Molecular Biology of Premalignant Lesions and Intestinal Carcinogenesis
Hereditary Colorectal Cancer Syndromes

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Graduate School of Biomedical Sciences
UT – M. D. Anderson Cancer Center

3 Simposio SEOM
Presidential Session
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Outline

1. ‘The Big Picture’: CRC incidence/mortality
2. From High-Risk patients to the general population
3. FAP as a model
4. Preclinical development of NSAIDs/COXibs
5. Clinical trials in FAP and translation to adenoma prevention in sporadics.
6. Future opportunities for chemoprevention: Systems Biology tools and NGS approaches
Cancer Statistics

Figure 4. Declines in Rates of Death from Major Noncommunicable Diseases in the United States, 1950 to 2010. Adapted from the National Center for Health Statistics, Centers for Disease Control and Prevention.18
## Colorectal Cancer Statistics

### Estimated New Cases*

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>241,740</td>
<td>29%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>116,470</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>73,420</td>
<td>9%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>55,000</td>
<td>7%</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>44,250</td>
<td>5%</td>
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<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,250</td>
<td>5%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>38,180</td>
<td>4%</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>28,540</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>26,830</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,090</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>848,170</td>
<td>100%</td>
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<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Breast</td>
<td>226,870</td>
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<tr>
<td>Lung &amp; bronchus</td>
<td>109,690</td>
<td>14%</td>
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<tr>
<td>Colon &amp; rectum</td>
<td>70,040</td>
<td>9%</td>
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<tr>
<td>Uterine corpus</td>
<td>47,130</td>
<td>6%</td>
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<tr>
<td>Thyroid</td>
<td>43,210</td>
<td>5%</td>
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<tr>
<td>Melanoma of the skin</td>
<td>32,000</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>31,970</td>
<td>4%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>24,520</td>
<td>3%</td>
</tr>
<tr>
<td>Ovary</td>
<td>22,280</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>21,830</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>790,740</td>
<td>100%</td>
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### Estimated Deaths

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<tr>
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<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>87,750</td>
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<tr>
<td>Prostate</td>
<td>28,170</td>
<td>9%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,470</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>16,850</td>
<td>6%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>13,980</td>
<td>5%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>13,500</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,040</td>
<td>4%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>10,510</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,320</td>
<td>3%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,650</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>301,820</td>
<td>100%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>72,590</td>
<td>26%</td>
</tr>
<tr>
<td>Breast</td>
<td>39,510</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>25,220</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>16,540</td>
<td>7%</td>
</tr>
<tr>
<td>Ovary</td>
<td>15,500</td>
<td>6%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>10,040</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>8,620</td>
<td>3%</td>
</tr>
<tr>
<td>Uterine Corpus</td>
<td>8,010</td>
<td>3%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>6,570</td>
<td>2%</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>5,980</td>
<td>2%</td>
</tr>
<tr>
<td>All Sites</td>
<td>275,370</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Siegel R et al, CA Cancer J Clin, 2012*
Colorectal Cancer Mortality

Role of technical developments: Surgery, Chemo, Targeted Drugs

Siegel R et al, CA Cancer J Clin, 2012
Colorectal Cancer Incidence
Causes of Hereditary Susceptibility to CRC

Sporadic (65%–85%)

Familial (10%–30%)

Hereditary

Causes of Hereditary Susceptibility to CRC

Causes of Hereditary Susceptibility to CRC

Adapted from Burt RW, Prevention and Early Detection of CRC (1996)

- Sporadic (65%–85%)
- Familial (10%–30%)
- Rare CRC syndromes (<0.1%)
- Familial Adenomatous Polyposis (FAP) (1%)
- Lynch Syndrome (3-5%)
Hereditary CRC Syndromes

**Polyposis Syndromes**
- **Adenoma predominant**
  - FAP (APC)
  - Attenuated FAP (APC)
  - MUTYH-polyposis (MUTYH)
- **Hamartoma predominant**
  - Peutz-Jeghers Sd (LKB1, STK11)
  - Juvenile polyposis Sd (BMPR1A, DPC4, PTEN)
  - Cowden Sd (PTEN)
- **Hyperplastic/Serrated adenoma**
  - Hyperplastic polyposis
  - Hereditary Mixed Polyposis Sd

**Non-polyposis Syndromes**
- **Mismatch repair deficient**
  - Lynch Syndrome (MLH1, MSH2, MSH6, PMS2)
- **Mismatch repair proficient**
  - Familial Colorectal Cancer Type X
  - Other Syndromes
From Cancer Genetics to the Sporadic Setting

Hereditary Cancer Syndromes

Sporadic Cancer
From Cancer Genetics to the Sporadic Setting

Hereditary Cancer Syndromes

Sporadic Cancer

FAP

Sporadic Adenomas
‘If I have seen further it is by standing on the shoulders of giants’
Isaac Newton

Chemoprevention in Hereditary Colorectal Cancer Syndromes

Ernest Hawk, M.D., M.P.H.¹
Ron Lubet, Ph.D.¹
Paul Limburg, M.D., M.P.H.²


Hawk, Cancer (1999); http://legomyphoto.wordpress.com/
Familial Adenomatous Polyposis

APC deletion of exon 4

Duodenal Ca 44
Familial Adenomatous Polyposis

- Prevalence 2.29-3.2 per 100,000 individuals
- 1 in 13,000 – 18,000 live births
- Autosomal dominant inheritance
- Caused by mutations in APC (5q21)
- Up to 30% with *de novo* mutations
- Complete penetrance (100%) for adenomas
- Genotype-Phenotype correlations

Familial Adenomatous Polyposis

Adenoma penetrance ~100%
Life-time risk of CRC ~100% if untreated
Familial Adenomatous Polyposis

Adenoma penetrance ~100%
Life-time risk of CRC ~100% if untreated
Familial Adenomatous Polyposis

Adenoma penetrance ~100%
Life-time risk of CRC ~100% if untreated
Familial Adenomatous Polyposis

- Adenoma penetrance ~100%
- Life-time risk of CRC ~100% if untreated
Familial Adenomatous Polyposis

**Extracolonic features**

- Duodenal Carcinoma (4-12%)*
- Desmoid Tumors (15%)*
- Thyroid Cancer - Papillary (2%)*
- Medulloblastoma (2%)*
- Hepatoblastoma (1.6%)*
- Adrenal mases (7.4%)
- Osteomas
- CHRPE
- Dental abnormalities (17%)
- Benign cutaneous – Epidermoid cysts

*Life-time risk

Adenoma-Carcinoma Sequence

- Loss of APC
- DNA hypomethylation
- KRAS activation
- Loss of 18q
- Loss of p53
- COX2

normal epithelium → hyperprolif epithelium → early intermediate adenoma → late carcinoma → invasion & metastases

Eberhart et al, Gastroenterology (1994); Williams et al, Gastroenterology (1996); Adapted from Vilar, Nature Reviews Clinical Oncology (2010)
Adenoma-Carcinoma Sequence

- Normal epithelium
- Hyperproliferative epithelium
- Early adenoma
- Intermediate adenoma
- Late adenoma
- Carcinoma
- Invasion & metastases

Key Events:
- Loss of APC
- DNA hypomethylation
- KRAS activation
- Loss of 18q
- Loss of p53
- Upregulation of COX2

References:
- Eberhart et al, *Gastroenterology* (1994);
- Williams et al, *Gastroenterology* (1996);
Adenoma-Carcinoma Sequence

- Normal epithelium → Hyperproliferative epithelium → Early adenoma → Intermediate adenoma → Late adenoma → Carcinoma → Invasion & metastases

- Loss of APC
- DNA hypomethylation
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- Loss of p53
- COX2 upregulation

Aspirin, NSAIDs, COXibs

Adenoma-Carcinoma Sequence

- Normal epithelium
- Hyperproliferative epithelium
- Early adenoma
- Intermediate adenoma
- Late adenoma
- Carcinoma

- Loss of APC
- KRAS activation
- Loss of 18q
- Loss of p53
- DNA hypomethylation

- Upregulated COX2

Aspirin, NSAIDs, COXibs

Glycerophospholipid

12-LOX, 15-LOX-1, 15-LOX-2 (mouse orthologue 8-LOX)

15-HETE, 12-HETE, 8-HETE

PGE\(_2\)

PG and TX synthase

COX2

PGD\(_2\)

PGI\(_2\)

TXA\(_2\)

PPAR\(\gamma\)

PPAR\(\delta\)

HETEs

HPETEs

EETs

Gupta and Dubois, *Nat Reviews Cancer* (2001)
NSAIDs in APCMin+ mice

Celecoxib

Sulindac

<table>
<thead>
<tr>
<th></th>
<th>+/-</th>
<th>Min</th>
<th>Min/sulindac</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice studied</td>
<td>5</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>No. of tumors/mouse</td>
<td>0</td>
<td>11.9 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Tumor distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>0 (0%)</td>
<td>0.8 ± 0.3 (6.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Jejunum + ileum</td>
<td>0 (0%)</td>
<td>10.7 ± 2.4 (90%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Large bowel</td>
<td>0 (0%)</td>
<td>0.4 ± 0.7 (3.5%)</td>
<td>1 (100%)</td>
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</table>

<sup>a</sup> p < .0001 compared to Min/sulindac.
NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

NSAIDs in APCMin+ mice

## Mouse models of FAP

<table>
<thead>
<tr>
<th>Mutation (gene symbol)</th>
<th>Tumor no./mouse&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor size and tumor histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Simple Apc&lt;sup&gt;+/−&lt;/sup&gt; mutations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apc&lt;sup&gt;Min&lt;/sup&gt; (or Apc&lt;sup&gt;Δ850&lt;/sup&gt;)</td>
<td>~30</td>
<td>~3</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;1638N&lt;/sup&gt;</td>
<td>~3</td>
<td>~0</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;Δ716&lt;/sup&gt;</td>
<td>~300</td>
<td>~3</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;Δ14&lt;/sup&gt;</td>
<td>~40</td>
<td>~4</td>
</tr>
<tr>
<td><strong>Cdx2</strong> (homeobox gene)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bub1b (Bub1R; yeast homologue)</td>
<td>SL, 1/6; Colon 6X</td>
<td>Same as in Apc&lt;sup&gt;+/−&lt;/sup&gt; mice</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;loxP&lt;/sup&gt; (or Apc&lt;sup&gt;580S&lt;/sup&gt;) × Adeno V-cre</td>
<td>Colon 10X</td>
<td>Tumors in infected colon</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;loxP&lt;/sup&gt; × Cdx2P-NLS-cre</td>
<td>Tumors predominantly in colon</td>
<td>Benign adenomas</td>
</tr>
<tr>
<td>Apc&lt;sup&gt;loxP&lt;/sup&gt; × Villin-cre</td>
<td></td>
<td>Light invasion in old mice</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated number of tumors per mouse.
NSAIDs

NSAIDs

NSAIDs

NSAIDs: Sulindac

COX-2 inhibition: Celecoxib

COX-2 inhibition: Celecoxib


~400 mg BID PO
Cross Species Comparison - Human

Sample status
- Normal
- Duodenum polyp
- Colon polyp

FAP polyp signature
- Upregulated
- Downregulated
Cancer In Silico Drug Discovery

Phase 1: CIDD hypothesis generation and experimental planning

1. Tumor gene expression profiling
   - (1) Tumor gene expression profiling
   - (2) Identify candidate drug compounds
   - (3) Match molecular signature to appropriate cell lines

Phase 2: Laboratory experimentation

- Tumor characteristic (e.g., BRAF V600E)
- Compound 1
- Compound 2
- Compound N1
- Cell line 1
- Cell line 2
- Cell line N2

San Lucas FA, …, Vilar E & Scheet P, MCT, 2014
Identification of Candidate Drugs

1. Identify samples with the phenotype of interest

2. Characterize gene expression changes associated with phenotype of interest

3. Connect tumor expression profile to candidate drugs

4. Annotate candidate compounds

5. Identify cell lines that most resemble tumors studied

Biologically interpretable candidate drug list

Cell lines for subsequent experimentation

TCGA
Clinical and experimental data

DrugBank
Drug annotations databases

Matador

KEGG Drug

Molecular gene expression signatures
MSigDB

Drug induced gene expression

cMap

Somatic mutations and gene expression

CCLE
Cell line experimental data

San Lucas FA et al, MCT, 2014
Identification of Candidate Drugs

Colorectal cancer BRAF V600E

BRAF V600E candidate drug compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enrichment score</th>
<th>Permutation P-value</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>gefitinib*</td>
<td>-0.995</td>
<td>0.016</td>
<td>0.000</td>
</tr>
<tr>
<td>2-deoxy-D-glucose</td>
<td>-0.977</td>
<td>0.051</td>
<td>0.022</td>
</tr>
<tr>
<td>S286656</td>
<td>-0.967</td>
<td>0.075</td>
<td>0.038</td>
</tr>
<tr>
<td>yohimbic acid</td>
<td>-0.901</td>
<td>0.003</td>
<td>0.000</td>
</tr>
<tr>
<td>amrinone</td>
<td>-0.884</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>trapidil*</td>
<td>-0.852</td>
<td>0.004</td>
<td>0.016</td>
</tr>
<tr>
<td>mycophenolic acid</td>
<td>-0.735</td>
<td>0.024</td>
<td>0.048</td>
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<tr>
<td>withaferin A</td>
<td>-0.679</td>
<td>0.026</td>
<td>0.054</td>
</tr>
<tr>
<td>MG-262*</td>
<td>-0.656</td>
<td>0.073</td>
<td>0.141</td>
</tr>
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</table>

BRAF V600E cell lines with matching gene expression signatures

Candidate Cell Lines
- RKO
- SNUC5
- CL34
- COLO205
- HT29

San Lucas FA et al, MCT, 2014
Identification of Candidate Drugs

San Lucas FA et al, MCT, 2014
## Identification of Candidate Drugs

### Colon and Duodenum Polyps

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enrichment score</th>
<th>Permutation P-value</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>spaglumic acid</td>
<td>-0.983</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>lycorine</td>
<td>-0.833</td>
<td>0.000</td>
<td>0.010</td>
</tr>
<tr>
<td>cloxacillin**</td>
<td>-0.779</td>
<td>0.000</td>
<td>0.010</td>
</tr>
<tr>
<td>quinpirole*</td>
<td>-0.817</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>yohimbic acid*</td>
<td>-0.905</td>
<td>0.010</td>
<td>0.000</td>
</tr>
<tr>
<td>arachidonyltrifluoromethane</td>
<td>-0.939</td>
<td>0.020</td>
<td>0.006</td>
</tr>
<tr>
<td>ketoconazole</td>
<td>-0.700</td>
<td>0.020</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>celecoxib</strong></td>
<td><strong>-0.652</strong></td>
<td><strong>0.020</strong></td>
<td><strong>0.000</strong></td>
</tr>
<tr>
<td>cefotiam</td>
<td>-0.653</td>
<td>0.040</td>
<td>0.026</td>
</tr>
<tr>
<td>quipazine</td>
<td>-0.653</td>
<td>0.040</td>
<td>0.016</td>
</tr>
</tbody>
</table>

San Lucas FA et al, *MCT*, 2014
Conclusions

1. Call for action: Increase efforts in early detection and chemoprevention of CRC
2. High-risk genetic populations as a model to understand the sporadic setting
3. NGS tools to get deep into the biology of premalignant lesions
4. Introduction of novel genomic and systems biology tools into preventive drug development
5. Development of precision preventive medicine
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