Bleyer[17,18], Eric S Lander[1,18] & Mark J Daly[1,2,18]

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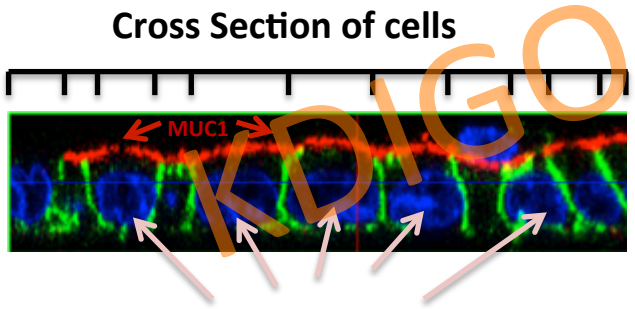
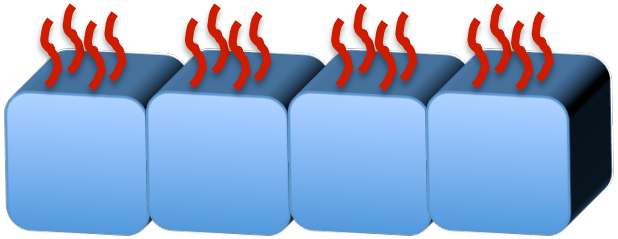
MUC1 is causal gene for MCKD1

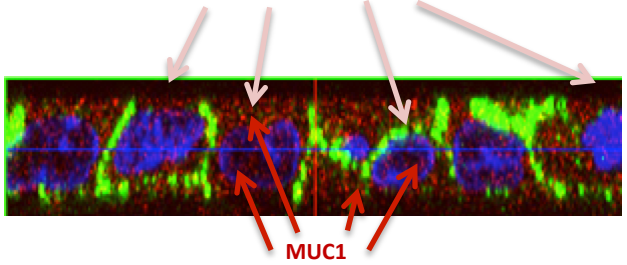
A mutation in MUC1 causes the protein to incorrectly localize

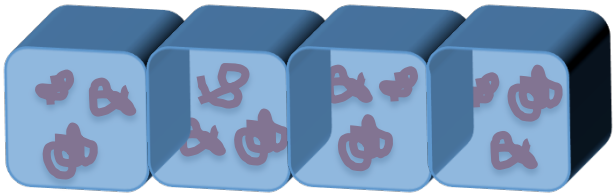
Healthy MUC1
on cell surface



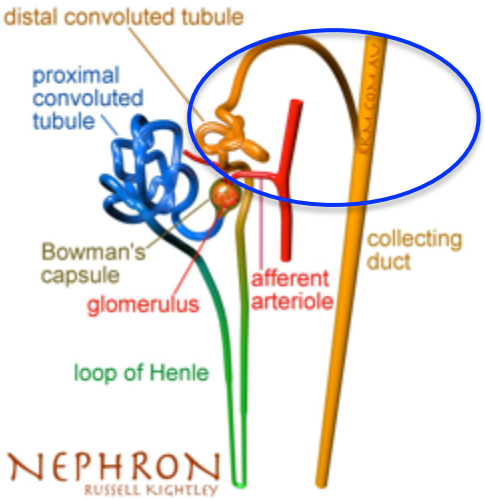


MCKD1 MUC1
stuck inside cell



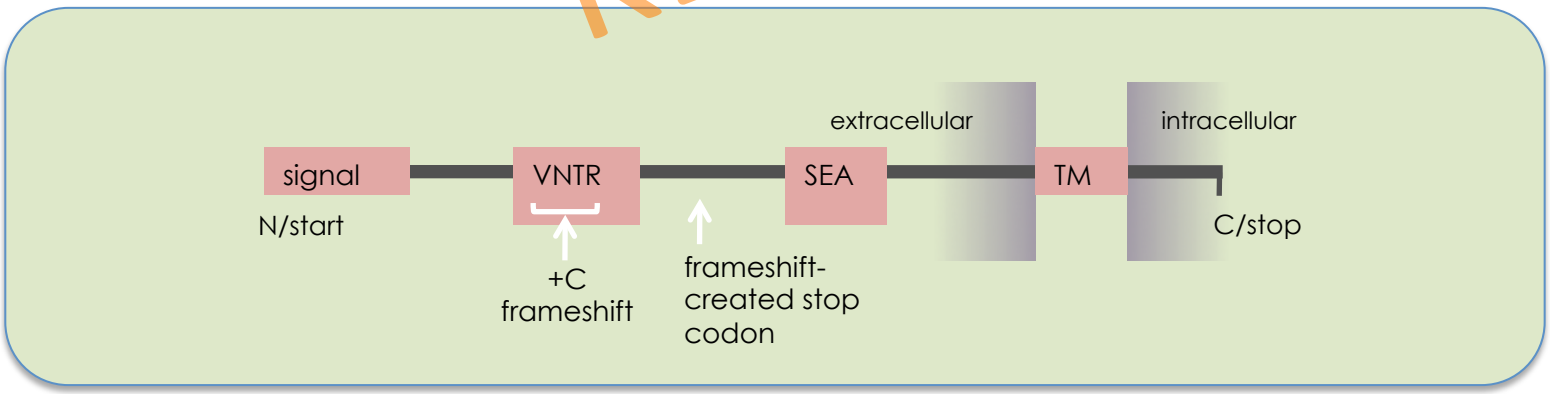


MUC1 in kidney



MUC1 expressed in distal convoluted tubule and collecting duct

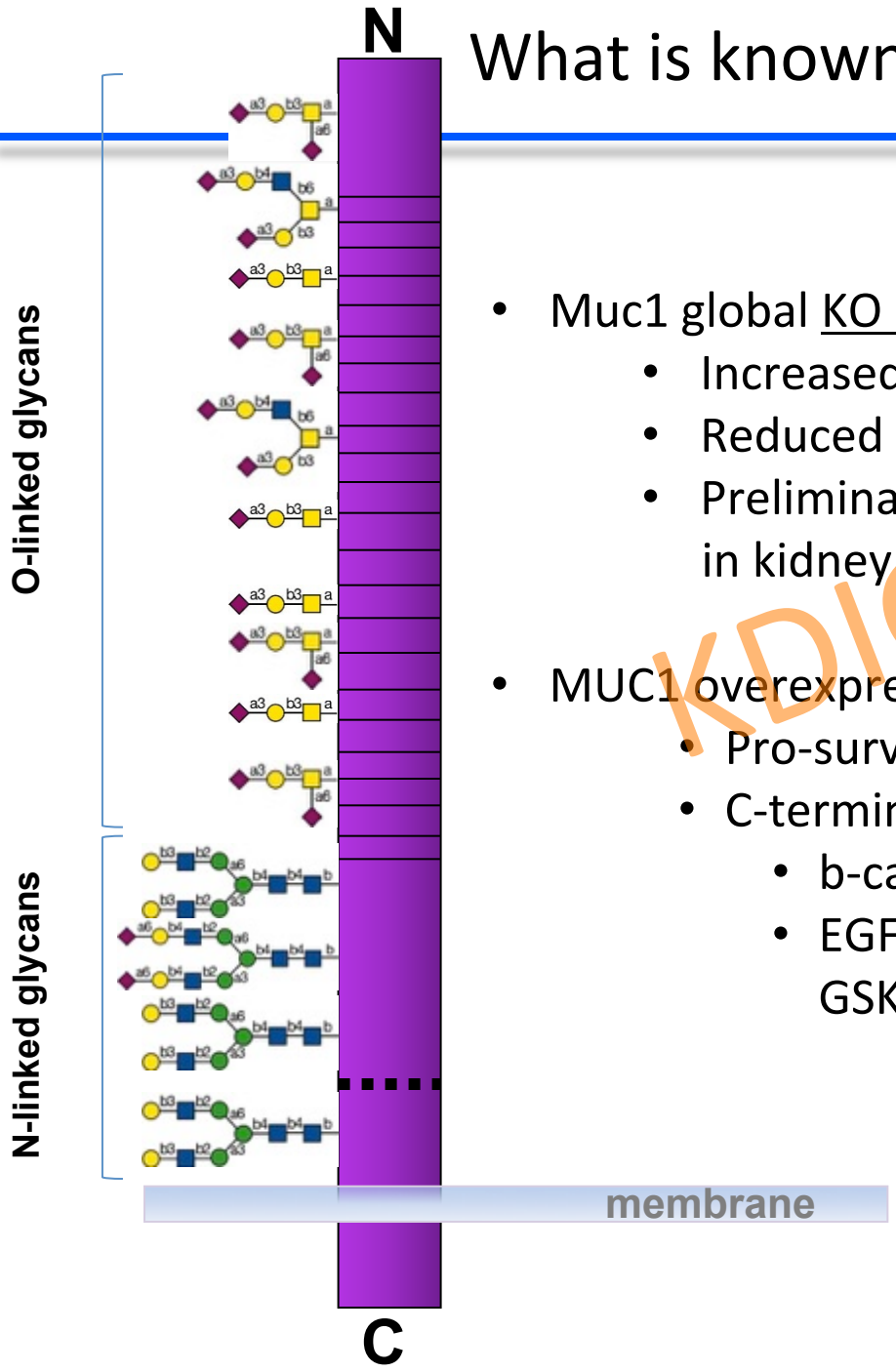
- +C mutation predicts neo-sequence and truncation
- Truncated neopeptide has pI of 12



Mutation produces many copies of altered VNTR unit

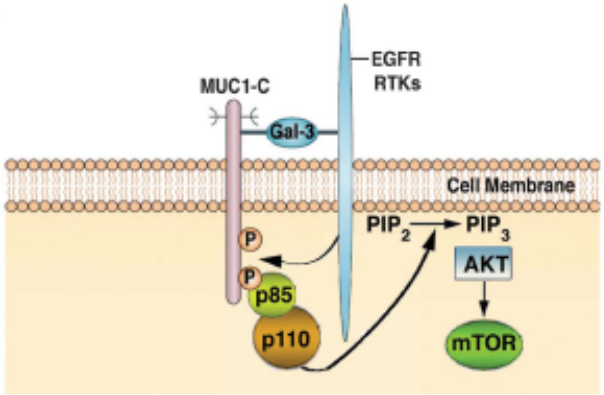
VTSAPDTRPAPGSTAPPAHG ⇨ CHLGPGHQAGPGLHRPPSPR

What is known about the function of MUC1?

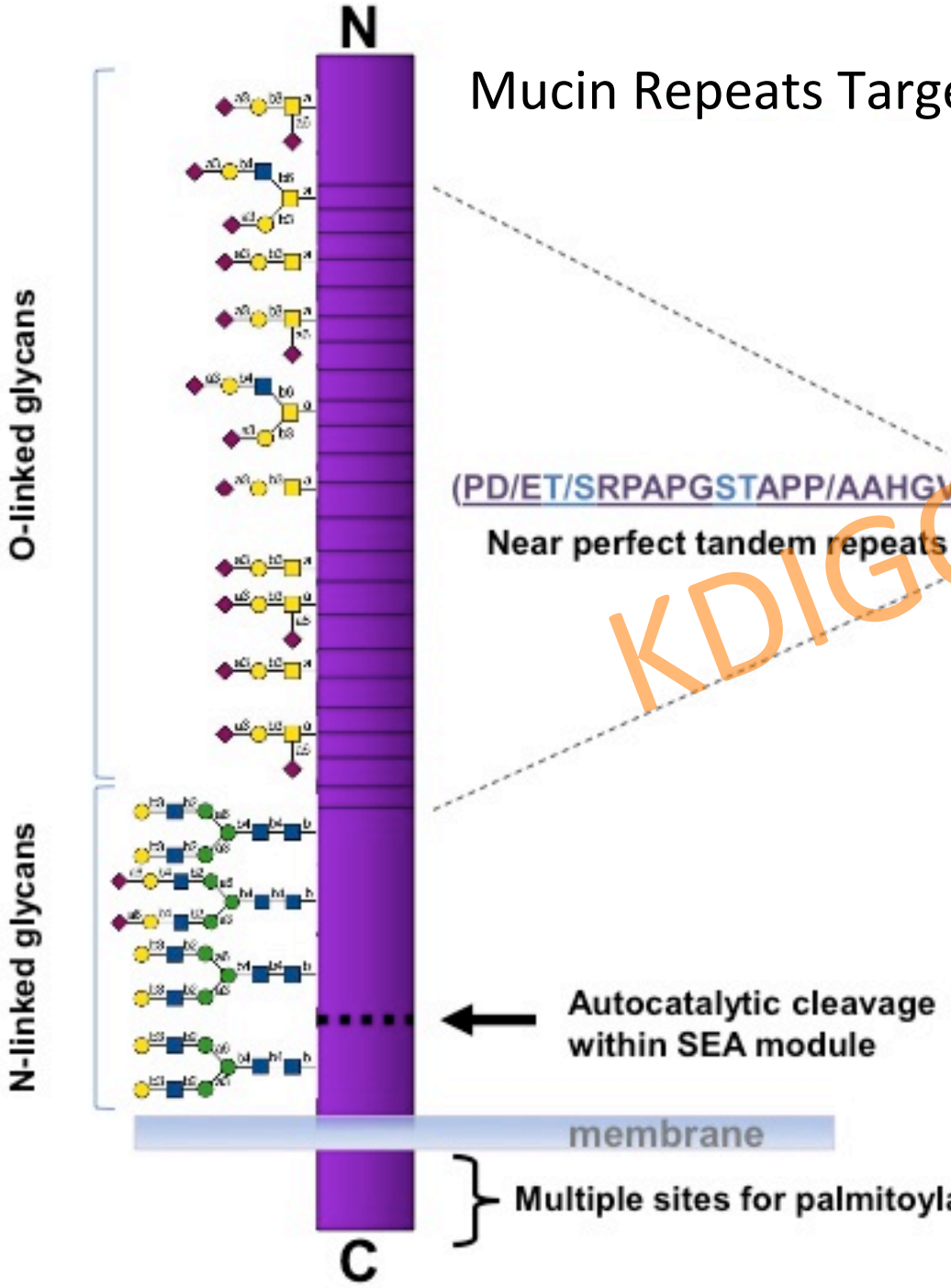


- Muc1 global KO mice have subtle phenotypes
  - Increased sensitivity to bacteria infections
  - Reduced intestinal mucus in CFTR -/- cross
  - Preliminary data supports a role in wound healing in kidney

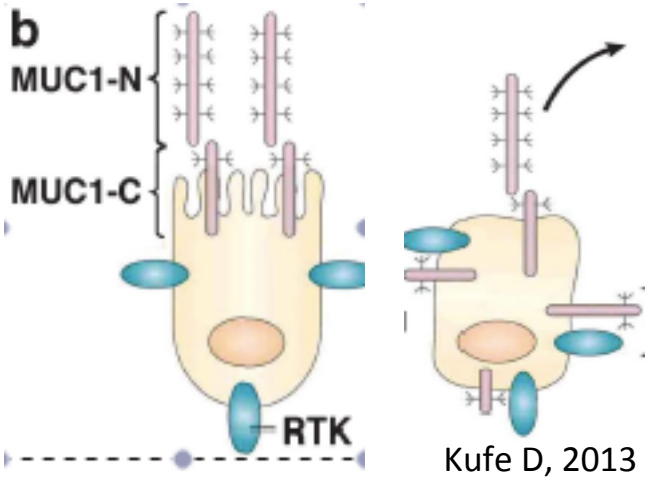
- MUC1 overexpressed in some cancers
  - Pro-survival and anti-apoptotic activities
  - C-terminus modulates:
    - b-catenin, p120 catenin, p53 and ER-a
    - EGF receptor activity, Src family kinases, GSK-3β, PKCδ



Mucin Repeats Target protein to Apical Membrane



Cleaved extracellular domain can remain associated with MUC1-C



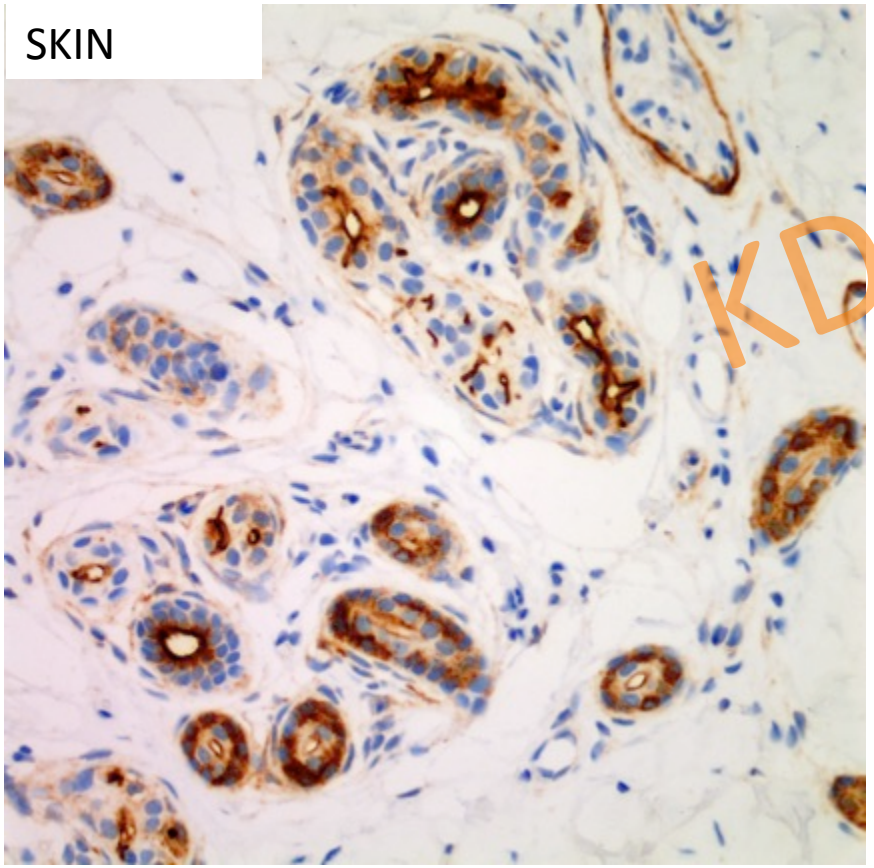
Kufe D, 2013

modified from Hughey, RP

MUC1 Extra-renal Involvement

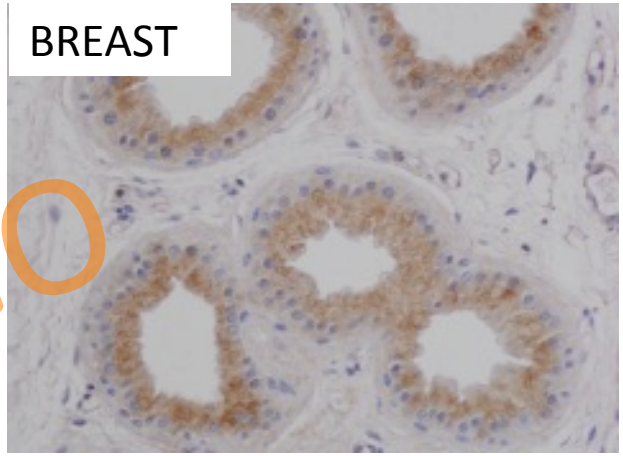
- +C fs muc1 is expressed in all tissues
- Mammary, Respiratory, GI tract function are normal

SKIN

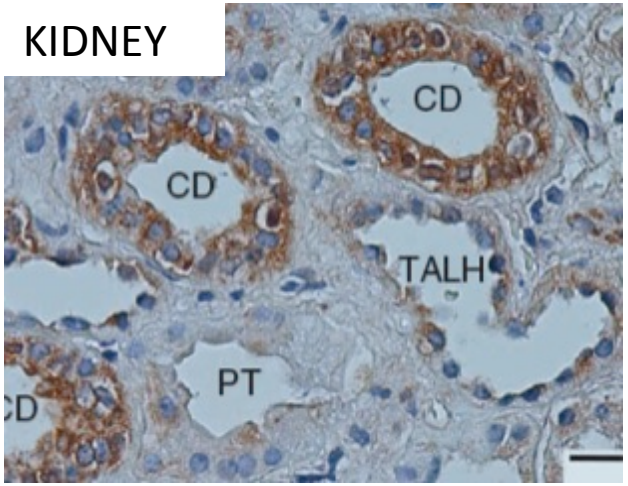


Courtesy of Stan Kmoch

BREAST



KIDNEY



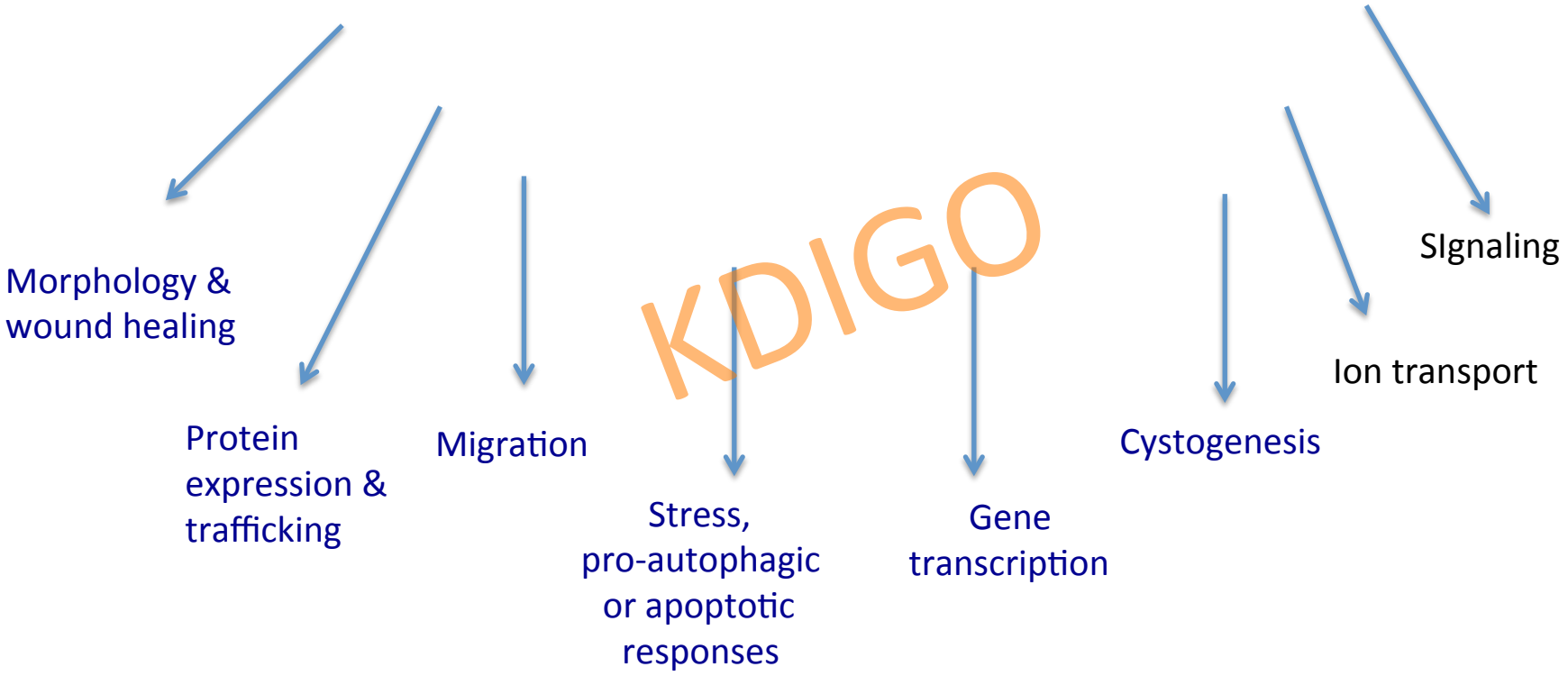
Complex biological questions remain.

How does fs *MUC1* cause disease?

- Current model: dominant gain-of-function toxic effect. What other model(s) is supported by the data?
- Why does fs MUC1, normally expressed in distal nephron epithelial cells, lead to pathology not only in those cells but also in interstitium and eventually in proximal tubule and glomerulus?
- Why does the widely expressed mutant MUC1 present clinically in a tissue-specific manner?
- in vivo models – mouse transgenics ongoing
  - Zebrafish? Others?

Exploring Muc1 biology and fs pathophysiology

Kidney differentiation and functional endpoints



BUT FIRST GENERATE THE REAGENTS!!

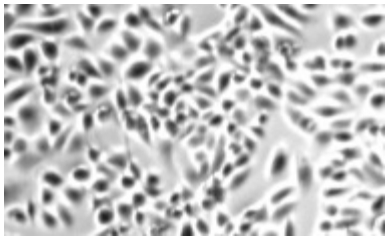
What do we need?

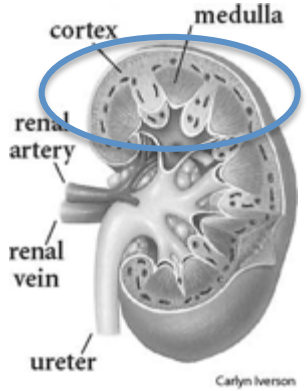
- MCKD1 patient-derived cell lines
  - hTERT / SV40 LgT and hTERT alone
- fs muc1 antibody development
  - Multiple attempts – peptides and phage display
- Reliable sequencing technology for VNTR
  - Many iterations w/ PacBio the winner
- Perfect cDNA expression constructs for WT and fs – round 2!
- Stable cell lines for WT and fs – round 2!
- Well characterized shRNA and CRISPR vectors

Building better models to study biology and for Rx Dx

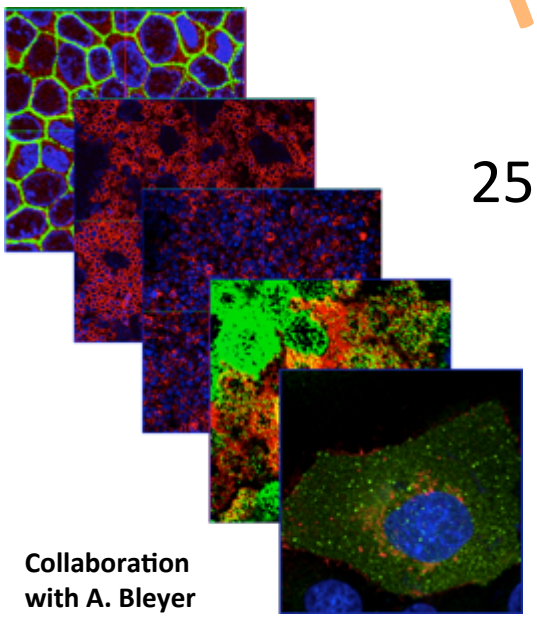
Source: a consented MCKD1 patient & a normal human kidney

Single renal epithelial cells





Infection with hTERT +/- SV40TAg

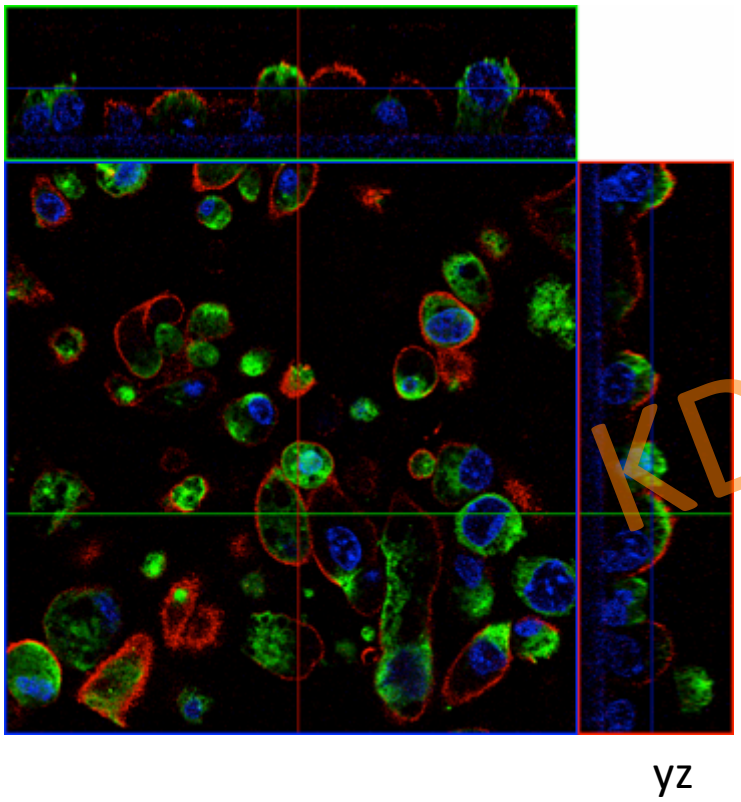


Collaboration with A. Bleyer and S Alper

25+ Patient clonal stable lines established

- Confirmed fsMUC1 presence and epithelial origin

hTERT-immortalized MCKD1 patient cells polarize *in vitro*

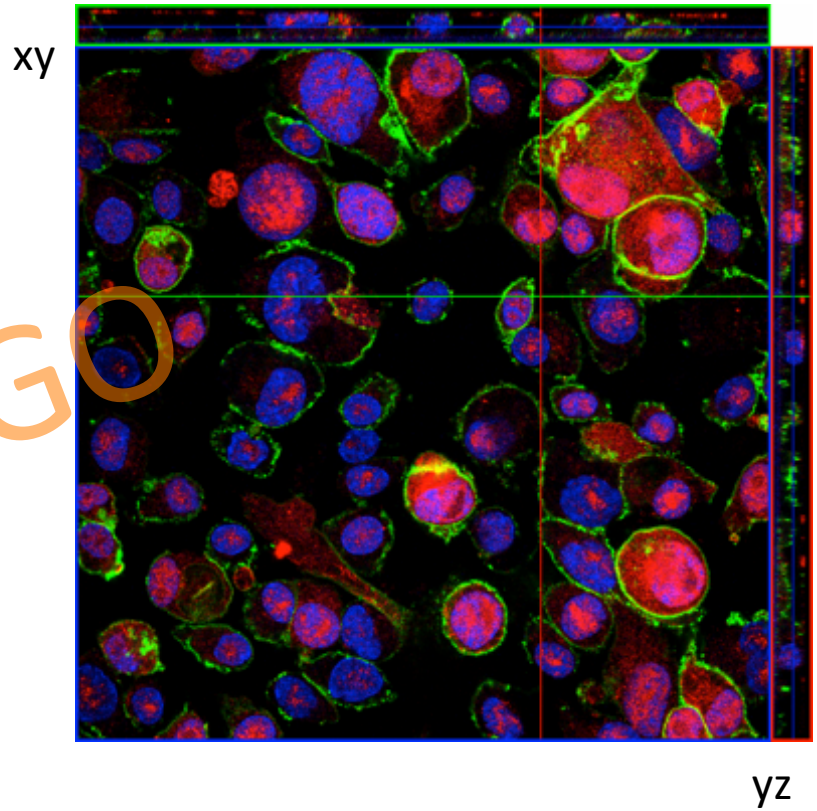


Wildtype MUC1

Tamm-Horsfall protein – UMOD

(TALH marker)

DAPI



NKCC2 (TALH marker)

NK-APTase (baso-lateral membrane)

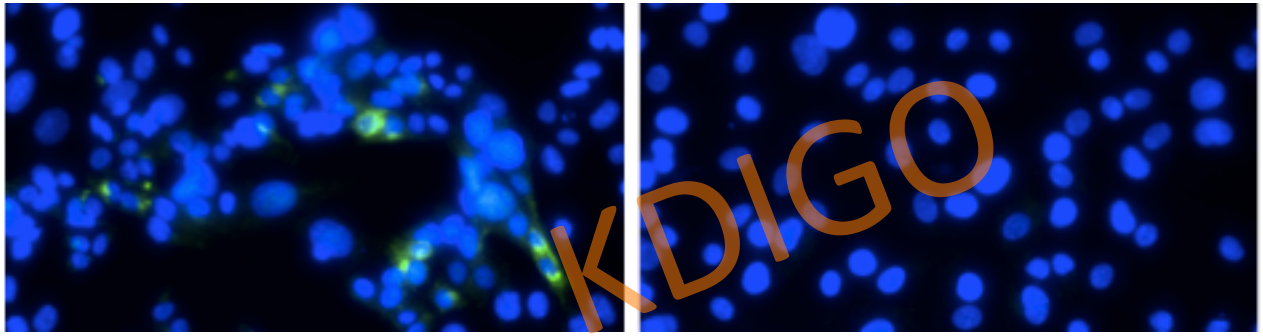
DAPI

Clone 1A8

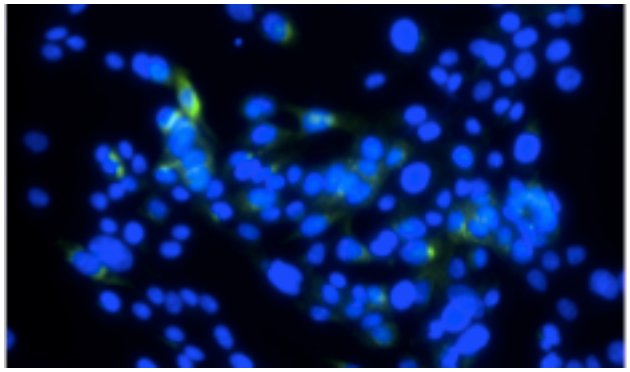
Jane Hsu, Juan Gutierrez, Savithri Kota, Seth Alper

fs muc1 in patient cell lines is intracellular

MCKD1 patient derived cells **Normal kidney derived cells**



Blue = nuclei
Green = fs muc1



hTERT + SV40 LgT lines
New phage display Ab #3

Doug Daniels and Juan Gutierrez

MCKD1 Mouse Model Development

- Pursuing three models
- Each model will use the full human MUC1 gene including extensive promoter regions
- HPRT locus targeting with MCKD1 mutant full human gene + promoter region
  - Create a mouse harboring a single additional human copy of gene on the X chromosome
- Knock-in of MCKD1 mutant full human gene + promoter region into mouse *Muc1* locus
  - Replaces the endogenous mouse gene + promoter region with the human locus
- Random insertion model (Seth Alper)
  - Further along (avoided re-cloning bottleneck)

Therapeutics Discovery and Development

- Therapeutic strategy focused on dominant gain-of-function hypothesis
  - HTS to identify compounds based on the hypothesis that reducing MUC1 protein levels could significantly reduce or delay disease progression
- Pursuing biological mechanism of disease studies in parallel
- Developing mouse models using WT and fs MUC1 in parallel with other studies
  - First random transgenic founders identified this week; characterization beginning
  - Two other models, including a knock-in replacement model, in progress

Therapeutic program goals

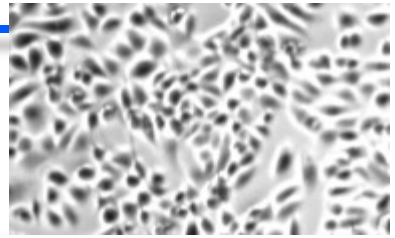
Stage 1 (complete)

- Identify disease gene
- Prove concept that reduction of MUC1 expression is feasible

Stage 2

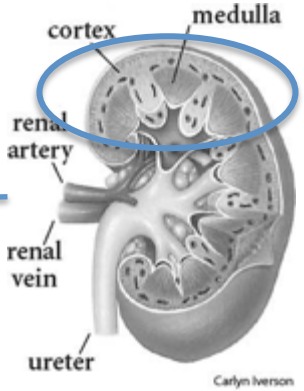
- Expand our knowledge of MUC1 role in normal kidney development and function
- Explore the role of fs *MUC1* in MCKD1
- Develop high-fidelity disease models to assess fs role
  - MCKD1 patient derived cell lines
  - animal (rat and murine) models
- Initiate a therapeutics program for MCKD1.

3 MUC1 focused HTS completed



MCKD1 kidney

lines in HTS



L1000 screen
MCF7
20K BioA/DOS

QPCR HTS
MCKD1 patient line
100K BioA/DOS/ML

KDIGO

HCS HTS
MCKD1 patient line
100K BioA/DOS/ML

40 BioA retested
15 cpds hit via QPCR
15 impact protein levels via IF

1200 cpds retested at dose
Very low retest rate
Overlap with L1000 data seen

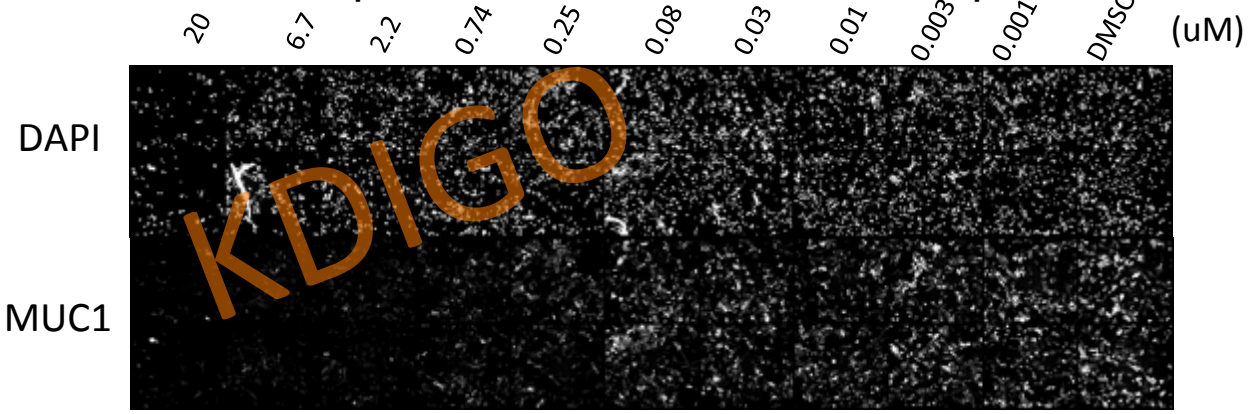
HTS Complete
Retests ongoing

HTS hits: Bioactives identified via L1000

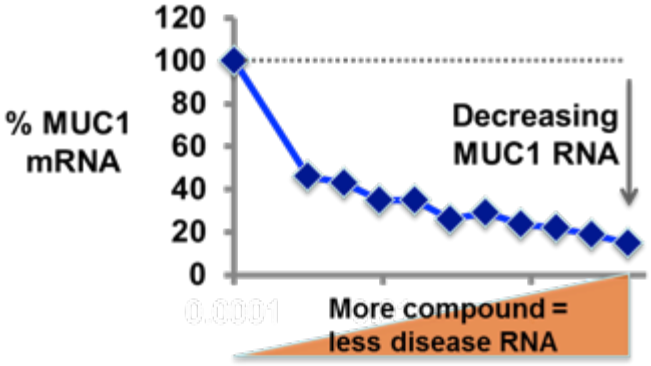
Hits identified via LINCS data troll in MCF7

- 8 known classes of drugs
- All 8 classes also identified as hits in QPCR HTS and further validated
- 4 classes validated to inhibit MUC1 protein via IF based dose response

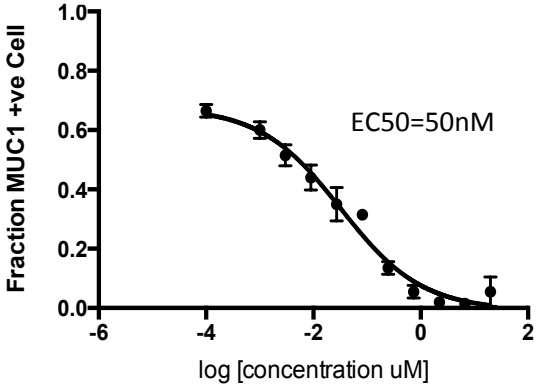
Immunofluorescent detection of surface WT MUC1



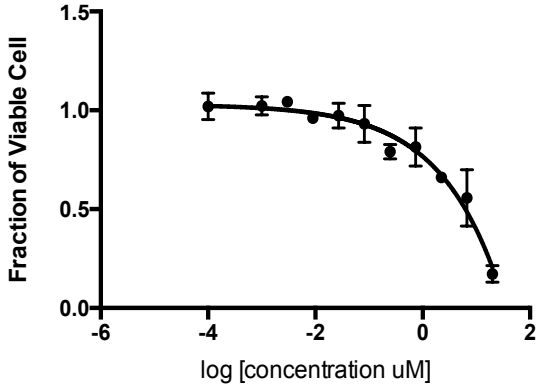
QPCR MUC1 mRNA levels



IF MUC1 cell surface protein

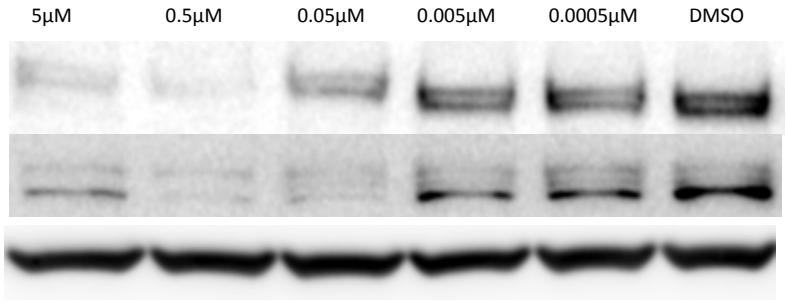


cell viability DAPI

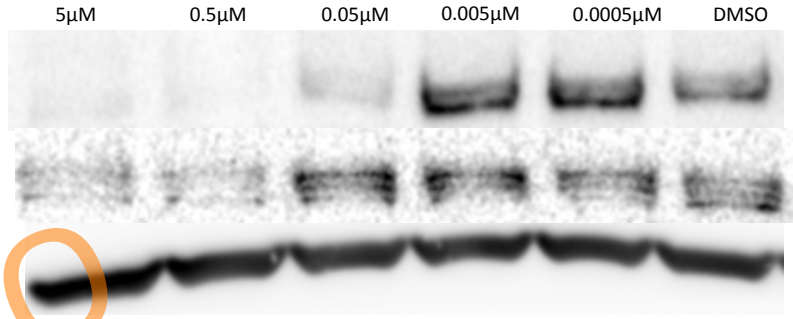


4 inhibitor classes reduce MUC1 protein levels

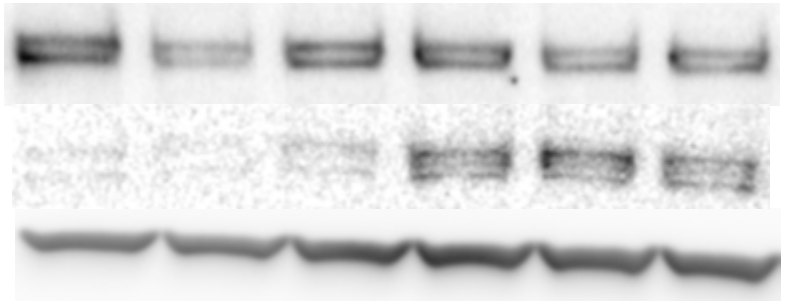
BRDXX29
Class 1



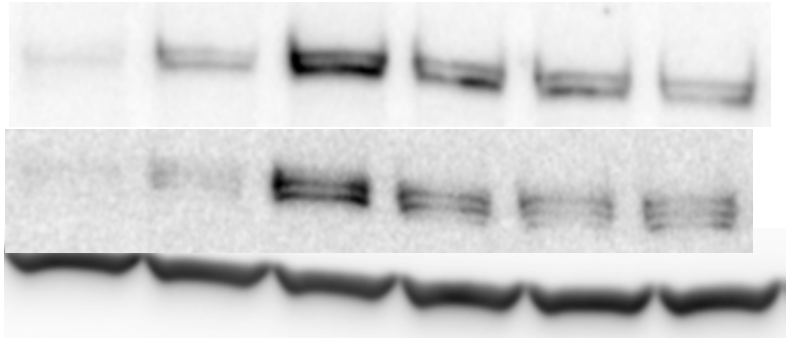
BRDXX88
Class 3



BRDXX16
Class 2



BRDXX19
Class 4



Clinical genomic data > Discovery > Translation> Therapy

Collaborators/ Advisors

Tony Bleyer
Stan Kmoch
Mark Daly
Martin Pollak
Ramnik Xavier

Biology/Nephrology Collaborators

Seth Alper
Johannes Schlondorff
Savithri Kota
David Doroquez

Broad

Andi Gnirke
Dave Jaffe
Chad Nusbaum
Iain MacCallum
Aravind Subramanian
Corey Flynn
John Doench
Melanie Donahue

Stephen Moss
Nature 2008

KDIGO

INSTITUTO Carlos Slim DE LA SALUD

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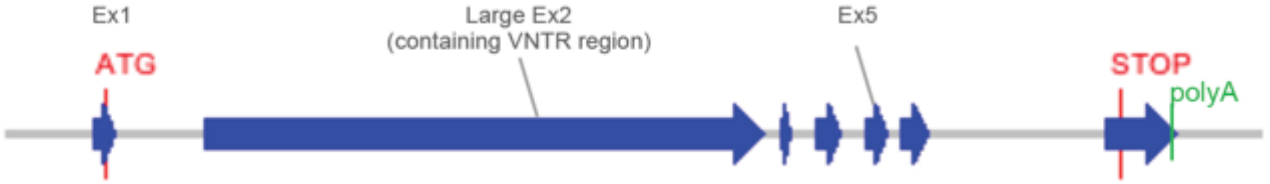
Paul Clemons
Steve Carr
Eric Kuhn
Todd Carter
Eric Lander

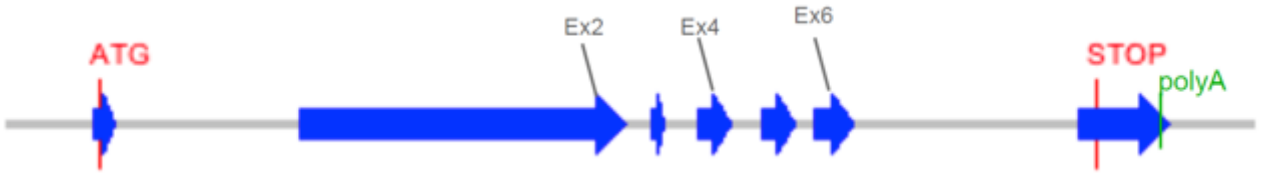
MUC1 Human and Mouse Gene Structures

Human



- Wide range of splice forms reported
- Exon 2 glycosylated repeat is variable
- Exon 2 is very GC rich

Mouse



- One transcript reported
- Exon 2 glycosylated repeat is not variable
- Exon 2 is less GC rich than human
- *Equivalent mutation in mouse TR would not result in extended novel protein*