TGFβ1 - Early Player in Mouse Colon Cancer: Suppresses IBD-Associated Colon Cancer by Preventing Pre-Clinical Inflammatory State of Readiness in Colon Mucosal Epithelium

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Characteristics of Adolescent and Young Adult CRC

Human CRC (~50,000 deaths/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


“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 \textit{TGFBR2} Mutation in CRC Subtypes

- **Sporadic CRC**
  - MSS & MSI-L
  - \textit{TGFBR2} 1.8%
  - MSI-H (14-32%)
  - 28-58%

- **HNPCC** (2-7%)
  - MSI-H (>90%)
  - ~90%

- **FAP** (1%)
  - MSS
  - 50-67%

- **UC-associated CRC** (1-2%)
  - MSI-H (8-24.5%)

Overall, the \textit{TGFBR2} mutation frequency in human CRC ranged from 8-25% up to 30% w/other TGFβ pathway mutations (\textit{TGFBR1, SMAD4, SMAD7}).

\textit{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

<table>
<thead>
<tr>
<th>Human</th>
<th>Mouse</th>
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<tbody>
<tr>
<td>Right-sided prevalence</td>
<td>Proximal prevalence</td>
</tr>
<tr>
<td>More likely to be flat-like than polypoid</td>
<td>More likely to be flat-like than polypoid</td>
</tr>
<tr>
<td>Earlier onset (44yrs vs. 65 average)</td>
<td>-</td>
</tr>
<tr>
<td>Faster progression</td>
<td>-</td>
</tr>
<tr>
<td>Predominantly mucinous</td>
<td>Predominantly mucinous</td>
</tr>
<tr>
<td>More likely to have inflam. infiltrates</td>
<td>More likely to have inflamm. infiltrates</td>
</tr>
<tr>
<td>More likely to be diploid</td>
<td>More likely to be diploid</td>
</tr>
<tr>
<td>Less likely to be metastatic</td>
<td>Less likely to be metastatic</td>
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</table>
TGFβ- and APC-Deficient Mouse CRCs are Quite Different

Expression profiles of mouse colon tumors

AOM  \( \text{Apc}^{\text{Min/+}} \)  Smad3\(^{-/-}\)  \( \text{Tgfb}^{1/-}\)
Frequency of Disease States in $Tgfb1$ $Rag2^{-/-}$ mice

Colon Tumor Progression in \( Tgf\beta1^{-/-} \) \( Rag2^{-/-} \) mice

- Normal Colon
- IBD-associated Hyperplasia
- Adenoma
- Mucinous Carcinoma

\( Tgf\beta1^{+/-} \) \( Rag2^{-/-} \)
\( Tgf\beta1^{-/-} \) \( Rag2^{-/-} \)
\( Tgf\beta1^{-/-} \) \( Rag2^{-/-} \)
\( Tgf\beta1^{-/-} \) \( Rag2^{-/-} \)

Infrequent Progression

Colitis- and lesion-free \textit{Tgfb1}^{-/-} \textit{Rag2}^{-/-} and \textit{Smad3}^{-/-} 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
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
Increased Expression of Oligopeptide Transporter in Inflammation-free *Tgfb1*^-/-* Rag2*^-/- and *Smad3*^-/- mice

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

### Colonic Epithelium

<table>
<thead>
<tr>
<th>Tgfb1^-/-</th>
<th>Smad3^-/-</th>
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<tbody>
<tr>
<td>Relative expression</td>
<td></td>
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</table>

### MEFs

<table>
<thead>
<tr>
<th>Tgfb1^-/-</th>
<th>Smad3^-/-</th>
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<tbody>
<tr>
<td>Relative transcripts <em>Pept1/b-actin</em></td>
<td></td>
</tr>
</tbody>
</table>

* Durga Cherukuri
Apical

Bacterial peptides

Di- and tri-peptides

Putrescine

Arg

DNA adducts

DNA damage

NO

NF-kB

Arg

PGE\textsubscript{2}

PEPT1

COX2

iNOS

Arg I/II

Ornithine

Polyamines

Cell proliferation, apoptosis

SLC7A7

SLC3A2

Putrescine

Arg

Inflammation

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Dysregulation of Prostaglandin Pathway in absence of Functional TGFβ1 Signaling

A. *Pgt* Prostaglandin transporter

B. **15-Pgdh**

C. **Cox2**

D. **Areg**

* *p > 0.05*
Plasma PGE$_2$ levels in Inflammation-free Tgf$\beta$1 Rag2$^{-/-}$ mice

PGEM / PGEM-tracer Competitive Immunoassay

Absorbance (415 nm)

PGEM standard (pg/ml)

Plasma samples

- ** 12 pg/ml
- ** >100 pg/ml
- 0
- 12.5
- 25
- 50

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Apical 

Bacterial peptides

Di- and tri-peptides

Basolateral

Putrescine

SLC7A7

NO

SLC3A2

PEPT1

Arg

PGE2

iNOS

DNA adducts

DNA damage

Arg I/II

Ornithine

Polyamines

Cell proliferation, apoptosis

Inflammation

PGE2

COX2

NF-kB

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

A. **Slc7a7** (Arginine transporter)

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

C. **Nos2** (iNOS)

Confirmed: S,Tmefs

Confirmed: S,Tmefs,Twb,Swb

*p<0.05*
“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

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

A. IL-6

B. IL-10

C. MCP1

D. IL-12
Are There Inflammatory Cytokines in Inflammation-free Smad3\(^{-/-}\) blood plasma

E. IFN-\(\gamma\)

F. TNF-\(\alpha\)

G. IL-17

minimum detection limit = 8pg/ml

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 presence of inflammatory stress, cancer progression ensues.
Colon Tumor Progression in $Tgfb1^{-/-}$ $Rag2^{-/-}$ mice

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

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

$Igfb1^{-/-}$ $Rag2^{-/-}$

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

$Igfb1^{-/-}$ $Rag2^{-/-}$

$Helicobacter$-pos

$Infrequent$ $Progression$

Normal Colon

IBD-associated Hyperplasia

Adenoma

Mucinous Carcinoma
Increased 1, $N^6$-ethenodeoxyadenosine ($\xi$dA) levels in Colon Cancer Susceptible Tissues from $Tgfb1^{-/-}$ $Rag2^{-/-}$ Mice with Colitis

<table>
<thead>
<tr>
<th>DNA adducts</th>
<th>$Tgfb1^{+/+}$ (Hyperplastic colon tissue)</th>
<th>$Tgfb1^{-/-}$ (Hyperplastic colon tissue)</th>
<th>Ratio KO/WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,$N^6$-ethenodeoxyadenosine/10$^8$ deoxyadenosine ($\xi$dA/10$^8$ dA)</td>
<td>0.9</td>
<td>0.5</td>
<td>0.55</td>
</tr>
<tr>
<td>3,$N^4$-ethenodeoxyctydine/10$^8$ deoxyctydine ($\xi$dC/10$^8$ dC)</td>
<td>1.3</td>
<td>10.7</td>
<td>8.23</td>
</tr>
</tbody>
</table>

Note: Patients of Ulcerative colitis have ~4 fold increase in $\xi$dC (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.
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