Dissecting the immune response to colorectal cancer

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Fred Hutchinson Cancer Research Center
April 25, 2013
Increasing evidence suggests that the immune system influences the natural history of solid tumors.

Colon cancer incidence - global

Lung (13%)
Female Breast (11%)
Colorectum* (10%)
Stomach (8%)
Prostate (7%)
Liver (6%)
Cervix (4%)
Oesophagus (4%)
Bladder (3%)
Non-Hodgkin Lymphoma (3%)
Leukaemia (3%)
Uterus (2%)
Pancreas (2%)
Kidney (2%)
Lip and Oral Cavity (2%)
Brain and CNS (2%)
Ovary (2%)
Thyroid (2%)
Malignant Melanoma (2%)
Larynx (1%)
Other Sites (12%)
Colon cancer staging
Colon cancer survival by stage

308,734 patients diagnosed with colorectal cancer in UK 1996-2006
Immune response to colorectal cancer?

Survival by TNM stage

Survival stratified by density of infiltrating CD3⁺CD45RO⁺ cells

↑ Density of tumor infiltrating T lymphocytes (TIL), in particular CD45RO⁺ memory T cells, correlates with lower rate of events associated with metastasis & improved prognosis

Pagès et al., NEJM 2005; 353:2654-2566
Galon et al., Science 2006; 313:1960-1964
T cell subsets in CRC TIL also correlate with prognosis

CD45RO, CD3, CD8, Th1, FOXP3+

Th17

Salama P, JCO 2009;
Tosolini M, Cancer Res, 2011;

Table 5. Multivariate Analysis Showing the Significant Prognostic Indicators in Stage II and Stage III CRC (n = 445)

<table>
<thead>
<tr>
<th>Feature</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC stage, III v II</td>
<td>3.29</td>
<td>2.25 to 4.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vascular invasion, yes v no</td>
<td>1.98</td>
<td>1.39 to 2.83</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FOXP3+ , high v low</td>
<td>1.51</td>
<td>1.07 to 2.13</td>
<td>.019</td>
</tr>
<tr>
<td>FOXP3+ , high v low</td>
<td>0.64</td>
<td>0.38 to 0.77</td>
<td>.001</td>
</tr>
</tbody>
</table>

NOTE. Cox proportional hazards regression model.
Abbreviations: CRC, colorectal cancer; HR, hazard ratio; AJCC, American Joint Committee on Cancer; N, normal tissue; T, tumor tissue.
Studies characterizing CRC TIL interactions with autologous tumor

- Hom *et al.*, *Cancer Immunol Immunother* 1993:
  3 of 9 TIL secreted cytokine in response to autologous, cryopreserved colon tumor
- Mulder *et al.*, *Cancer Immunol Immunother* 1995:
  3 of 8 TIL secreted cytokine in response to autologous colon tumor
- Marits *et al.*, *Br J Cancer* 2006:
  1 of 6 TIL secreted IFN-γ in response to autologous tumor lysate from colon tumor
- Sarrabayrouse *et al.*, *Int J Cancer* 2011:
  0 of 4 CD4+ and CD8+ TIL, 3 of 4 DP CD4+CD8+ TIL secreted cytokine in response to autologous colon tumor
[Some of the] Challenges in studying the autologous TIL response to CRC

- Tumor digests are heterogeneous mixtures of cancer cells, stroma, and TIL
- Small numbers of infiltrating lymphocytes from specimens
- Difficulty propagating autologous CRC tumor cells from surgical specimens \textit{in vitro}
Cell line-derived xenografts bear little resemblance to primary human CRC

SW480 colorectal carcinoma cell line
NOD/Scid/IL2Rγ−/− mice are excellent hosts for human CRC

Dalerba et al., PNAS 2007; 104:10158-10163
Todaro et al., Cell Stem Cell 2007; 1:389-402
Dangles-Marie et al., Cancer Res 2007; 67:398-407

NIDDK Core Center of Excellence, Xenograft Core P30 DK056465 (B. Torok-Storb)
Dissecting the autologous adaptive immune response to colorectal cancer

Establish xenograft

NOD/Scid/IL2-Rγ−/−

Tumor cells

10-50-fold amplification per generation

Lymphocytes (TIL)

Lymphocytes

>1000-fold amplification

Expand TIL:

• “REP”
• αCD3/αCD28 beads
• KT64/BBL cells

Histology and IHC
Immunophenotyping
Transcriptional profiling
Methylation analysis
Establish new xenografts!

Immunophenotyping

TCR repertoire analysis
Functional analysis
Transcriptional profiling
Expansion of CRC tumor and TIL for study of autologous anti-tumor responses

- Implant tumor cells
- Digest tumor
- Expand T cells
- Isolate T cell subsets/clones
- Flow cytometry, TCRβ repertoire analysis

- 275 cGy
- Serial implants for expansion
- In vitro studies
- In vivo studies
- Harvest & digest tumors
- CD8⁺
- CD4⁺
Most $\alpha\beta$ T cells in CRC TIL are CD4$^+$CD45RO$^+$

D61540
Normal Adjacent Colon

D61540 T1

D61540 T2
Case #1: TIL from adjacent pieces of tumor

Tumor piece #1 (21%)

Tumor piece #2 (47%)

Normal colon (59%)

Normal colon (67%)

Deep sequence TCRβ CDR3

(%) = Percentage of sequences unique to the indicated population
Case #1: TIL from adjacent pieces of tumor

<table>
<thead>
<tr>
<th>Normal colon</th>
<th>Tumor piece 1</th>
<th>Tumor piece 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(59%)</td>
<td>(21%)</td>
<td>(59%)</td>
</tr>
<tr>
<td>(67%)</td>
<td>(47%)</td>
<td>(24%)</td>
</tr>
</tbody>
</table>

(%) = Percentage of sequences unique to the indicated population
Case #2: TIL from 4 non-contiguous liver metastases

48 year old woman with stage IV colon cancer metastatic to liver with 4 non-contiguous lesions. Treated with 4 cycles of FOLFOX without bevacizumab prior to surgery.

Collected 4 liver metastases, 2 normal liver samples, and a sample of peripheral blood for study of T cells in each compartment.

[Ray Yeung, UW Surgery]
(%): Percentage of sequences unique to the indicated population.

T cell phenotype:
- **CD4**
- **CD8**

**Graphical Data**:
- **Normal liver #1 (24%)**
  - $r^2=0.72$  
  - # shared=596

- **Normal liver #2 (23%)**
  - $r^2=0.42$  
  - # shared=305

- **Liver metastasis#1 (69%)**
  - $r^2=0.81$  
  - # shared=1241

- **Liver metastasis#2 (52%)**
  - $r^2=0.46$  
  - # shared=328

- **PBMC (68%)**
  - $r^2=0.72$  
  - # shared=596

- **Liver metastasis#1 (68%)**
  - $r^2=0.42$  
  - # shared=305

- **Liver metastasis#1 (41%)**
  - $r^2=0.46$  
  - # shared=328

---

**Legend:**
- Normal liver
- Liver metastasis
- PBMC
- T cell phenotype
- CD4
- CD8
Case #2: the αβ TCR repertoire of TIL is quite distinct from that in PBMC or adjacent liver.
Case #3: TIL from primary colon tumor and synchronously resected liver metastasis

78 year old man with stage IV colon cancer metastatic to liver. Treated with CapeOX with bevacizumab prior to surgery.

Collected tissues from colon tumor and liver metastasis as well as uninvolved normal colon, normal liver, and blood, for study of T cells in each compartment.

[Ray Yeung, UW Surgery]
(%) = Percentage of sequences unique to each comparator population
Case #3: TIL from colon tumor and synchronous hepatic metastasis demonstrate largest overlap
The majority of CRC TIL are $\alpha\beta$ TCR$^+$, CD4$^+$, CD45RO$^+$

The TCR$\beta$ CDR3 sequence repertoire of TIL from primary and metastatic CRC tumors:
- shows limited diversity
- Is quite distinct from that of adjacent normal tissue

Some clones that are highly prevalent in TIL are not found in normal adjacent tissue or blood

TCR repertoires of TIL from synchronously resected, noncontiguous colon tumors and hepatic metastases are closely related
Expansion of CRC tumor and TIL for study of autologous anti-tumor responses

275 cGy

Implant tumor cells

Serial implants for expansion

Harvest & digest tumors

Digest human tumor

Expand T cells

Isolate T cell subsets/clones

In vitro studies

In vivo studies
<table>
<thead>
<tr>
<th>CK20</th>
<th>CE</th>
<th>HLA-ABC</th>
<th>EpCAM</th>
<th>H&amp;E</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="primary_tumor" alt="Image" /></td>
<td>![Image](1° xenograft)</td>
<td>![Image](2° xenograft)</td>
<td>![Image](3° xenograft)</td>
<td>![Image](7° xenograft)</td>
</tr>
<tr>
<td>![Image](1° xenograft)</td>
<td>![Image](2° xenograft)</td>
<td>![Image](3° xenograft)</td>
<td>![Image](7° xenograft)</td>
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<td>![Image](3° xenograft)</td>
<td>![Image](7° xenograft)</td>
<td>![Image](7° xenograft)</td>
</tr>
</tbody>
</table>
Transcriptional analysis of tumors and xenografts

50-nucleotide paired-end sequence reads on Illumina HiSeq 2000

- Paired in Mm but not Hs?
  - Y: Mm
  - N

- Hits mouse with ≤1 mismatch?
  - Y: Mm
  - N

- Fewer mismatches in Mm than Hs?
  - Y: Mm
  - N: Hs
High reproducibility of RNA-seq data within a primary colon tumor and peritoneal metastases

D61540.T1

D61540.T2

2726 Ovarian met

2726 Omental met

r = 0.985, 0.990

r = 0.975, 0.997
Significant changes in the transcriptome characterize the transition from human to murine host.

D61540.T2/2726 Ovarian met → 1° xenograft → 2° xenograft

Primary

Secondary

Ovarian Met

Secondary

D61540.T2

$\text{r}=0.86$

$\text{r}=0.99$

$\text{r}=0.94$

$\text{r}=0.99$
Expression of epithelial and stromal genes

Human

Mouse

EpCAM

Vimentin

Tumor

Xenograft

Tumor

Xenograft
<table>
<thead>
<tr>
<th></th>
<th>E-cadherin</th>
<th>Vimentin</th>
<th>Fibronectin</th>
<th>CD31 (PECAM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D61540 human tumor</td>
<td><img src="D61540_human_tumor_E-cadherin.png" alt="Image" /></td>
<td><img src="D61540_human_tumor_Vimentin.png" alt="Image" /></td>
<td><img src="D61540_human_tumor_Fibronectin.png" alt="Image" /></td>
<td><img src="D61540_human_tumor_CD31_PECAM.png" alt="Image" /></td>
</tr>
<tr>
<td>M202 Xenograft (1°)</td>
<td><img src="M202_Xenograft_E-cadherin.png" alt="Image" /></td>
<td><img src="M202_Xenograft_Vimentin.png" alt="Image" /></td>
<td><img src="M202_Xenograft_Fibronectin.png" alt="Image" /></td>
<td><img src="M202_Xenograft_CD31_PECAM.png" alt="Image" /></td>
</tr>
</tbody>
</table>
The transcriptional profile of successive CRC xenografts is reasonably stable.
Unsupervised cluster analysis confirms expected relationships amongst transcriptional profiles.
Retention of human stroma in CRC xenografts

<table>
<thead>
<tr>
<th></th>
<th>HLA-ABC</th>
<th>E-cadherin</th>
<th>Vimentin</th>
<th>Fibronectin</th>
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<tbody>
<tr>
<td>P2750 human tumor</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td>M149 xenograft</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Generation of stroma-dominant tumor from malignant ascites fluid

2726 Ovarian met
2726 Omental met

2726 Ascites fluid

10 xenograft

HLA-ABC  E-cadherin  Vimentin  Fibronectin
Identification of genes preferentially expressed in parental tumors or in xenografts
Expression of lymphoid genes in colon cancer

BodyMap

D55949

2726

D61540

Lymph node
Colon
2nd xenograft
3rd xenograft
3rd xenograft
4th xenograft
7th xenograft
Ovary met
Omental met
1st xenograft
2nd xenograft
Tumor piece 1
Tumor piece 2
1st xenograft
2nd xenograft

LCK
CD3E
SYK
CD20

Expression levels for different xenografts and tumor pieces.
D61540 tumor cells express multiple T-cell associated genes

Human tumor

1. Xenograft

PD1
• Xenografts faithfully retain the histological characteristics of the human tumors from which they are derived
• Human stroma and vasculature are rapidly replaced by murine analogues in almost all CRC xenografts
• The transition from human tumor to mouse xenograft is marked by broad, reproducible transcriptional changes that reflect the replacement of human by murine stroma
• After initial establishment of a xenograft, the transcriptome remains largely stable with serial transplantation
• CRC cells express genes numerous lymphoid-specific genes
Expansion of CRC TIL and tumor for study of autologous anti-tumor responses

- Implant tumor cells
- Digest tumor
- 275 cGy
- Serial implants for expansion
- Harvest & digest tumors
- Isolate T cell subsets/clones
- Expand T cells
- In vitro studies
  - IFN-γ ELISpot
  - In vivo studies
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient dermal fibroblasts</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>EpCAM+ xenograft (tumor) cells</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>EpCAM− xenograft cells</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Murine liver cells</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Murine spleen cells</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Summary (3)

• TIL from primary (2) or metastatic (4) tumors contain T lymphocytes that are selectively reactive with autologous tumor cells but not fibroblasts

• Studies underway:
  – Identification of the antigens recognized by CD8^+ autologous tumor-reactive T cells is underway
  – Phenotypic characterization of CD4^+ autologous tumor-reactive T cells
Mining RNA-seq data for [immuno]therapy targets

- Cancer-testis genes
- Consensus immunotherapy targets
- Mesothelin
- Human endogenous retrovirus products
- Cancer-specific protein products

“I’ll pause for a moment so you can let this information sink in.”
Expression of potential immunotherapy targets in parental tumors and xenografts

Cancer-testis genes

CITN priority immunotherapy targets
Protein products of human endogenous retroviruses (HERVs) as therapeutic targets

A Human Endogenous Retroviral Sequence Encoding an Antigen Recognized on Melanoma by Cytolytic T Lymphocytes

Francesca Schiavetti, Joëlle Thonnard, Didier Colau, Thierry Boon, and Pierre G. Coulie

Cellular Genetics Unit, Christian de Duve Institute of Cellular Pathology, Université de Louvain, B-1200 Brussels [F.S., J.T., T.B., P.G.C.], and Ludwig Institute for Cancer Research, Brussels Branch, B-1200 Brussels [D.C., T.B.], Belgium

Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells

Yoshiyuki Takahashi, Nanae Harashima, Sachiko Kajigaya, Hisayuki Yokoyama, Elena Cherkasova, J. Philip McCoy, Ken-ichi Hanada, Othon Mena, Roger Kurlander, Tawab Abdul, Ramaprasad Srinivasan, Andreas Lundqvist, Elizabeth Malinzak, Nancy Geller, Michael I. Lerman, and Richard W. Childs

1Hematology Branch and 2Flow Cytometry Core Facility, National Heart, Lung, and Blood Institute (NHLBI), 3Surgery Branch, National Cancer Institute (NCI), 4Department of Clinical Pathology, Clinical Center, 5Office of Biostatistics Research, NHLBI, and 6Center for Cancer Research, NCI, NIH, Bethesda, Maryland, USA.
Primer on HERV genomics

Transposable Elements, ~45%; 3x10^6

Transposons, 2.8%; 0.3x10^6
Retroelements, 42.2%; 2.7x10^6

RNA intermediate

non LTR, 33.9%; 2.4x10^6
SINE (Alu, 10.6%; MIR, 2.5%)
LINE (L1, 16.9%; L2, 3.2%)
processed pseudogenes, <1.0%

LTR, 8.3%; 0.3x10^6
class I ERV + MER4, 2.8%
class II ERV, 0.3%
class III ERV, 4.6%
others (MST, MLT, etc.), 0.6%

SINEs (80-630 bp)
e.g. human Alu

LTR AAAAA

e.g. human SINE-R

(6-8 kbp)

LINE

Orf 1 Orf 2 (pol)
e.g. human LINE-1 (L1)

AAAAA

Retrotransposon

(4-8 kbp)

OrfA (gag) OrfB (pol)
e.g. Drosophila Copia, Yeast Ty1

Endogenous Retrovirus

(9-10 kbp)
e.g. HERV-K

A

Provirus

LTR gag prot

pol

env U3 RUS

Transcripts

full length

env

rec

“1.5 kb”

B

HERV-K, type 2 env and 3’-LTR sequence

HERV-K, type 1 env and 3’-LTR sequence

SD „b” SA

SD „a” SA

1

8.6 kb

3.3 kb

1.8 kb

1.5 kb

np9

A292 bp
HERVs & repeats expressed in cancer

HERV and repeat sequences over-expressed in ovarian cancer
SOURCE: McIntosh, Tewari
(POCRC Ovarian SPORE; Project 2)

RT-PCR for HERV-K env in colon cancers and xenografts

IHC for HERV-K env protein in 1° breast cancers
Cancer-selective proteins from alternative splicing

**Observed splice junctions**

- Somatic Tissue RNA-seq
- EST Libraries from Somatic Tissues
- Cancer RNA-seq

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal</th>
<th>Cancer</th>
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<tbody>
<tr>
<td>brain</td>
<td>666467</td>
<td>37798</td>
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<td>testis</td>
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<td>1059</td>
</tr>
<tr>
<td>placenta</td>
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<td>82100</td>
<td>0</td>
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<tr>
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<td>0</td>
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</tbody>
</table>

NCI 1 U01 CA176270-01
Cancer Target Discovery and Development Network
“Profiling cancer neoantigen repertoires and validating immunotherapy targets”
Pis: McIntosh, Warren

**Source:** McIntosh (NCI HHSN261200800001E, DOD W81XWH-12-1-0349), work by N. Clegg and M. Fitzgibbon.
Abundant evidence suggests that the immune system influences the natural history of CRC

Propagation of CRC in immune-deficient mice is enabling dissection of tumor immunobiology

Analysis of CRC genetic & genomic data identifies potential targets, but none to date that are widely expressed in tumors from many patients
Acknowledgements

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Sheila Ojeaburu

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Matt Fitzgibbon
Martin McIntosh

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Melissa Upton (UW Path)

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Cyd Nourigat
Melissa Comstock
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NIDDK Core Center of Excellence, Xenograft Core P30 DK056465 (B. Torok-Storb)
Thanks for your attention!
γδ T cells in CRC immune responses

Peripheral blood

Microbial and endogenous phosphoantigens: e.g., *M. tuberculosis*, *P. falciparum*, HMB-PP, IPP

GI tract

Vγ9/Vδ2 TCR

Stress-induced NKG2D ligands such as MICA, MICB (Groh, Spies; Strong)

CD3+ CD45RO+

Vγ2/Vδ1 TCR

CD45RO+ cells in CRC