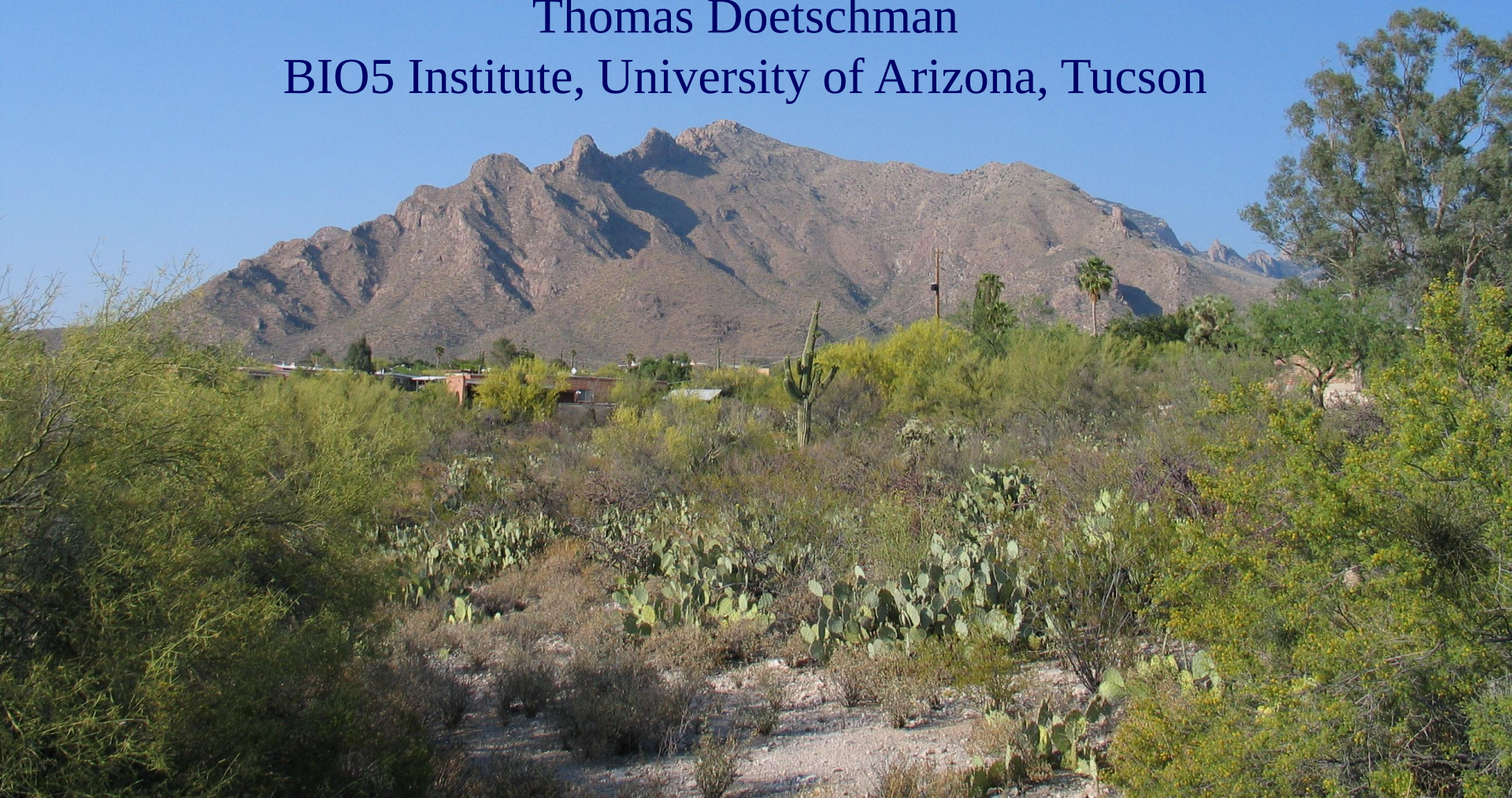
TGFβ1 - Early Player in Mouse Colon Cancer:

Suppresses IBD-Associated Colon Cancer by Preventing Pre-Clinical Inflammatory State of Readiness in Colon Mucosal Epithelium

Thomas Doetschman
BIO5 Institute, University of Arizona, Tucson



Characteristics of Adolescent and Young Adult CRC

Human CRC (~50,000deaths/Yr in US; 10% of all cancer deaths)

↓

Under 40 CRCs (2-6%)

↓

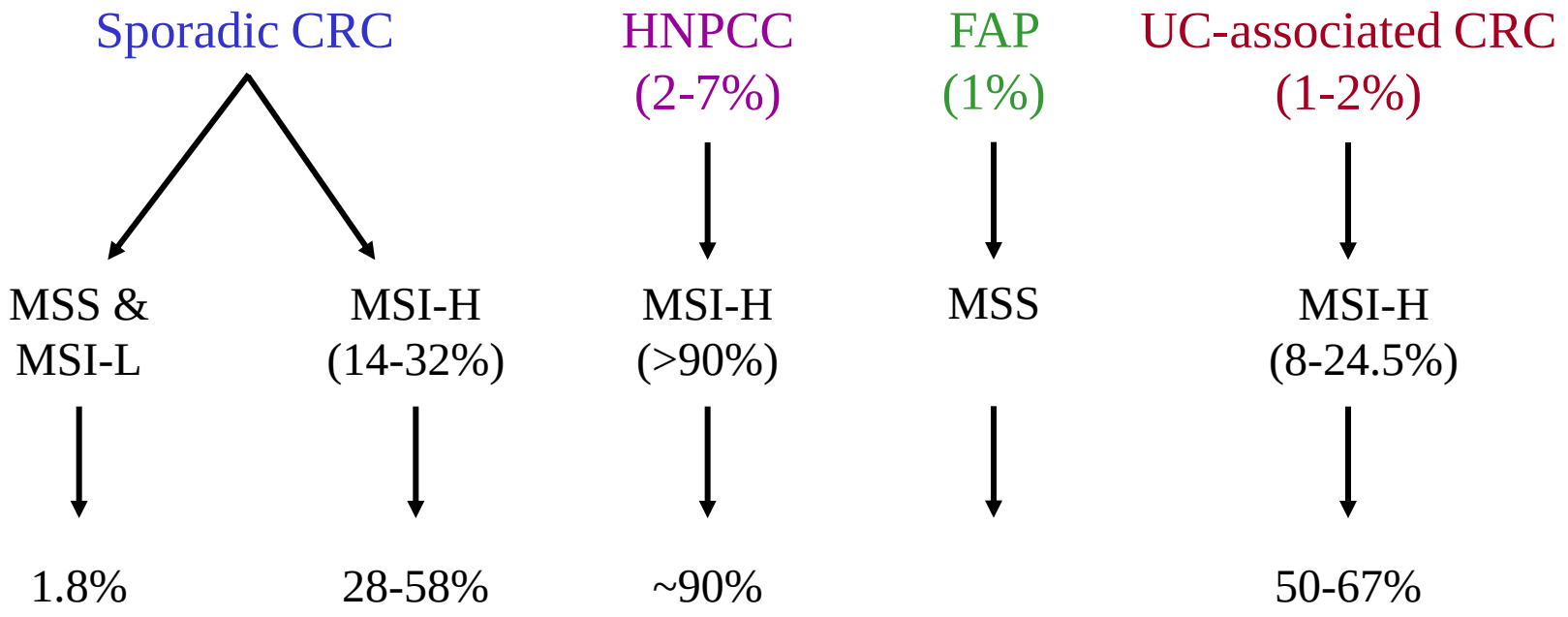
Aggressive Poor Prognosis	Poorly Differentiated	Right-sided Prevalence	Inf. Lymphocytes & Colitis	Mucinous Carcinoma

Okuno et al, *Am.Surg.*, 1988; Itzkowitz & Yio, *Am.J Physiol Gastrointest.Liver Physiol.*, 2004; Lin et al, *J Gastroenterol.Hepatol.*, 2005; Jenkins et al, *Gastroenterol.*, 2007; Lutgens et al, *Gut*, 2008

"Colitis-associated [CRC] affects individuals at a younger age than the general population. They more often have a mucinous or signet ring cell histology…in some studies, they demonstrate a more proximal distribution in the colon…these same features are found in CRCs arising in individuals with HNPCC."

Itzkowitz & Yio, *Am.J Physiol Gastrointest.Liver Physiol.*, 2004

HNPCC, MSI and *TGFBR2* Mutation in CRC Subtypes



Overall, the *TGFBR2* mutation frequency in human CRC ranged from 8-25%
up to 30% w/other TGFβ pathway mutations (*TGFBR1*, *SMAD4*, *SMAD7*)
APC mutations account for about 70% of all human CRC

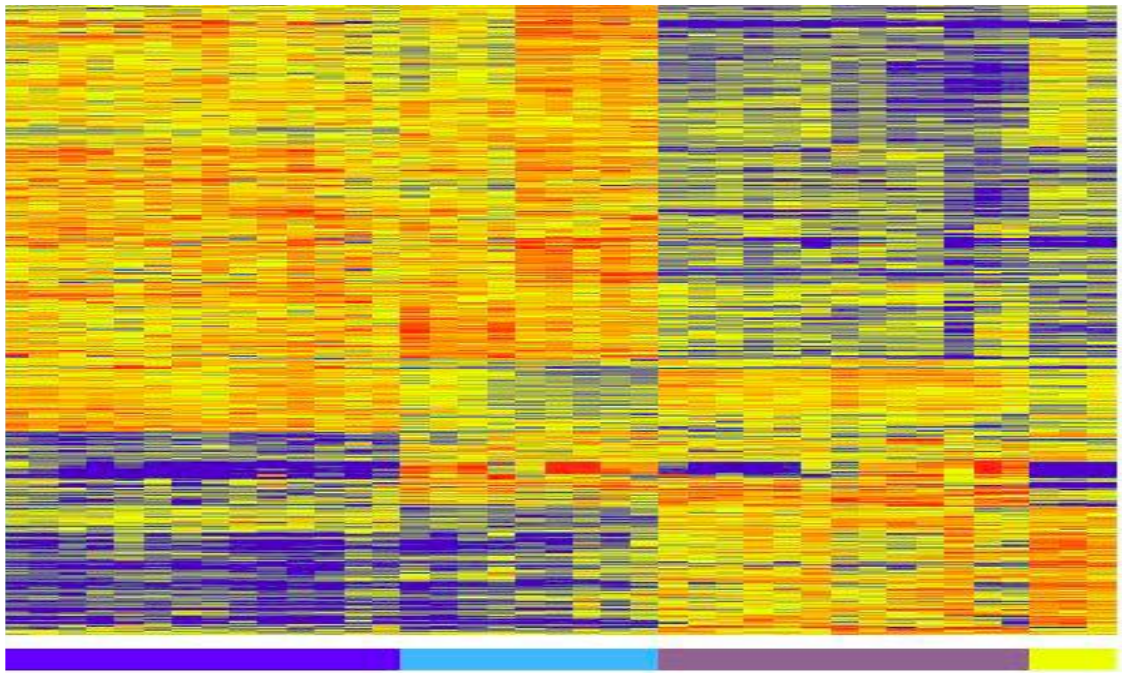
MSS=microsatellite stable; MSI-L=microsatellite instability-low;
MSI-H-microsatellite instability-high

Comparison: MSI in Human CRC and CRC in Mice with TGFβ Deficiency

Human	Mouse
Right-sided prevalence	Proximal prevalence
More likely to be flat-like than polypoid	More likely to be flat-like than polypoid
Earlier onset (44yrs vs. 65 average)	-
Faster progression	-
Predominantly mucinous	Predominantly mucinous
More likely to have inflam. infiltrates	More likely to have inflamm. infiltrates
More likely to be diploid	More likely to be diploid
Less likely to be metastatic	Less likely to be metastatic

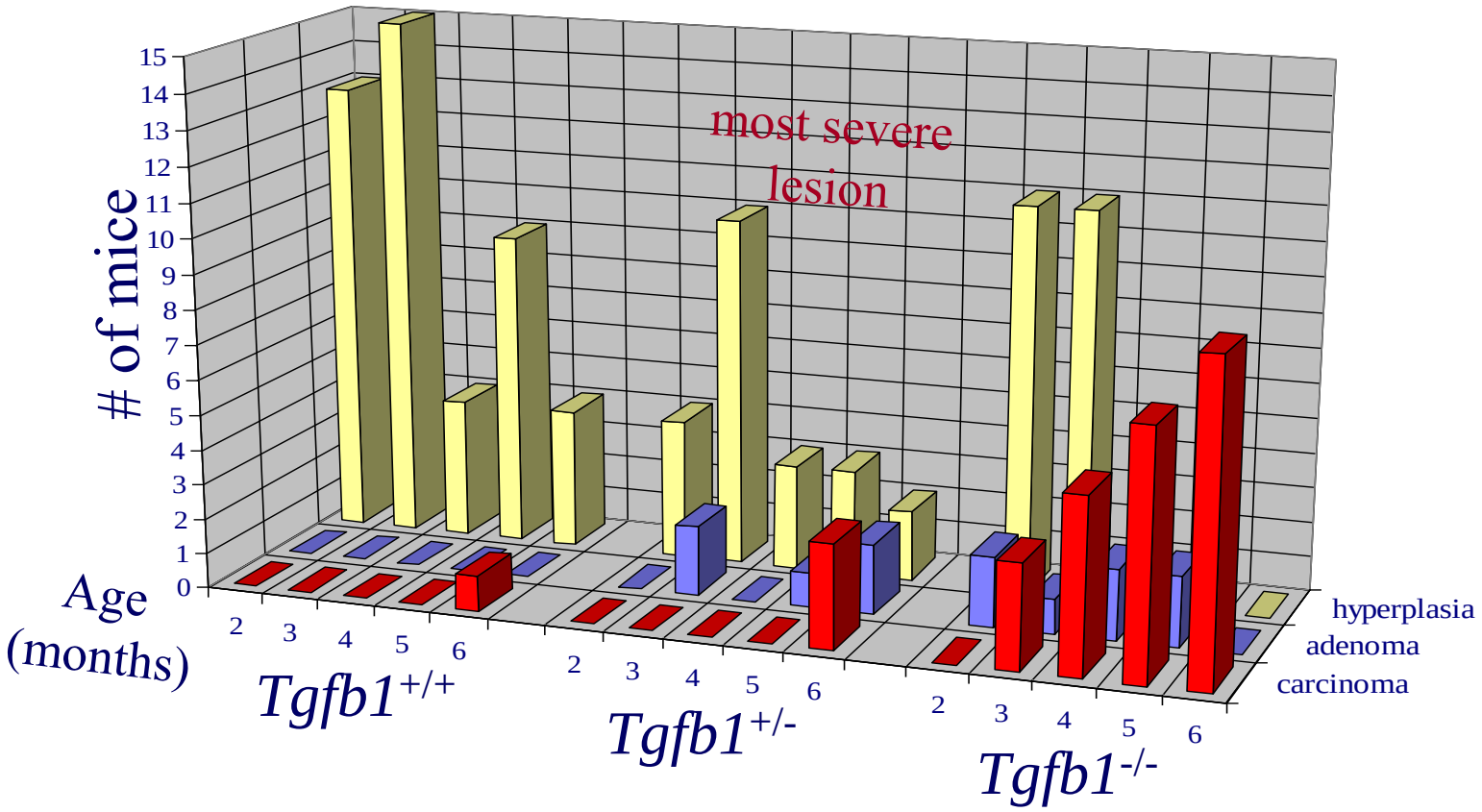
TGFβ- and APC-Deficient Mouse CRCs are Quite Different

Expression profiles of mouse colon tumors



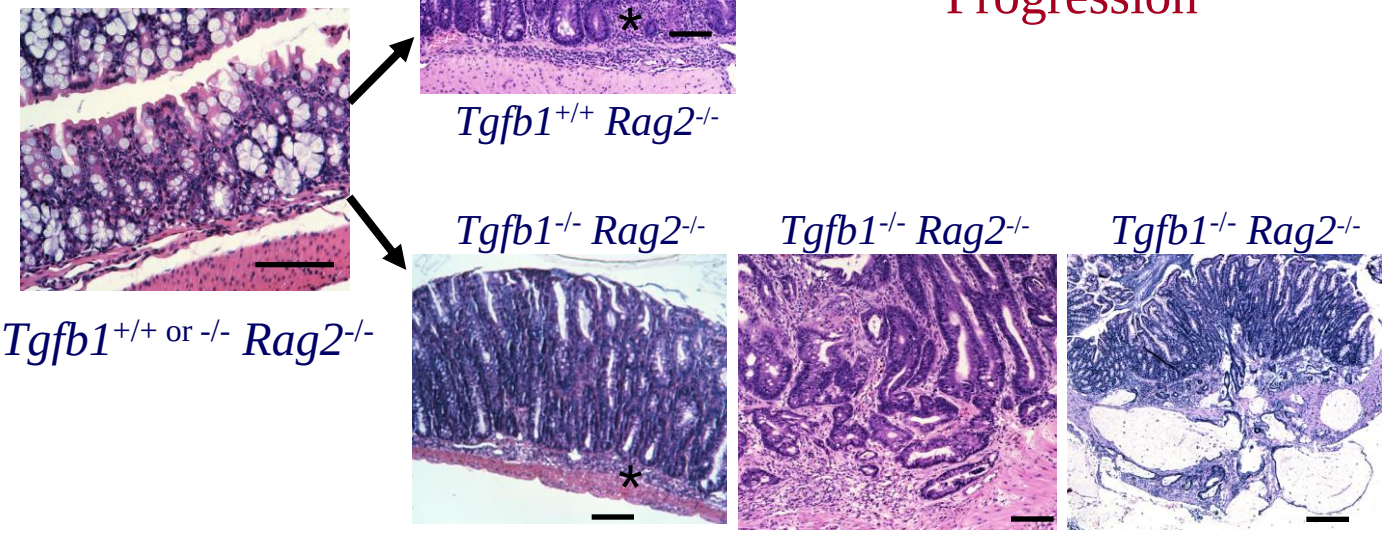
Aronow *et al*, *Genome Biology*, 2007

Frequency of Disease States in *Tgfb1 Rag2*$^{-/-}$ mice



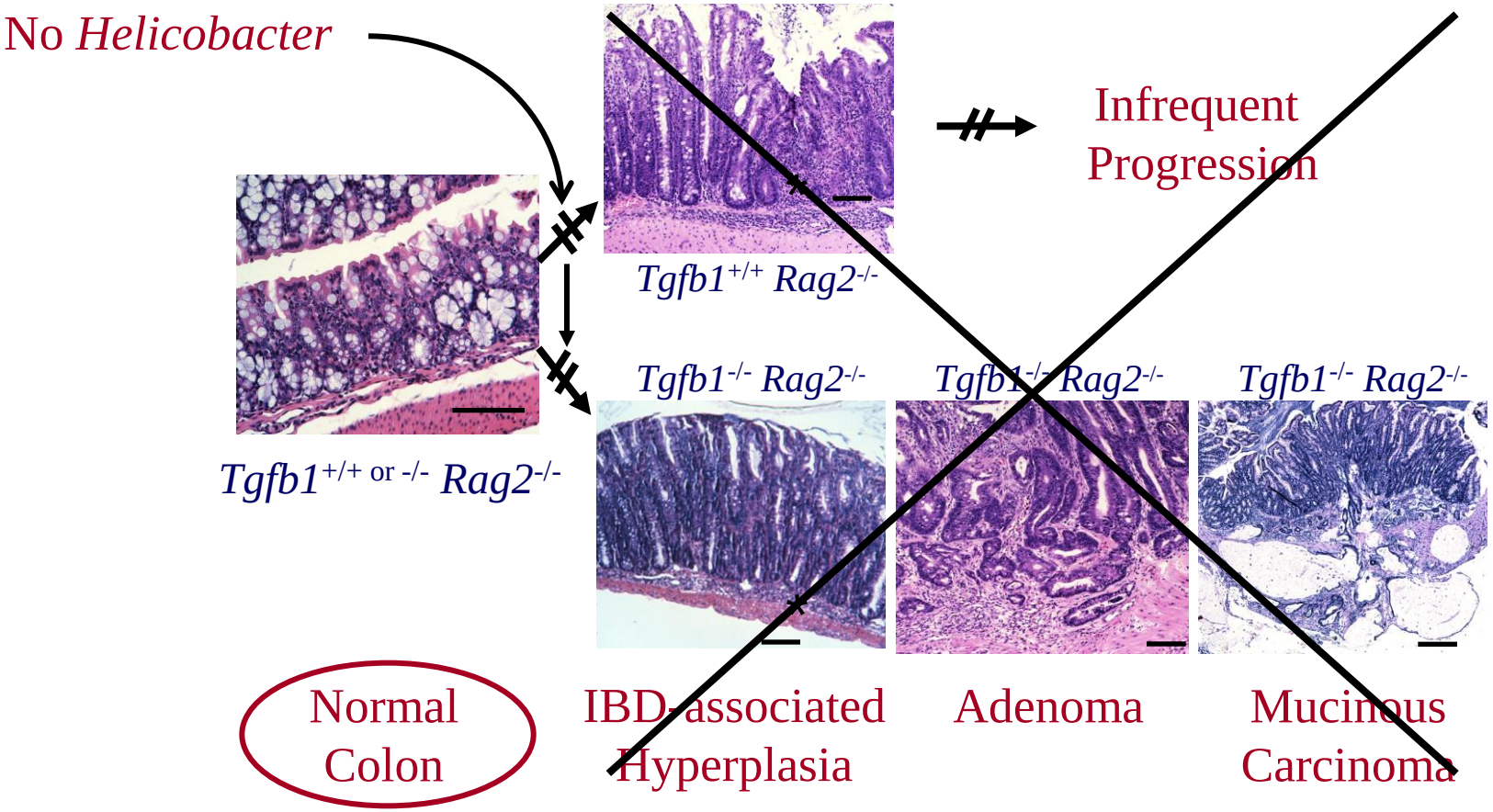
Engle *et al* (1999) *Cancer Res.*

Colon Tumor Progression in *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice



Sandi Engle *et al* (1999) *Cancer Res.*

Colitis- and Lesion-free *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ and *Smad3*$^{-/-}$mice



Sandi Engle *et al* (2002) *Cancer Res.*

SMAD3: Maggio-Price et al (2006) *Cancer Res.*

Differentially Expressed Genes in Colons of Inflammation-free *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice

Microarray study:

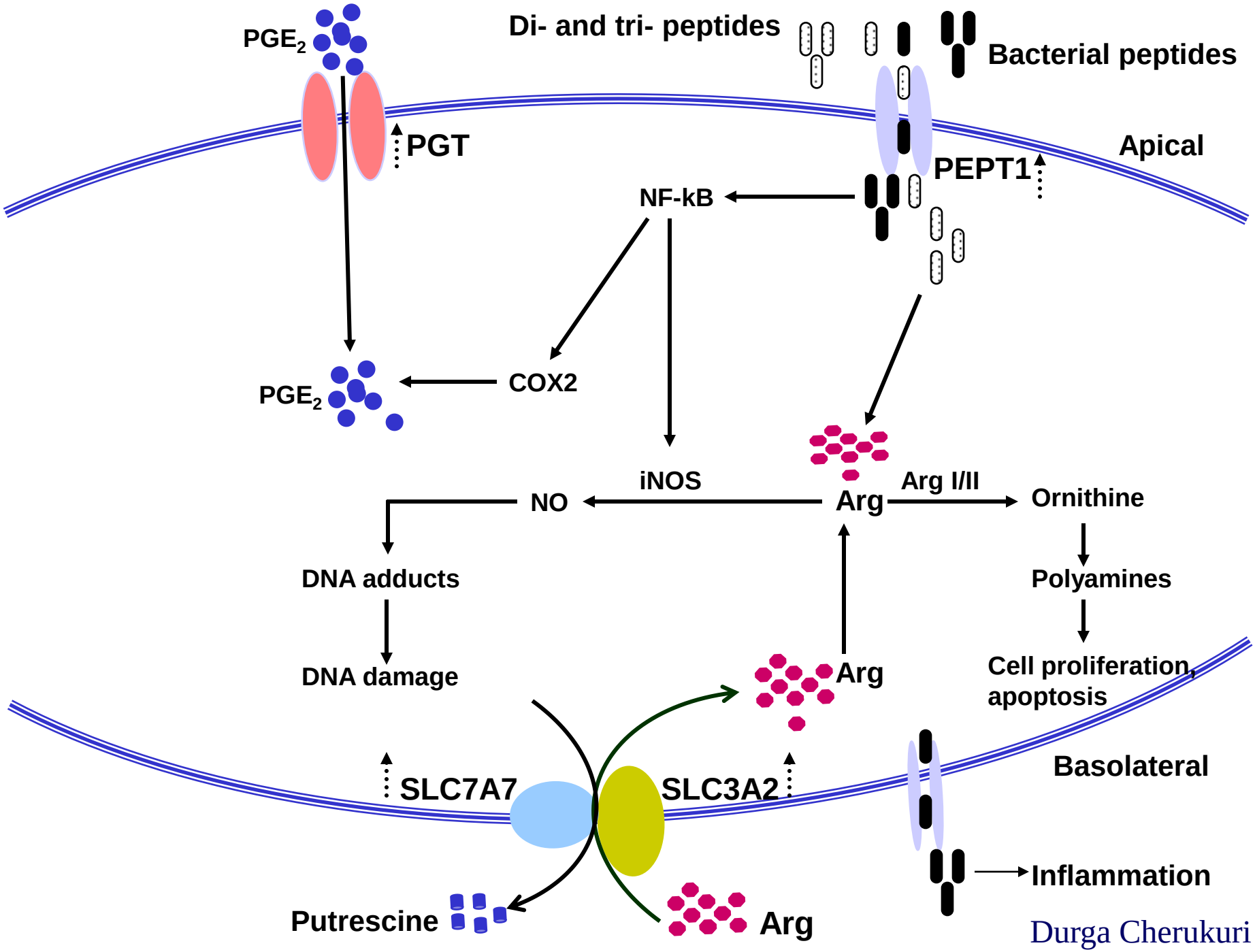
- Altered expression of 927 genes in *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice compared to *Tgfb1*$^{+/+}$ *Rag2*$^{-/-}$ mice (n=3)
- Functional association of differentially expressed genes
  - Transport 24 genes
    (inflammation, lipid & energy metab., antigen processing, flora sensing)
  - Inflammation 9 genes
  - Cell adhesion 9 genes
  - Cell matrix 10 genes
  - Lipid metabolism 20 genes

Bruce Aronow, Mohamad Azhar, Durga Cherukuri, Eyad Nusayr

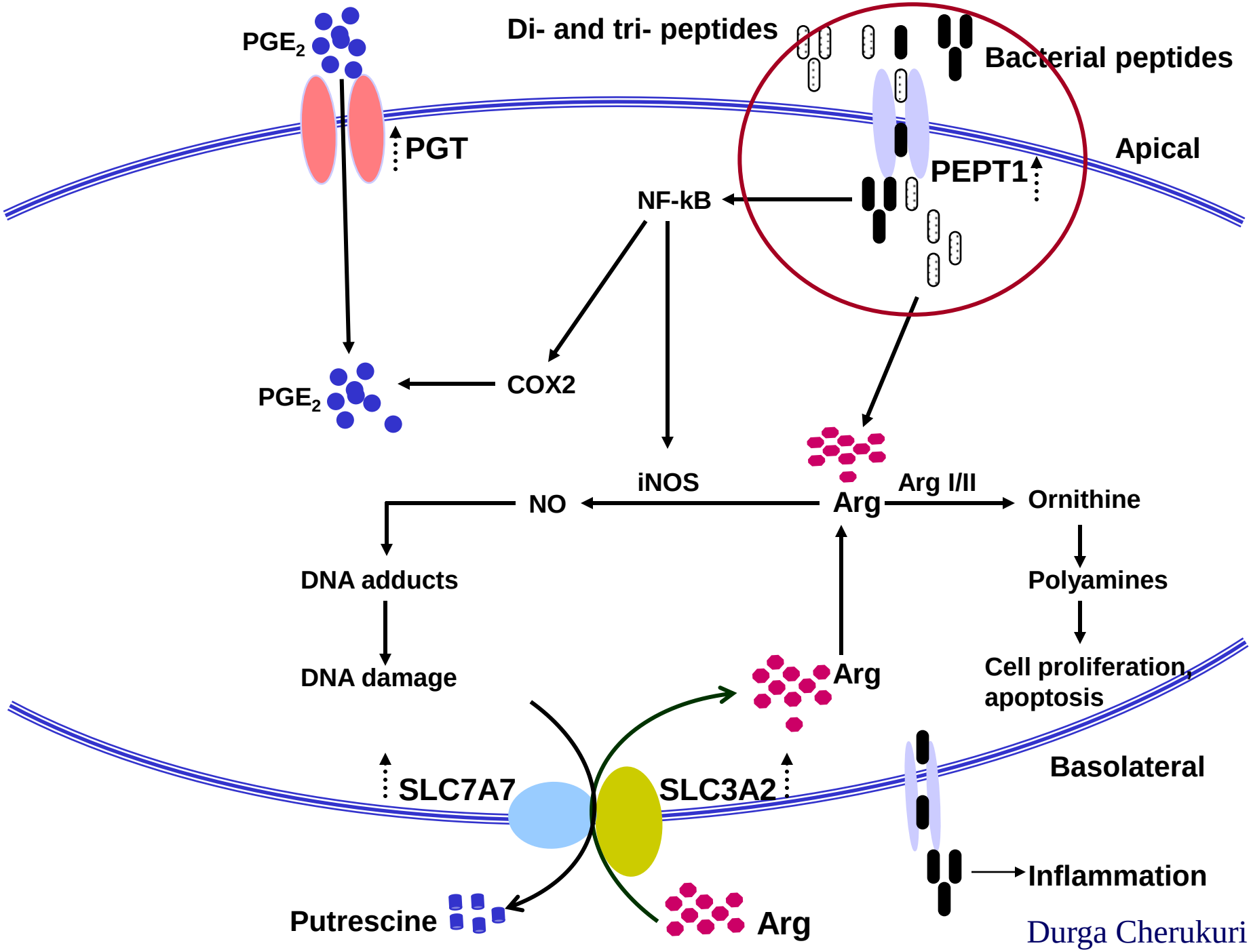
Differentially Expressed Genes in Colons of Inflammation-free *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice

Microarray study:

- Altered expression of 927 genes in *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice compared to *Tgfb1*$^{+/+}$ *Rag2*$^{-/-}$ mice (n=3)
- Functional association of differentially expressed genes
  - Transport 24 genes
    (inflammation (4 genes) , lipid & energy metab., antigen processing, flora sensing)
  - Inflammation 9 genes
  - Cell adhesion 9 genes
  - Cell matrix 10 genes
  - Lipid metabolism 20 genes



Durga Cherukuri

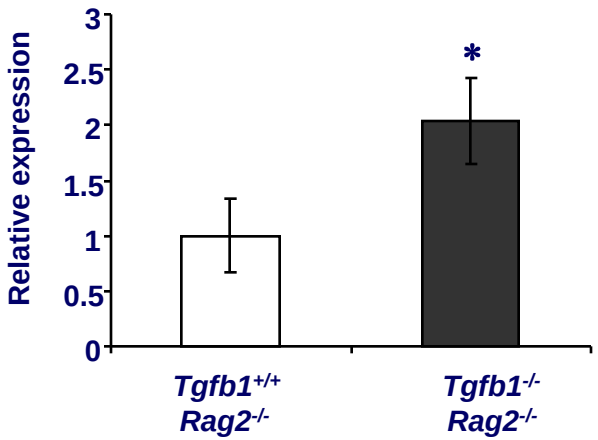


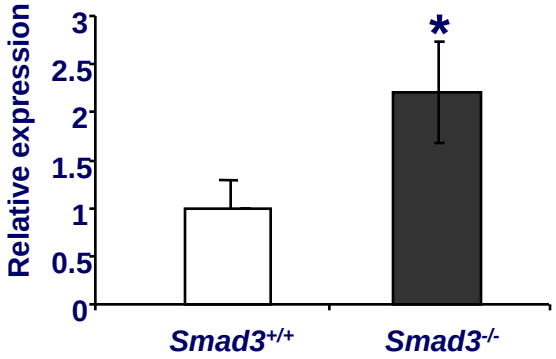
Durga Cherukuri

Increased Expression of Oligopeptide Transporter in Inflammation-free *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ and *Smad3*$^{-/-}$ mice

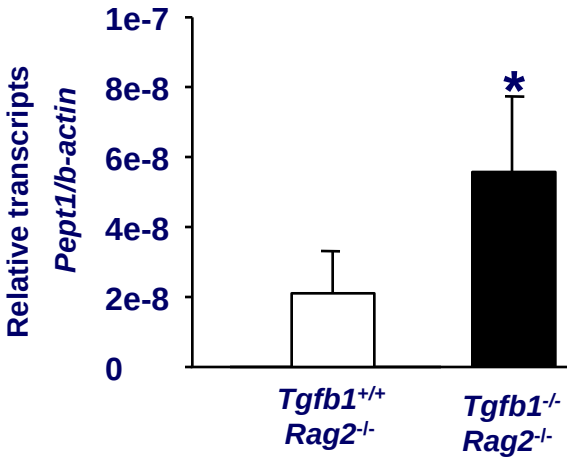
Slc15a1 **(PEPT1) di- and tri-peptide transporter**

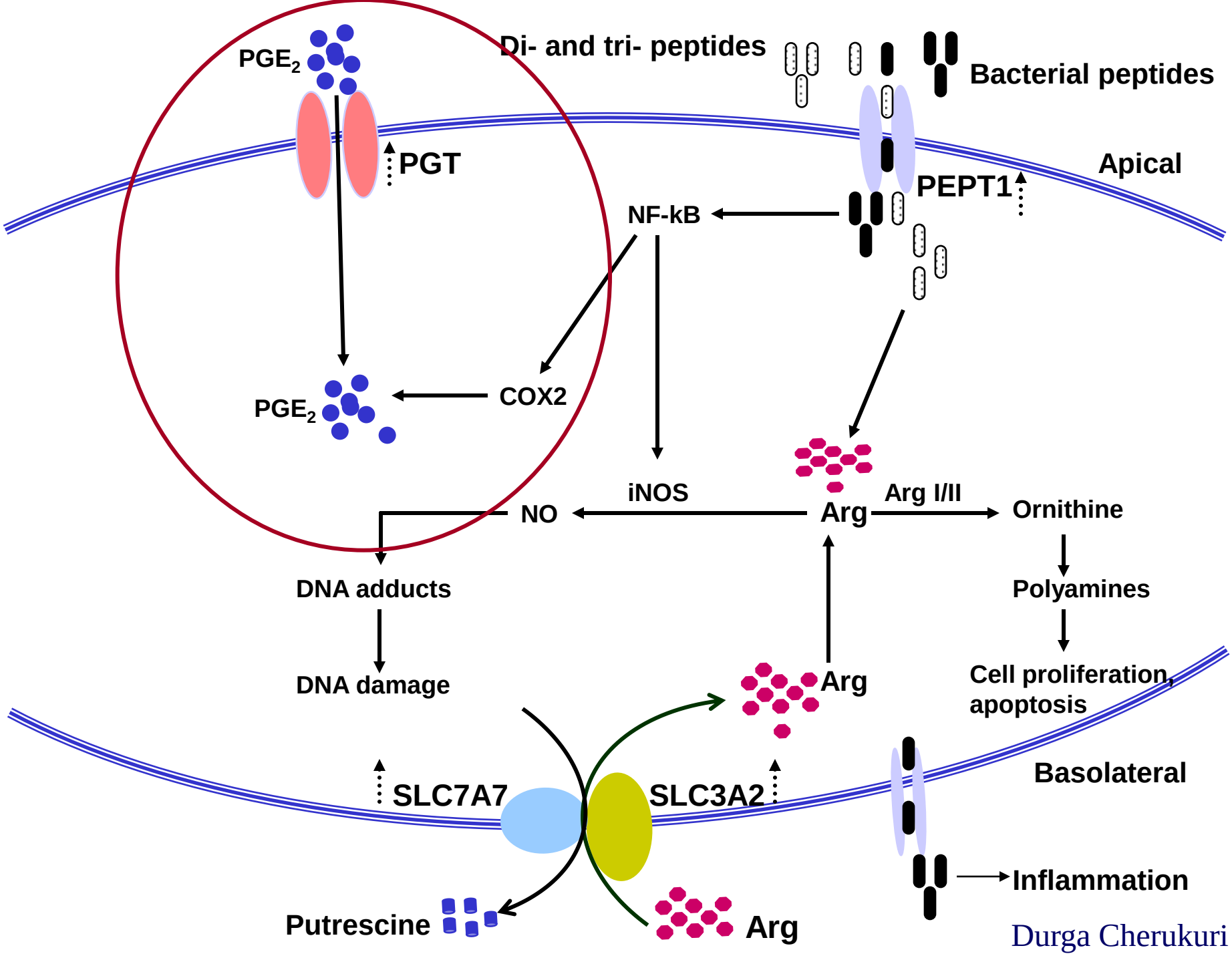
Colonic Epithelium





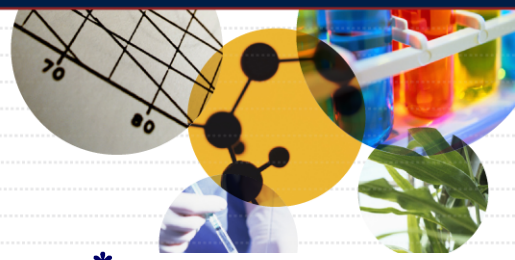
MEFs



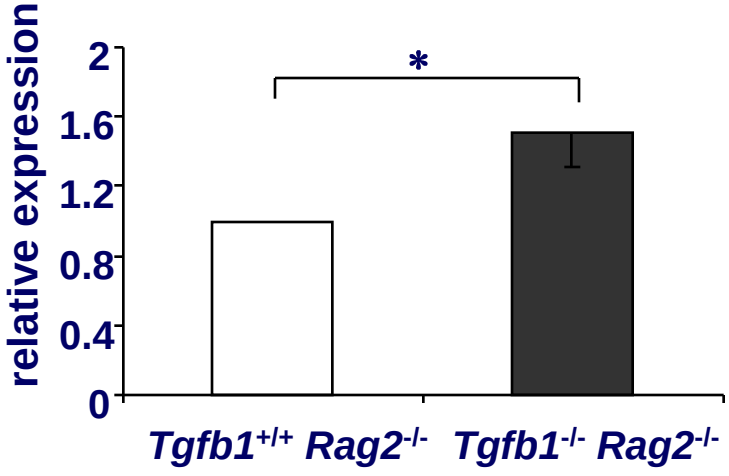


Durga Cherukuri

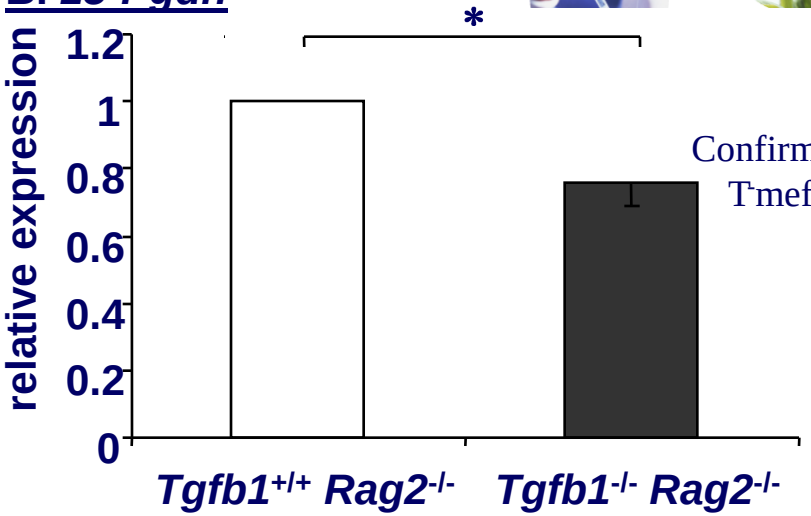
Dysregulation of Prostaglandin Pathway in absence of Functional TGFβ1 Signaling



A. *Pgt* Prostaglandin transporter

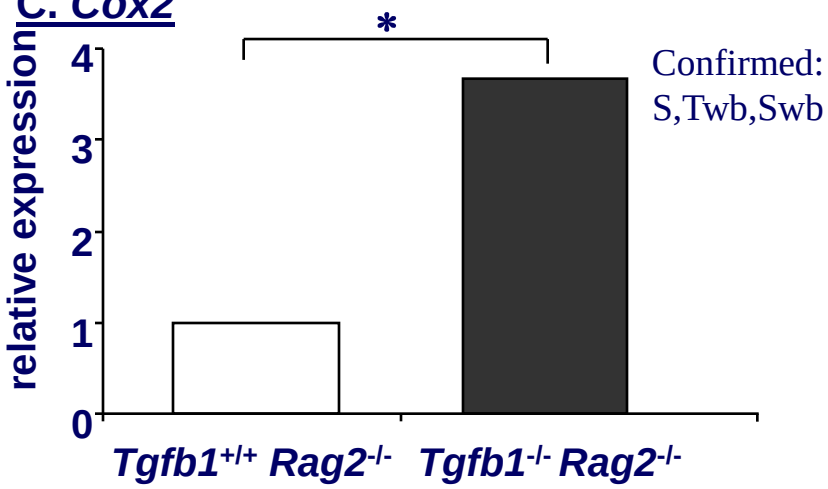


B. *15-Pgdh*



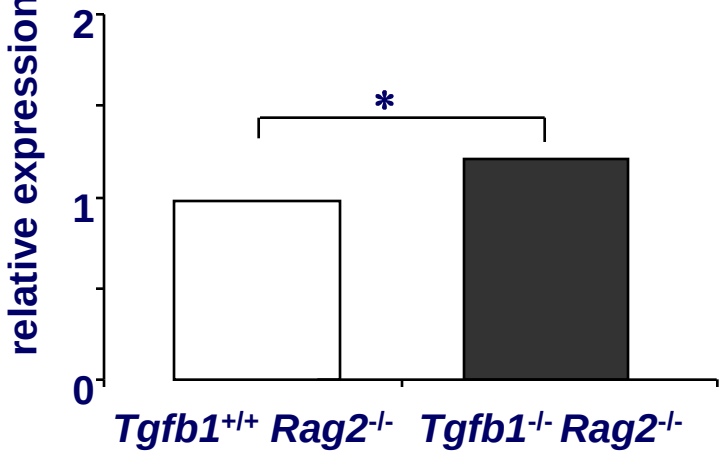
Confirmed: Tmefs

C. *Cox2*



Confirmed: S,Twb,Swb

D. *Areg*

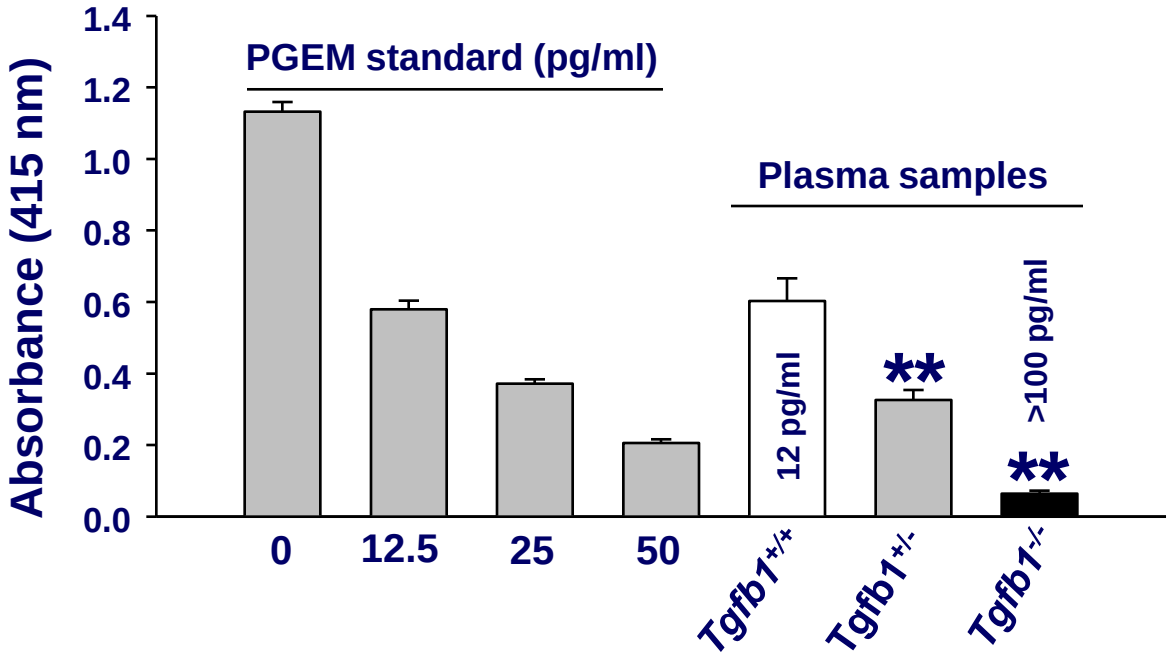


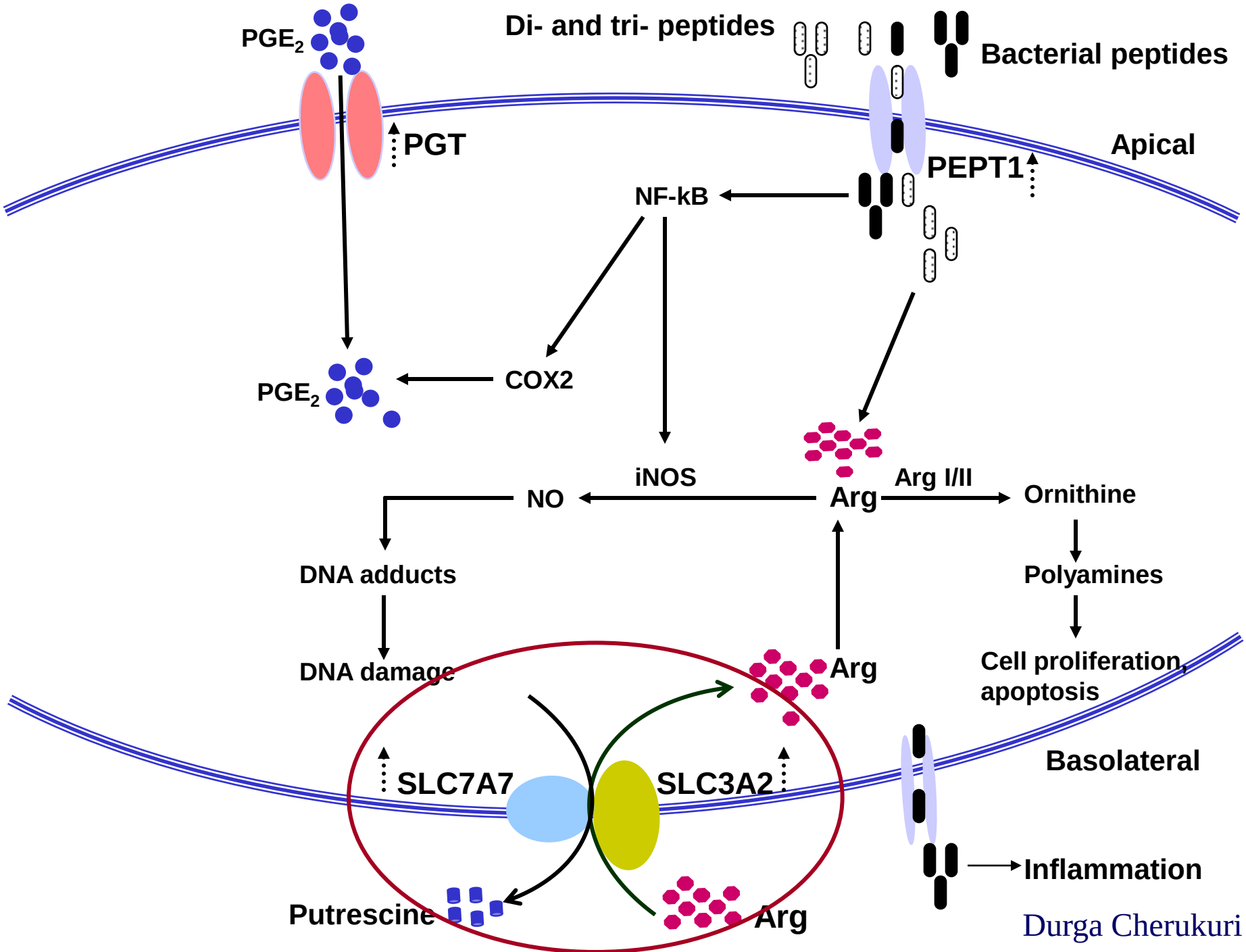
*$p>0.05$

Durga Cherukuri

Plasma PGE_2 levels in Inflammation-free *Tgfb1 Rag2*$^{-/-}$ mice

PGEM / PGEM-tracer Competitive Immunoassay

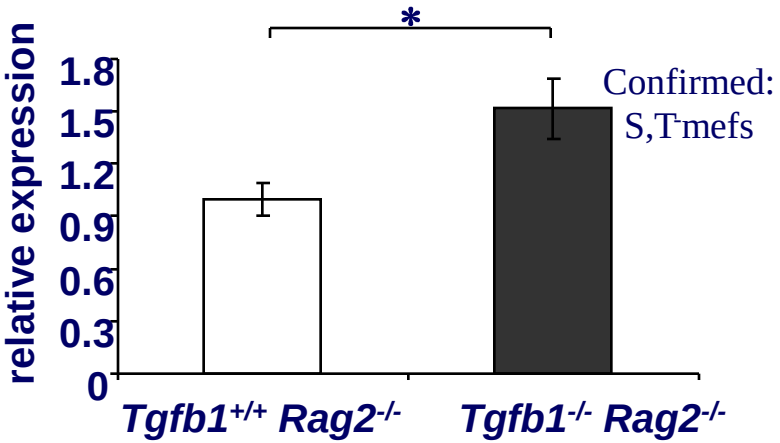




Durga Cherukuri

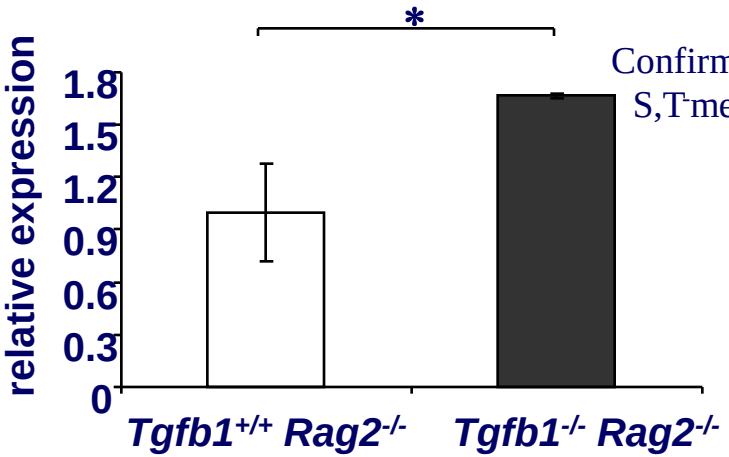
Dysregulation of Nitric Oxide (NO) Pathway in Absence of Functional TGFβ1 Signaling

A. *Slc7a7* (Arginine transporter)



Confirmed: S,T-mefs

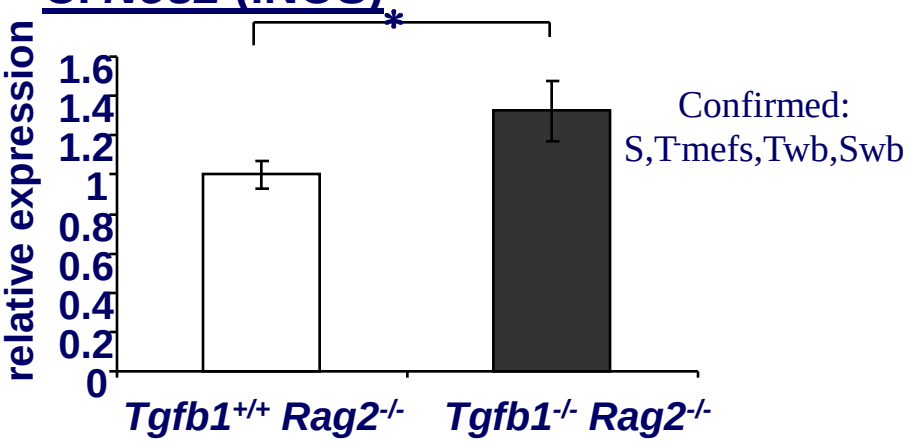
B. *Slc3a2* (CD98) Dibasic & neutral AA transporter)



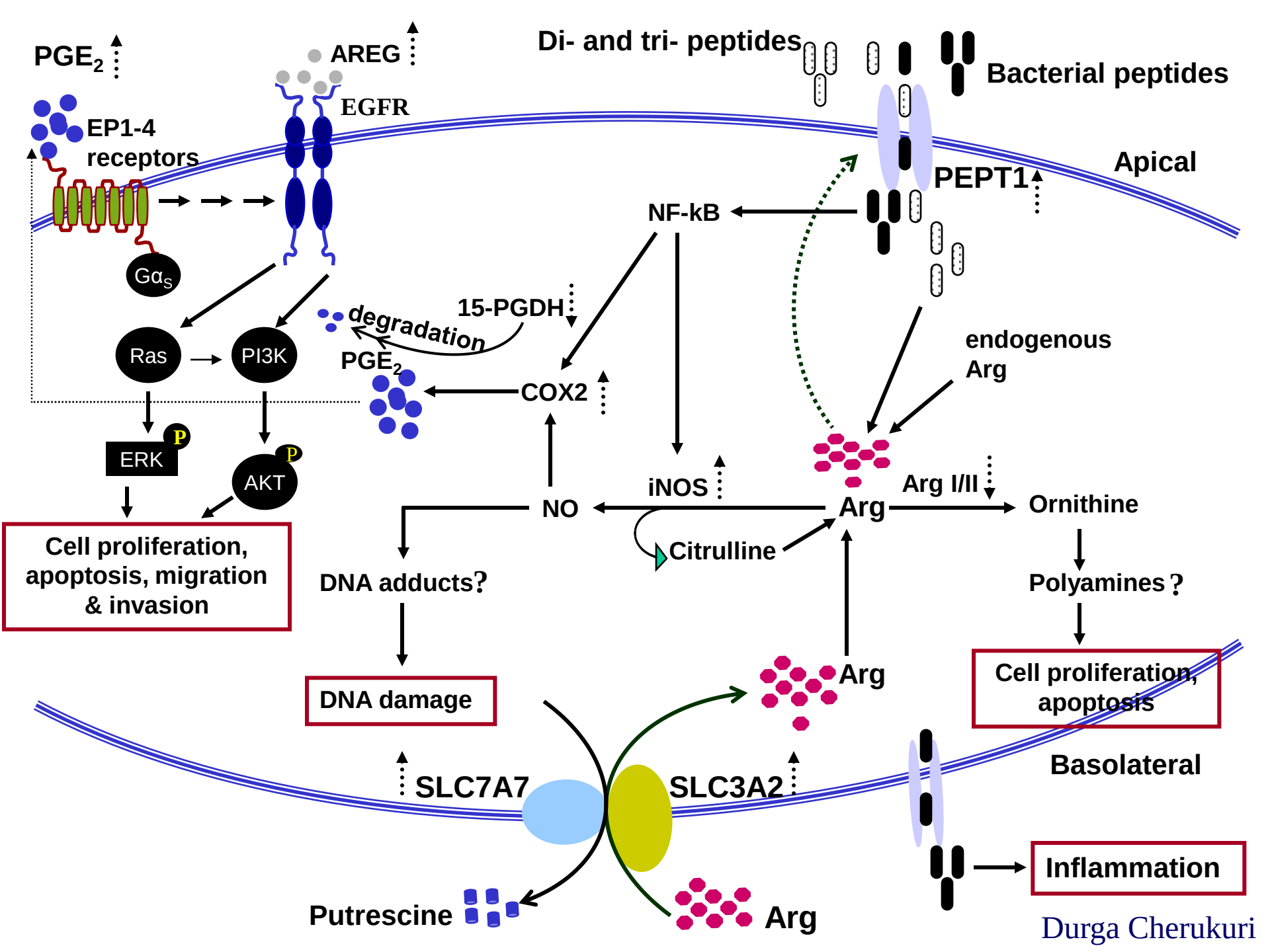
Confirmed: S,T-mefs

*p<0.05

C. *Nos2* (iNOS)



Confirmed: S,T-mefs,Twb,Swb



Durga Cherukuri

“Death by a Thousand Cuts”

Lalage Wakefield,NCI

Cancer is a Complex Disease

In *TGFBR2** CRCs, 84% have mutations
in combinations of 5 other genes

Calin et al, (2000) *Int J Cancer*

Some GWAS studies have been to some degree frustrating
perhaps because different combinations of differences in multiple genes,
each of which can lead to small expression differences,
may confer differential cancer susceptibilities

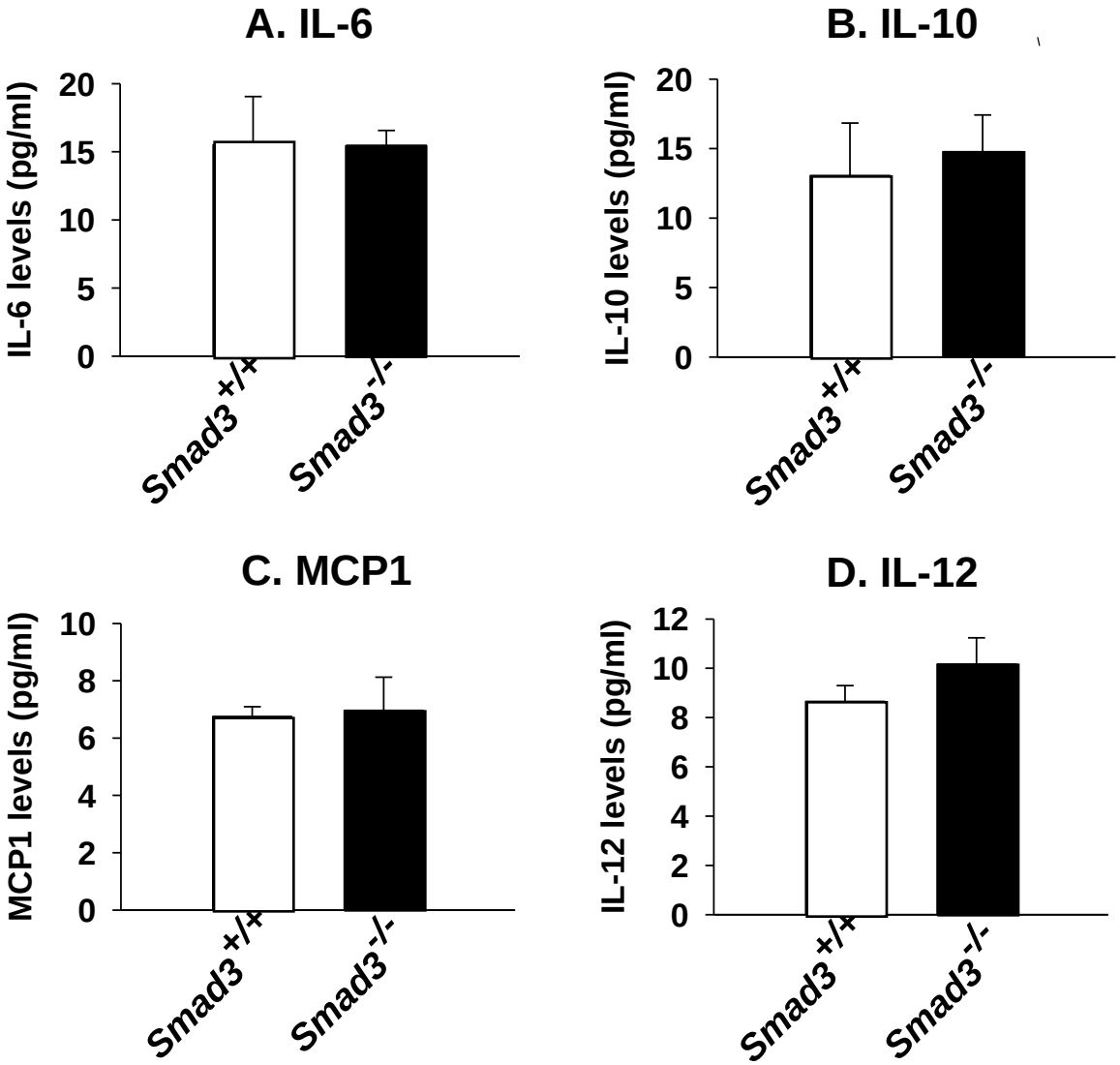
Hypothesis

In absence of TGFβ signaling there exists in the colon mucosal epithelium a

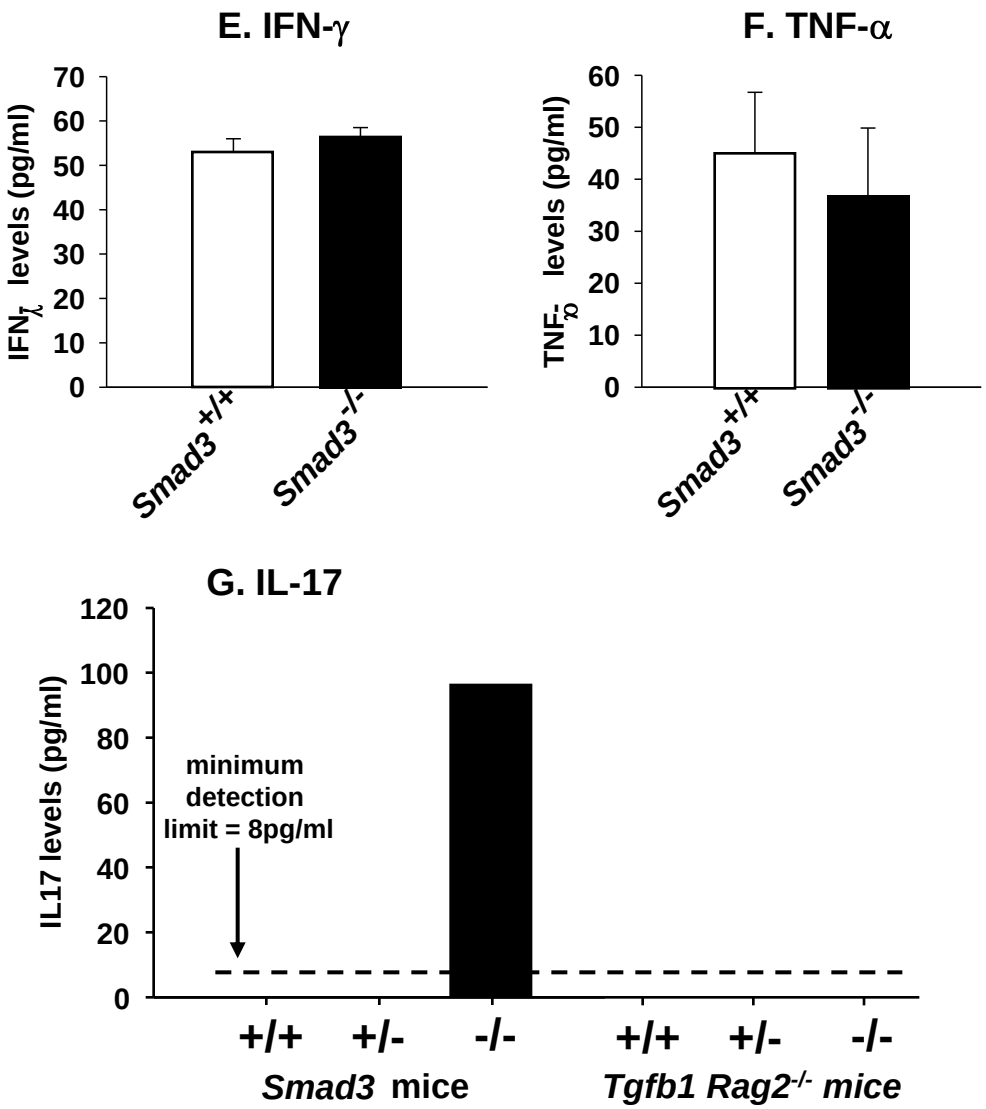
"Sub-clinical state of inflammatory readiness"

such that in the presence of inflammatory stress, cancer progression ensues

Are There Inflammatory Cytokines in Inflammation-free *Smad3*$^{-/-}$ blood plasma



Are There Inflammatory Cytokines in Inflammation-free *Smad3*$^{-/-}$ blood plasma



Durga Cherukuri

Conclusion

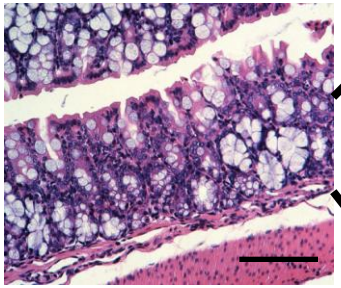
In absence of TGFβ signaling there exists in the colon mucosal epithelium a

"Sub-clinical state of inflammatory readiness" such that

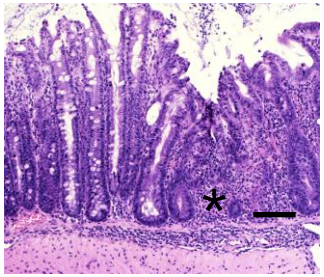
in the presences of inflammatory stress, cancer progression ensues

Colon Tumor Progression in *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice

Helicobacter-pos



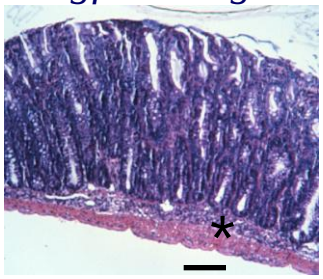
Tgfb1$^{+/+}$ or $^{-/-}$ *Rag2*$^{-/-}$



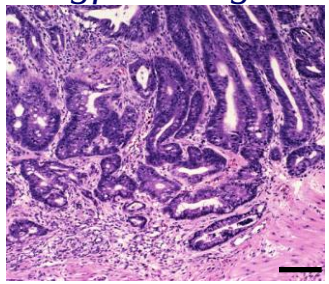
Tgfb1$^{+/+}$ *Rag2*$^{-/-}$

Infrequent Progression

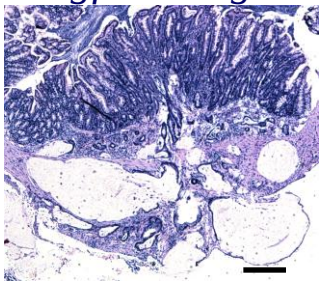
Tgfb1$^{-/-}$ *Rag2*$^{-/-}$



Tgfb1$^{-/-}$ *Rag2*$^{-/-}$



Tgfb1$^{-/-}$ *Rag2*$^{-/-}$



Normal Colon

IBD-associated Hyperplasia

Adenoma

Mucinous Carcinoma

Increased 1, N^6-ethenodeoxycytidine (εdC) levels in Colon Cancer Susceptible Tissues from *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ Mice with Colitis

DNA adducts	*Tgfb1*$^{+/+}$ (Hyperplastic colon tissue)	*Tgfb1*$^{-/-}$ (Hyperplastic colon tissue)	Ratio KO/WT
1,N^6-ethenodeoxyadenosine/ 10^8 deoxyadenosine (εdA/10^8 dA)	0.9	0.5	0.55
3,N^4-ethenodeoxycytidine/ 10^8 deoxycytidine (εdC/10^8 dC)	1.3	10.7	8.23

Note: Patients of Ulcerative colitis have ~4 fold increase in εdC (Bartsch and Nair 2005 *Mut. Res*.)

Summary

TGFβ-deficient mice model prevalent aspects of CRC patients under 40 yrs of age.

Their cancer has a proximal preference, often colitis associated, less differentiated, more flat-like and often mucinous.

These pre-tumor tissues reveal a sub-clinical state of inflammatory readiness, such that in the face of inflammatory stress, susceptibility for progression to CRC is increased.

Acknowledgements

Lab

Marcia Shull
Sandra Engle
Jay Hoying
Ilona Ormsby
Tom Mast
Mohamad Azhar
Durga Cherukuri

Collaborators

Joanna Groden, OSU
Bruce Aronow, Cincinnati
Greg Boivin, Cincinnati
Ed Balish, Wisconsin
Helmut Bartsch, DKFZ, Heidelberg
Jagadeesan Nair, DKFZ, Heidelberg
Dave Besselsen, Arizona
Mark Nelson, Arizona
Gene Gerner, Arizona

NIH NCI
BIO5 Institute