Breast & Colon Cancer: Molecular Alterations & Therapeutic Targets
Part 2

Nancy Hynes
FMI

June 8, 2007
Germ-line mutations in genes involved in DNA repair contribute to inherited colorectal cancers (CRC)

- Familial Adenomatous Polyposis (FAP)
  - APC
    - In 1991 the APC gene was cloned; germ line & sporadic mutations were described.

- Hereditary Nonpolyposis Colorectal Cancer (HNPCC)
  - Lynch syndrome
  - DNA mismatch repair genes - next slide
Model for DNA mismatch repair

Mutations in *hMSH2, hMLH1* account for ~50% of HNPCC families.

Recognition

IDL=insertion/deletion loops

Recruitment of MLH1 & PMS2 activate downstream repair events.

Wei et al 2002 Vol 8 346
Colorectal cancer develops in defined stages

Aberrant Crypt Focus - earliest lesion

Polyps

Adenomas of various stages

In addition to APC that is mutated in most CRC, other genes encoding important signaling proteins are mutated.
**Familial Adenomatous Polyposis - FAP**

- FAP is relatively rare, accounting for <1% CRC in US; affects 1 in 7000 individuals.
- Patients develop hundreds of polyps or adenomas at an early age.
- Benign tumors are not individually life threatening, however, considering their large numbers, it is inevitable that some will progress to invasive lesions.
- Rate limiting step in progression is loss of the WT APC allele.

**APC** = adenomatous polyposis coli
**Familial Adenomatous Polyposis - FAP**

- First described in the 18\textsuperscript{th} century.
- In 1986 a deletion on chromosome 5q was cytogenetically described. 
- The gene was localized to 5q21 in 1987. 
  Bodmer et al Nature 328; Leppert et al Science 238
- In 1991 the \textit{APC} gene was cloned; germ line & sporadic mutations described. 

\textit{APC} = adenomatous polyposis coli
Domain structure of APC

- APC is a large protein with many different domains; APC is now known to interact with many proteins - coming up.

- In 1993 β-catenin was shown to bind APC, providing the 1st functional clue for APC’s role in cancer. Rubinfeld et al Science 262; Su et al Cancer Res 2728.
E-cadherin binds β-catenin

G. Christofori 2006 Nature 441:444

β-catenin binds E-cadherin linking adhesion complexes to the cytoskeleton.

E-cadherin mediates cell-cell interactions

- Genetic studies in Drosophila placed Armadillo, the β-catenin homologue, on the wingless pathway.
In 1996 it was shown that β-catenin binds TCF (T cell factor). Behrens et al Nature 382 (Birchmeier lab); Molenaar et al Cell 86 (Clevers lab).

TCFs are a family of transcription factors that form a sub-group of the high mobility box (HMG) family; (in vertebrates 4 genes: TCF-1, Lef-1, TCF-3 & TCF-4.

TCFs bind the DNA as monomers & facilitate the binding of proteins that activate transcription - like β-catenin.

TCFs are the ultimate mediators of Wnt signaling
β-catenin is a key protein in the Wg/Wnt pathway

- Wg/Wnt signaling is one of the primary signaling pathways used during metazoan development; the pathway is conserved throughout evolution.
- In the fly, mutations in Fzd, the Wg/Wnt receptor, lead to planar polarity defects, e.g., in wings.

Cadigan & Nusse 1997 Genes & Develop 11: 3286
APC has functions distinct from controlling β-catenin stability

Review
Non-traditional roles for the Adenomatous Polyposis Coli (APC) tumor suppressor protein

Gene 361 (2005) 1–12

Caroline A. Hanson, Jeffrey R. Miller *

EB1 = microtubule +end binding protein

EB1 = cell polarity
**APC has functions distinct from controlling β-catenin stability**

Review

Non-traditional roles for the Adenomatous Polyposis Coli (APC) tumor suppressor protein

Caroline A. Hanson, Jeffrey R. Miller *

(A) - APC is enriched at the leading edge of a migrating cell, where it colocalizes with tips of MTs (C).
APC has functions distinct from controlling β-catenin stability

The inability of truncated APC to bind, e.g., EBI or MTs might also contribute to cancer.
**The Wnt pathway: from the cell surface to the nucleus**

- In the absence of Wnt, free β-catenin is rapidly targeted for degradation by a "destruction complex".
- In the presence of Wnt, DSH blocks GSK3β activity, β-catenin accumulates & enters the nucleus where it activates TCF-mediated transcription.
The Wnt pathway: from the cell surface to the nucleus

- Wnt activates other pathways - termed "Non-canonical"
The Go sub-unit mediates pathways transduced by Fz receptors in the fly.

Trimeric G Protein-Dependent Frizzled Signaling in Drosophila

Vladimir L. Katanaev,¹ Romina Ponzielli,² Michel Sémériva,² and Andrew Tomlinson¹,*

Fz receptors & GPCRs have similar structures; both contain seven transmembrane helices with an extracellular N-terminus and an intracellular C-terminus.

The paper shows that the Gα subunit (Go) mediates the planar cell polarity pathway in the fly.
The Wnt pathway is hyperactive in CRC

G. Christofori 2006 Nature 441:444

- In the majority of colorectal cancers (CRCs) APC is deleted/mutated & can no longer bind β-catenin.

- This results in stabilization of β-catenin leading to constitutive activation of the β-catenin/TCF pathway.
**β-catenin is mutated in some human cancers**

- β-catenin’s destruction box is also a target for mutation (aa 32-45); these mutations lead to stabilization & transcriptional activation of TCF.

### Amino acids 32-45 of β-catenin

| WT sequence | D | S | G | I | H | 37 | S | G | A | T | 41 | T | T | A | P | 45 | Δe3 | Missense mutation frequency | Exon 3 deletion frequency |
|-------------|---|---|---|---|---|----|---|---|---|---|----|---|---|---|---|----|---------------------------|--------------------------|
| Colorectal adenoma & carcinoma | E | V |   |   |   | A  |   |   |   |   | F  |   |   |   |   | F  | yes | 14/300                   | 8/98                     |
| Endometrial carcinoma | C | F | A | C | F | A  | I |   |   |   | C  | F |   |   |   |   | no  | 5/35                     | 0/35                     |
| Hepatocellular carcinoma | N | V | P | E | V | Y  | A |   |   |   | F  | P |   |   |   | F | yes | 17/101                   | 3/101                    |
| Malignant fibrous histiocytoma | Cu | Y |   |   |   |   |   |   |   |   |   |   |   |   |   | F | -  | 1/21                      | -                        |
| Medulloblastoma | C | Y |   |   |   | C  |   |   |   |   |   |   |   |   |   |   | -  | 3/67                      | -                        |
| Ovarian carcinoma | G | Y | F | C | V | C  | A | T | A | P |   |   |   |   |   |   | no | 25/160                   | 0/210                    |
| Pilomatrixoma | G | Y | F | C | Y | F  | E | C | F |   |   |   |   |   |   |   |   | -  | 12/16                     | -                        |
| Prostate | Y | F |   |   |   |   | A | Δ |   |   |   |   |   |   |   |   |   | -  | 5/104                     | -                        |
| Synovial sarcoma | Y |   |   |   |   |   |   | A | C | A |   | F |   | C | F |   |   | 1/7 | -                          | -                        |
| Uterine | F |   |   |   |   |   |   | A | C | A | F |   |   | C | F |   |   | 10/76 | -                       | -                        |

*Miller et al 1999 Oncogene 18, 7860*
The β-catenin destruction complex is multimeric

Scaffold proteins:
APC & Axin

Kinases:
GSK3B & CK1

Ubiquitin ligase:
β-TrCP

Tandem affinity purification (TAP) recently performed to identify novel proteins in the complex.

BAIT:
β-catenin
AXIN
APC
β-TrCP
Novel regulators of β-catenin signaling

- Blue - known interactors
  - GSK3- axin1
  - β-catenin-Tcf4, Lef1

- Red - novel interactors

- WTX
  - Binds β-catenin & APC
  - Recently identified in another context

M. Major et al 2007 Science Vol 316, p1043
WTX is a novel tumor suppressor - kidney cancer

Performed a genome-wide, large scale array CGH screen for DNA copy number changes in 51 kidney cancers - novel LOH at Xq11.1 found.

An X Chromosome Gene, WTX, Is Commonly Inactivated in Wilms Tumor

Miguel N. Rivera,¹²³ Woo Jae Kim,¹ Julie Wells,¹ David R. Driscoll,¹ Brian W. Brannigan,¹ Moonjoo Han,² James C. Kim,² Andrew P. Feinberg,⁴ William L. Gerald,⁵ Sara O. Vargas,⁶ Lynda Chin,⁷ A. John Iafrate,² Daphne W. Bell,¹* Daniel A. Haber†

2 FEBRUARY 2007 VOL 315 SCIENCE
**WTX is a novel tumor suppressor - kidney cancer**

- WTX is inactivated in ~30% Wilms tumors - kidney cancer.

![Graph and diagram]

Region is subject to X-chromosome inactivation.

In females with Del in WTX, the WT allele was always present on the inactive chromosome-covered with Xist.
**WTX is a novel tumor suppressor - kidney cancer**

An X Chromosome Gene, *WTX*, Is Commonly Inactivated in Wilms Tumor

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2 FEBRUARY 2007   VOL 315   SCIENCE

Report is interesting for 2 reasons:

First example of a tumor suppressor on the X-chromosome-(mention Knudson’s 2-hit hypothesis)

Links aberrant activation of the Wnt pathway to kidney cancer.
The Wnt pathway is altered in many human tumors

Barker & Clevers
Nature Reviews Drug Discovery
Vol 5 pg 997

Mining the Wnt pathway for cancer therapeutics
Nick Barker and Hans Clevers

Alterations in breast cancer
**Wnt-1 was the first mammary gland specific oncogene**

- First identified MMTV integration site activated int-1 (Wnt-1).

- To date no activating mutations of Wnt pathway proteins have been identified in human breast tumors. *(APC, AXIN, β-catenin)*

- Various lines of evidence suggest that the pathway is active in breast cancer.
Evidence for canonical Wnt pathway activity in breast cancer

- Nuclear β-catenin can be detected in the majority of breast tumors. 
  *Li et al PNAS 2001; Johnson et al 2000; Ryo et al 2001*

- Multiple Wnts & Fz receptors are expressed in breast tumors.

- sFRP1 a negative Wnt regulator is down-regulated in many primary breast tumors via promoter methylation. See next slide  
  *Ugolini et al 2001 Oncogene; Veeck et al 2006 Oncogene*

- Active Dvl & β-catenin in many breast cancer cell lines.  
  *Schlange et al 2007 submitted*
sFRP1 & other tumor suppressors are hypermethylated in cancer

A stem cell–like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing

NATURE GENETICS | VOLUME 39 | NUMBER 2 | FEBRUARY 2007

Joyce E Ohm¹, Kelly M McGarvey¹,², Xiaobing Yu³, Linzhao Cheng³,⁴, Kornel E Schuebel¹, Leslie Cope⁴, Helai P Mohammad¹, Wei Chen¹,⁵, Vincent C Daniel¹, Wayne Yu¹, David M Berman⁶, Thomas Jenuwein⁷, Kevin Pruitt¹, Saul J Sharkis¹,², D Neil Watkins³, James G Herman¹,⁶ & Stephen B Baylin¹,²
Conclusion
The Wnt signaling pathway is active in many types of human tumors, which results in constitutive transcription of TCF target genes.

Activation of TCF target genes is the primary transforming event in CRC.

What are the important target genes & how do they contribute to cancer?

This information should help explain how constitutive TCF signaling induces colorectal cancer.
How does constitutive TCF signaling contribute to CRC?

- Clevers & colleagues identified a number of genes whose expression is either induced or blocked after disruption of constitutive TCF signaling in CRCs. (TCF4)

  (Van de Wetering et al 2002 Cell 111:241)

- How did they accomplish this?

- Took a colorectal cancer cell line growing in tissue culture and introduced an inducible construct that expressed a dominant-negative* (dn) TCF4 protein & then identified genes whose expression was altered.

*dnTCF is deleted in its β-catenin binding domain, but still binds DNA - thus blocking β-catenin/TCF induced transcription.
What genes are affected by dnTCF4?
Van de Wetering et al 2002 Cell 111:241

- 120 down-regulated genes: *CD44, c-MYC, EPHB2 etc.*

These genes are normally expressed in the proliferative compartment of the intestines.

- 115 genes were stimulated: *p21, EphrinB2 etc.*

These genes are expressed in the differentiated compartment of the intestines.

*p21 & c-Myc are particularly interesting!*
c-Myc controls p21 transcription in CRC

- c-Myc expression is high in CRC cancer cells.
- siRNA KD of c-Myc induces p21 expression.

High Myc represses MIZ1 activity

MIZ1 is a transcriptional activator of p21 when Myc levels are low.
What genes are affected by dnTCF4?
Van de Wetering et al 2002 Cell 111:241

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These genes are expressed in the differentiated compartment of the intestines.

**Hypothesis**
Expression of the CDK inhibitor p21 in CRCs leads to a block in proliferation - this induces differentiation.

Explain basics of colon differentiation
Anatomy of the small intestinal epithelium

Radtke & Clevers 2005 Science 205: 1904

Normal mucosa contains large numbers of invaginations.

As proliferating cells move up the length of the fold they differentiate & become specialized to form various cell types that absorb nutrients & perform other functions.

TCF4 is expressed in stem cells at the base of the crypt.

Wnt is probably expressed in stromal cells at the base of the crypt.

Wnt signaling is active in a limited compartment of the intestines.
Anatomy of the small intestinal epithelium

Stem cells - give rise to the differentiated cells.

Radtke & Clevers 2005 Science 205: 1904
Mouse models with aberrant Wnt activity

Min = multiple intestinal neoplasia - mutation in APC

Germline ENU mutagenesized B6 males X AKR females - strain with an autosomal dominant mutation that produced multiple adenomas throughout the length of the GI tract.

(Moser et al 1990 Science 247: 322- W. Dove lab)

Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration

Owen J. Sansom,1 Karen R. Reed,1 Anthony J. Hayes,1 Heather Ireland,2 Hannah Brinkmann,1 Ian P. Newton,3 Eduard Batlle,4 Patricia Simon-Assmann,5 Hans Clevers,4 Inke S. Nathke,3 Alan R. Clarke,1,6 and Douglas J. Winton2

(Genes Dev. 18: 1385-90, 2004)

Inducible model

Cyp1A1-Cre X APC fl/fl

Cre expression induced with β-napthoflavone-leading to rapid APC loss

Cyp1A1 = cytochrome p450 1A1
120 genes were down-regulated in the cancer cells expressing dn TCF4: CD44, c-MYC, EPHB2 etc.

These genes are normally expressed in the lower portion of the intestines - the compartment with high TCF4 activity.

Van de Wetering et al 2002 Cell 111:241
The genes that are normally expressed in the lower, proliferating portion of the intestines are also expressed in the earliest detectable stage of cancer - aberrant crypt foci.

Van de Wetering et al 2002 Cell 111:241
The aberrant crypt foci & normal mucosa were examined for expression EphB2 a RTK that is normally expressed in the proliferating cells at the crypt bottom.

Van de Wetering et al 2002 Cell 111:241

Aberrant crypt focus (ACF)

EphB2 staining + proliferative marker

β-catenin staining

EphB2 staining - cells at the bottom of the crypts

ACF in the intestines of Min mice express EphB2.
Eph receptors & ephrin ligands

Eph receptors are members of the RTK superfamily;

Ephrin ligands are membrane bound: GPI anchored or membrane-spanning.

Eph receptors - activated by membrane-attached ligands.

Eph activation requires cell-cell contact.
Eph receptors & ephrin ligands

- The Eph/ephrin interaction frequently results in repulsion.
Model: Role of Eph-ephrin in the intestines

- Repulsive Eph-ephrin interactions establish the boundary between proliferating & differentiating cells.

- Role of Eph-ephrin is to position cells properly along the crypt-villus axis.

Villus - Differentiated cells

Crypt - Proliferating cells

Gradient of Ephrin expression in the villus.

Gradient of EphB2 expression in the crypt.

High Wnt activity leads to high levels of EphB2 expression.

M. Peifer 2002 Nature 420, 274
**What goes wrong in early stages of CRC?**

- Normally, the Wnt source at the crypt base maintains TCF activity in well defined regions of the crypt-villus axis.
- EphB is high in the base, but low at the junction where ephrin levels start to rise.

![Diagram showing cell cycle arrested differentiated cells, proliferating undifferentiated precursors, high EphB-low ephrin, and high EphB-low ephrin regions.](image)
What goes wrong in early stages of CRC?

- Normally, the Wnt source at the crypt base maintains TCF activity in well defined regions of the crypt-villus axis.

- When TCF is constitutively active due, e.g., to mutant APC, EphB is abnormally high in cells close to the repulsive Ephrin ligand.
Intestinal architecture in APC mutant mice

- In the presence of constitutive TCF activity abnormal structures are evident.

Out-pocketing pouches form at the proliferative zone of the crypt & protrude into the neighboring villi.

Microadenoma formation within single villi.

- Abnormal positioning of cells is likely to play a role in cancer progression: normal cell-cell contact or cell-basement membrane contact is affected; these types of alterations are known to contribute to the cancer phenotype.

Intestinal epithelium is thought to be the simplest mammalian model for studying tissue self-renewal.

This model features multipotent stem cells, amplifying compartments, lineage decisions & programmed cell death.

Mice with APC mutations can be studied to understand how deregulation of Wnt signaling effects normal tissue architecture ultimately leading to cancer.
Important implications

- Data from Clevers paper suggests that blocking TCF activity forces cancer cells to arrest in G1 & undergo differentiation.

- Targeting the APC/β-catenin/TCF pathway with directed therapeutics should have a major impact on treatment of CRC & other cancer types.

What are the possibilities for intervention?

Could loss of c-Myc "rescue" colon cancer? This was tested in a mouse model of the disease.
**Mouse models with aberrant Wnt activity**

**Min** = multiple intestinal neoplasia - mutation in APC

*Germline ENU mutagenesized B6 males X AKR females - strain with an autosomal dominant mutation that produced multiple adenomas throughout the length of the GI tract.*

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**Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration**

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**Inducible model**

*Cyp1A1-Cre X APC fl/fl*  
Cre expression induced with β-napthoflavone

*Cyp1A1 = cytochrome p450 1A1*

Owen J. Sansom¹, Valerie S. Meniel², Vanesa Muncan³, Toby J. Phesse², Julie A. Wilkins¹, Karen R. Reed², J. Keith Vass¹, Dimitris Athineos¹, Hans Clevers³ & Alan R. Clarke²

Cyp1A1-Cre X APC fl/fl X Myc fl/fl

“Dramatic changes conferred by APC loss (cell #, mitoses/crypt, EphR levels) are entirely dependent on functional c-Myc.”
Myc deletion rescues Apc deficiency in the small intestine


Owen J. Sansom¹, Valerie S. Meniel², Vanesa Muncan³, Toby J. Phesse², Julie A. Wilkins¹, Karen R. Reed², J. Keith Vass¹, Dimitris Athineos¹, Hans Clevers³ & Alan R. Clarke²

\[ \text{Cyp1A1-Cre} \times \text{APC fl/fl} \times \text{Myc fl/fl} \]

“Dramatic changes conferred by APC loss (cell #, mitoses/crypt, EphR levels) are entirely dependent on functional c-Myc.”

Role of c-Myc in Apc Mutant
Intestinal Phenotype: Case Closed or Time for a New Beginning?
Cancer Cell 2007 11:391
Guido T. Bommel² and Eric R. Fearon¹²A*

Concern that c-Myc loss might have other effects - 20 days after ablation of both genes intestinal cells were lost.
**Important implications**

- Data from Clevers paper suggests that blocking TCF activity forces cancer cells to arrest in G1 & undergo differentiation.

- Targeting the APC/β-catenin/TCF pathway with directed therapeutics should have a major impact on treatment of CRC & other cancer types.

**What are the possibilities for intervention?**

- Could loss of c-Myc "rescue" colon cancer? This was tested in a mouse model of the disease.

- RNAi or AS approaches to target Myc are a long-term option but are currently not feasible for treating humans with CRC.

**Other options?**

- NSAIDS, specific inhibitors of β-catenin/TCF, etc
Blocking the β-catenin/TCF interaction is an attractive strategy - this could be via peptides or small molecules.

Attempts have been made in this direction.
Screen for antagonists of Tcf4/β-catenin association

- Molecules that disrupt the Tcf4/β-catenin complex yield reduced alkaline phosphatase (AP) levels.

M Lepourcelet et al 2004 Cancer Cell vol 5 pg 91
Screen for antagonists of Tcf4/β-catenin association

Molecules that disrupt the Tcf4/β-catenin complex were tested in a reporter gene assay for blockade of transcription.

![Graph showing IC50 (μM) of compounds](image)

**TOP-Flash reporter assay**
- TCF responsive promoter linked to a luciferase reporter gene.
- Increasing conc (μM) of cpds

Reporter gene is transiently expressed in cells - e.g. HEK293, cells are treated with inhibitors & luciferase activity is measured.

M Lepourcelet et al 2004 Cancer Cell vol 5 pg 91
Screen for antagonists of Tcf4/β-catenin association

- The same molecules that disrupt the Tcf4/β-catenin complex & reporter gene activity also block proliferation of cancer cells.

Proliferation of HCT116 colon cancer cell line is blocked by PKF118-310 at an IC50 of 0.35μM

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 in MTS assay HCT116</th>
<th>IC50 in MTS assay HT29</th>
<th>IC50 in MTS assay PC-3</th>
<th>IC50 in MTS assay DU-145</th>
<th>IC50 ratio PC3:HCT116</th>
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<td>0.17 ± 0.01</td>
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<td>PKF118-744</td>
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<tr>
<td>PKF115-584</td>
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<td>0.40 ± 0.05</td>
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<td>2.88 ± 1.60</td>
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<tr>
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<td>0.64 ± 0.20</td>
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<td>NPDDG1.024</td>
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<td>0.60</td>
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</tr>
</tbody>
</table>

M Lepourcelet et al 2004 Cancer Cell vol 5 pg 91
Inhibitors of Wnt signaling

Three natural compounds that scored as potent inhibitors of the TCF/β-catenin interaction in secondary assays -

blocking Wnt induced axis duplication in xenopus embryos.

M Lepourcelet et al 2004 Cancer Cell vol 5 pg 91
Blocking TCF activity for CRC therapy

What might go wrong?

- An undesirable side-effect would be blocking β-catenin’s interaction with E-cadherin in normal cells.

- Disruption of β-catenin’s interaction with APC would not be desirable in normal cells.
Colorectal Cancer Therapy

- Antimetabolite 5-Fluorouracil (5-FU)

5-FU has been widely used in cancer treatment since the 1960s; this drug has had an impact on CRC survival.

- Novel therapies:
  - NSAIDs & COX2 inhibitors
  - EGFR kinase inhibitors
  - VEGF blocking antibody
Nonsteroidal anti-inflammatory drugs (NSAIDs)

Cyclooxygenase (Cox) inhibitors

- In 1988 it was reported that persons who regularly used aspirin had a 40% lower risk of incidence of CRC compared to those who did not use aspirin. (Kune et al 1988 Cancer Res 48: 4399)

- Clinical studies indicated that the chemoprotective effects of aspirin & other NSAIDs are particularly evident in familial forms of CRC - FAP patients. (Giardiello et al 1993 NEJM 328:1313)

- NSAIDs have multiple effects, but suppression of COX enzymatic activity is clearly an important one.
Cox 1 & 2 are involved in prostaglandin production

- Cox enzymes catalyze key steps in the production of short-lived, bioactive lipid molecules, the prostaglandins.

**Tracking the Cyclooxygenase Pathway**

- Cell membrane phospholipids
- Phospholipase A₂
- Arachidonic acid
- Cyclooxygenase 1 and 2
- Prostaglandin H₂
- Thromboxane A₂
- Prostaglandin D₂
- Prostaglandin E₂
- Prostaglandin F₂
- Prostacyclin
Cox-2 & Colorectal Cancer

Cyclooxygenase (COX) Enzyme Isoforms

- COX-1, the “homeostatic” isoform, is constitutively expressed in many tissues and produces the prostaglandins involved in gastric cytoprotection and vascular homeostasis
- COX-2 is an inducible isoform expressed in inflamed tissue, but also constitutively expressed in brain and kidney
- Cytokines, endotoxin, hormones, growth factors, and mitogens are all capable of inducing COX-2

Cox-2 levels are elevated in many cancers including CRC.
**Prostaglandins act via autocrine & paracrine mechanisms**

- Prostaglandins are overproduced in CRC & stimulate a variety of processes - proliferation, cell motility & angiogenesis.

PG receptors are G protein coupled (Gs).

EP2 is the main receptor in CRC.

EP2 activation leads to an increase in β-catenin levels & TCF activity.

Bind intracellular receptors

- PPAR

Effects on transcription
Prostaglandin E2 stimulates Wnt pathway

Prostaglandins have multiple effects - including a direct activation of Wnt signaling - mechanism still unclear - important point is that β-catenin/TCF activity is elevated.

Prostaglandin E₂ Promotes Colon Cancer Cell Growth Through a Gₛ-Axin-β-Catenin Signaling Axis

Maria Domenica Castellone,¹ Hidemi Teramoto,² Bart O. Williams,³ Kirk M. Drusy,⁴ J. Silvio Gutkind ⁵*

Science 2005 vol 310: 1504-1510.

Prostaglandin E₂ Stimulates the β-Catenin/T Cell Factor-dependent Transcription in Colon Cancer *


Strong interest in developing Cox-2 inhibitors
**Cox inhibitors**

Many, but not all, NSAIDs are COX inhibitors

<table>
<thead>
<tr>
<th>Aspirin (Acetylsalicylic acid)</th>
<th>Salicylic acid</th>
<th>Phenylbutazone</th>
<th>Oxyphenbutazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meclofenamic acid</td>
<td>Flufenamic acid</td>
<td>Mefenamic acid</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Flurbiprofen</td>
<td>Ketoprofen</td>
<td>Naproxen</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Diclofenac</td>
<td>Piroxicam</td>
<td></td>
</tr>
</tbody>
</table>

Blocks Cox 1 & 2

unacceptable toxicity - intestinal bleeding & kidney damage

thought to be due to Cox1 blockade

RJ Flower March 2003
Specific Cox-2 inhibitors have been developed

Block Cox 2
APC mutant mice and COX-2 inhibitors

- APC mutant mice have been used for testing effects of COX2 specific inhibitors - polyp growth & for tumor prevention.

Chemoprevention of Intestinal Polyposis in the ApcΔ716 Mouse by Rofecoxib, a Specific Cyclooxygenase-2 Inhibitor

Masanobu Oshima, Naomi Murai(Hata), Stacia Kargman, Meztli Arguello, Pauline Luk, Elizabeth Kwong, Makoto M. Taketo, and Jilly F. Evans

Number of polyps in intestines of APC mutant mice

Dose dependent decrease with Vioxx

Not a COX-1 or COX-2 inhibitor
Clinical Trial with a COX-2 inhibitor - FAP patients

- 83 FAP patients were treated twice daily (BID) for 6 months with 100 or 400 mg celecoxib.

Phillips et al 2002 Gut 50: 857

- There was a significant reduction in polyp appearance in the FAP patients treated with the high dose Cox-2 inhibitor.
COX-2 inhibitors & pain control


- Cox-2 inhibitors have become the established standard treatment for relieving pain from conditions such as rheumatoid arthritis.

- Older treatments including aspirin and other NSAIDs also block COX-2 but also target COX-1, which is the likely reason for increased gastric bleeding.

- Unfortunately, the large APPROVe trial, designed mainly to see whether Vioxx would prevent the formation of polyps (benign lesions) revealed that patients receiving Vioxx had twice as many cardiovascular events.

- Starting in October 2004, Vioxx and other Cox-2 inhibitors were withdrawn from the market.

**Conclude** - there is not enough knowledge about how the inhibitors function at a molecular level - for pain control & for cancer prevention.
**APPROVe Clinical Trial** - tested rofecoxib (Vioxx) a COX-2 inhibitor for polyp prevention

- Large trial* to evaluate the hypothesis that 3 yrs of treatment with Vioxx would reduce the risk of recurrent polyps among patients with a history of CRC adenomas. (*n = 2586 patients) (Bresalier et al 2005 N Engl J Med 352: 1092)

<table>
<thead>
<tr>
<th>Table 2. Incidence of Adjudicated Thrombotic Adverse Events.*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adverse Event</strong></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Cardiac events</td>
</tr>
<tr>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Fatal myocardial infarction</td>
</tr>
<tr>
<td>Sudden death from cardiac causes</td>
</tr>
<tr>
<td>Unstable angina pectoris</td>
</tr>
<tr>
<td>Cerebrovascular events</td>
</tr>
<tr>
<td>Fatal ischemic stroke</td>
</tr>
<tr>
<td>Ischemic stroke</td>
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<tr>
<td>Transient ischemic attack</td>
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<tr>
<td>Peripheral vascular events</td>
</tr>
<tr>
<td>Peripheral arterial thrombosis</td>
</tr>
<tr>
<td>Peripheral venous thrombosis</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
</tr>
</tbody>
</table>

- The trial was stopped due to adverse clinical events; the most common were hypertension (25 vs 7 in placebo), increased blood pressure (6 vs. 1 in placebo) & peripheral edema (7 vs 1 in placebo).
Chemopreventive action of NSAIDs - other targets

Sulindac targets nuclear β-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines.

- FAP patients were examined for nuclear β-catenin after 6 mos of Sulindac treatment - decreased staining.

- In CRC cell lines lacking COX2 activity, sulindac treatment led to a decrease in β-catenin/TCF reporter gene activity.

- Sulindac blocks activity of phosphodiesterase D5, a major isoform of cGMP phosphodiesterases. B. Zhu et al., 2005 J Cell Biochem, 94:336
Sporadic adenomatous polyp regression with exisulind is effective but toxic: a randomised, double blind, placebo controlled, dose-response study

N Arber, S Kuwada, M Leshno, R Sjodahl, R Hultcrantz, D Rex, for the Exisulind Study Group

Gut 55: 367-373

Unfortunately although exisulind does cause significant regression of sporadic colonic polyps in FAP patients, it also showed strong side-effects including abdominal pain & liver-related problems.

These side effects would prevent long-term use of exisulind as a chemopreventive agent.

Exisulind = sulindac sulfane
**Colorectal Cancer Therapy**

- **Antimetabolite 5-Fluorouracil (5-FU)**

  5-FU has been widely used in cancer treatment since the 1960s; this drug has had an impact on CRC survival.

- **Novel therapies:**
  - NSAIDs & COX2 inhibitors
  - **EGFR kinase inhibitors**
  - VEGF blocking antibody
EGFR as a therapeutic target in CRC

- Coexpression of the epidermal growth factor receptor (EGFR) & some of its ligands (TGFα) has been reported in CRC.
  (Salomon et al 1995 Crit Rev Oncol Hematol 19: 183)

- This would lead to “autocrine” receptor activation.

- EGFR has an important role in intestinal tract development.
  (Meittinen et al 1995 Nature 376: 337)
The Min model has been used to show that EGFR activity is important in polyp development

- *Egfr*^wa2^ mice have a mutant EGFR with impaired kinase activity.

In the *Egfr*^wa2^ background the number of polyps is dramatically lower.

Roberts et al. 2002 PNAS 99:1521

Waved 2 mice have a mutation in the kinase domain of the EGFR. *Egfr*^wa2^
Blocking EGFR activity lowers tumor growth

- Xenograft models of human cancer cells growing in immune-compromised nude mice.

HCA-7 human CRC cells

HCT-116 human CRC cells

Growth curves of tumor cells in nude mice treated with EKI-785, an EGFR kinase inhibitor. (Doses are in mg/kg body weight)

An EGFR targeted antibody was approved for treatment of CRC in 2004.
Drugs used for CRC Therapy

- In the past decade the median time of survival among patients with metastatic CRC has increased from 12 months to ~ 18-21 months mainly owing to the introduction of irinotecan and oxaliplatin.

- These inhibitors are used in combination with fluorouracil (5-FU), which was shown in the 1960s to prolong survival.

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**Irinotecan** - topoisomerase inhibitor
Binds the topo2-DNA complex & prevents replication of ss breaks.

**Oxaliplatin** - a platinum based cpd that intercalates in DNA.

**5-FU** - anti-metabolite that blocks essential biosynthetic processes &/or is incorporated into macromolecules including DNA & RNA.

**Leucovorin** - an active form of B complex vitamin folate; given in combination with 5-FU to protect healthy tissue from decreased folic acid levels.
Clinical trial to test an EGFR targeted antibody for CRC therapy

Cetuximab in combo with irinotecan increased the median survival time from 6.9 mos to 8.6 mos,

Targeting the EGFR does not cure metastatic CRC.

Approved by the FDA in Feb 2004 for therapy of metastatic CRC.
Herceptin®/Trastuzumab & Breast Cancer

- Herceptin® does not cure metastatic breast cancer - at this stage tumor cells have spread to distant organs.

\[
\begin{align*}
\text{Median time to disease progression} & \quad 7.4 \text{ mos.} \\
\text{Chemotherapy plus trastuzumab} & \quad 4.6 \text{ mos.} \\
\text{Chemotherapy alone} & \\
\end{align*}
\]

\[
\begin{array}{cccccc}
\text{NO. AT RISK} & \text{Chemotherapy plus trastuzumab} & \text{Chemotherapy alone} \\
235 & 234 \\
152 & 103 \\
63 & 25 \\
15 & 6 \\
\end{array}
\]

15 patients-CR
6 patients-CR

Slamon et al NEJM 2001 vol 344

June 1st lecture
Addition of trastuzumab reduced mortality rate by one third.

These results suggest that trastuzumab acts as a chemosensitizer when given together with chemotherapy directly after surgery.
Major goal of lectures

Present studies from the area of breast & colon cancer to show how molecular analyses can be used to achieve the ultimate goal of providing better therapies for patients.

- Described some of the biology & important molecules involved in the development of each cancer.

- Where possible, I tried to give you a mechanism which might explain how specific alterations contribute to cancer development.

- Described approaches to use our knowledge of these specific molecular alterations for developing novel targeted therapies.