Interaction of Radiation and Cytotoxic Drugs in Treatment of Colorectal Cancer

Alesia Ivashkevich

Radiation Oncology
Canberra Hospital
Australia

DoReMi Lecture Series
“Molecular Radiation Carcinogenesis”
22/04/2015
Colorectal cancer

• Third most common type of cancer making up about 10%

• 75-95% occurs in people with little or no genetic risk

Risk factors

Lifestyle, diet

Older age

Genetic disorders (small fraction affected)

Chronic inflammation contributes to approximately 20% of all CRC cases
• About 20% cases of CRC have a family history

**Syndromes (<5%)**

• Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome accounts for about 3% of people with CRC

• Gardner syndrome and familial adenomous polyposis (FAP) are about 1%
Most CRC start with the polyps in epithelial lining of the colon or rectum. Progression from benign colorectal adenoma to malignant carcinoma arises from accumulation of several events such as:

- chromosomal abnormalities
- genetic mutations
- epigenetic changes

Benign (hyperplastic polyp) → Pre-malignant (tubular adenoma) → Malignant (colorectal adenocarcinoma)
Molecular Pathogenesis

• Chromosomal instability pathway (CIN) is the most common phenotype accounting for 85% of all sporadic CRCs. Malignant cells are aneuploid and reveal large scale chromosomal rearrangements.

• Microsatellite instability (MSI) phenotype represents 15% of all CRCs and is caused by various deficiencies in the DNA mismatch-repair system, leading to a large increase in the mutation rate.

• Cancers with CpG island methylator phenotype (CIMP) exhibit aberrant DNA methylation leading to concordant promoter hypermethylation of multiple genes.
Molecular Pathogenesis

- Sequencing of colon cancer genomes identified mutations in the coding sequences of approximately 67 genes in an average colon cancer genome, of which a subset of 12 genes were proposed to be the genes most likely to be involved with cancer formation.

- Not clear which of the mutations are pathogenic (driver mutations) and which are the consequences (passenger mutations).

- About 15 alterations are thought to be driver mutations that are functionally important and positively selected for during CRC carcinogenesis.

- Driver mutations affect a wide range of cellular functions from proliferation, migration, differentiation, adhesion, cell death, DNA stability and repair.
Mutations in the Wnt signaling pathway occur in epithelial cells

- APC gene is most frequently mutated in colorectal cancer – APC protein prevents the accumulation of β-catenin

- Without APC, β-catenin accumulates to high levels and translocates into the nucleus, binds to DNA and activates transcription of proto-oncogenes
Molecular Pathogenesis

- DNA MMR deficiency has been linked to hereditary colon cancer and to an increasing number of sporadic cancers.

- MMR factors (MSH2, MLH1, PMS2) were shown to be involved in:
  
  - repair of DNA damage (alkylation, etc)
  
  - mediation of X-ray induced apoptosis in a p53-independent fashion

Apart of APC and MMR genes, most commonly altered genes in CRC:

- TP53
- KRAS
- PI3CA
- BRAF
- PTEN

Simultaneous mutations in all three genes (APC, K-ras and p53) are uncommon in the same tumor.
Colorectal cancer treatment

- Combination of surgery with radiation therapy, chemotherapy and targeted therapy

- Chemotherapy treatment of CRC includes oxalaplatin, 5-fluouracil (5-FU), irinotecan, capecitabine

- 5 year survival rate in the US is around 65%
Fluoropyrimidines

• Are antimetabolites

• 5-Fluorouracil after conversion into its active metabolites (FdIMP, FdUTP, FUTP) induces RNA and DNA damage

• Inhibition of thymidylate synthase (TS) and DNA synthesis

5-FU  
5-fluorouracil

FdUrd  
5-fluoro-2′-deoxyuridine
Thymidylate Synthase is one of the key enzymes controlling DNA replication and is a target enzyme for numerous anticancer drugs.

- However, only 10-15% of advanced CRC tumors treated with 5-FU/leucovorin respond.
Numerous colon cancer cell lines were established from tumour tissues.

Used for studying mechanisms, modelling and predicting treatment response.
<table>
<thead>
<tr>
<th>Cell line</th>
<th>Patient</th>
<th>Organ</th>
<th>Disease</th>
<th>Stage</th>
<th>Derived from</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco-2</td>
<td>77-Year-old female</td>
<td>Colon</td>
<td>Colorectal carcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Caro et al.,\textsuperscript{41}</td>
</tr>
<tr>
<td>CO-115</td>
<td>55-Year-old female</td>
<td>Colon ascendens</td>
<td>Colorectal adenocarcinoma</td>
<td>Primary tumor</td>
<td>primary tumor</td>
<td>Carrel et al.,\textsuperscript{57}</td>
</tr>
<tr>
<td>COLO 320</td>
<td>Male</td>
<td>Colon sigmoid</td>
<td>Colorectal adenocarcinoma</td>
<td>Primary tumor</td>
<td>misclassified</td>
<td>Quinn et al.,\textsuperscript{58}</td>
</tr>
<tr>
<td>DLD-1</td>
<td></td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>HCT-15/DLD-1</td>
<td>misclassified</td>
<td>Chen et al.,\textsuperscript{59} and Dexter et al.,\textsuperscript{60,61}</td>
</tr>
<tr>
<td>EB</td>
<td></td>
<td>Colon</td>
<td>Colonic carcinoma</td>
<td>Primary tumor</td>
<td></td>
<td>Brattain et al.,\textsuperscript{61} and Chantret et al.,\textsuperscript{61,62}</td>
</tr>
<tr>
<td>FRI</td>
<td></td>
<td>Colon</td>
<td>Colonic carcinoma</td>
<td>Primary tumor</td>
<td></td>
<td>Chen et al.,\textsuperscript{59} and Tibbetts et al.,\textsuperscript{63} and Dexter et al.,\textsuperscript{64}</td>
</tr>
<tr>
<td>HCT-15</td>
<td>Male</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>HCT-15/DLD-1</td>
<td>misclassified</td>
<td>Caro et al.,\textsuperscript{41}</td>
</tr>
<tr>
<td>HCT-116</td>
<td>48-Year-old male</td>
<td>Colon ascendens</td>
<td>Colorectal carcinoma</td>
<td>Dukes’ D</td>
<td>Primary tumor</td>
<td>Caro et al.,\textsuperscript{41}</td>
</tr>
<tr>
<td>HT-29</td>
<td>44-Year-old female</td>
<td>Colon ascendens</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Caro et al.,\textsuperscript{41}</td>
</tr>
<tr>
<td>IS1</td>
<td></td>
<td>Colon</td>
<td>Colonic carcinoma</td>
<td>Dukes’ C</td>
<td>Peritoneal metastasis</td>
<td>Fogh\textsuperscript{65}</td>
</tr>
<tr>
<td>IS3</td>
<td></td>
<td>Colon</td>
<td>Colonic carcinoma</td>
<td>Dukes’ C</td>
<td>Peritoneal metastasis</td>
<td>Cajot et al.,\textsuperscript{68}</td>
</tr>
<tr>
<td>LoVo</td>
<td>56-Year-old male</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Left supraclavicular region</td>
<td>DREWINKO et al.,\textsuperscript{69}</td>
</tr>
<tr>
<td>LS1034</td>
<td>54-Year-old male</td>
<td>Cecum</td>
<td>Cecal carcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Suardet et al.,\textsuperscript{70}</td>
</tr>
<tr>
<td>LS-174T</td>
<td>58-Year-old female</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Subcultured LS 180</td>
<td>Tom et al.,\textsuperscript{71}</td>
</tr>
<tr>
<td>NCIH509</td>
<td>55-Year-old male</td>
<td>Cecum</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Abdominal wall metastasis</td>
<td>Park et al.,\textsuperscript{72}</td>
</tr>
<tr>
<td>RKO</td>
<td>73-Year-old male</td>
<td>Colon</td>
<td>Colonic carcinoma</td>
<td>Dukes’ A</td>
<td>Primary tumor</td>
<td>Brattain et al.,\textsuperscript{60,61}</td>
</tr>
<tr>
<td>SW1116</td>
<td>83-Year-old female</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Leibovitz et al.,\textsuperscript{73}</td>
</tr>
<tr>
<td>SW48</td>
<td>50-Year-old male</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Lymph node metastasis</td>
<td>Leibovitz et al.,\textsuperscript{73}</td>
</tr>
<tr>
<td>SW480</td>
<td>51-Year-old male</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Lymph node metastasis</td>
<td>Leibovitz et al.,\textsuperscript{73}</td>
</tr>
<tr>
<td>SW948</td>
<td>81-Year-old female</td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Leibovitz et al.,\textsuperscript{73}</td>
</tr>
<tr>
<td>TC71</td>
<td></td>
<td>Colon sigmoid</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ C</td>
<td>Primary tumor</td>
<td>Bras-Goncalves et al.,\textsuperscript{74}</td>
</tr>
<tr>
<td>V9P</td>
<td>67-Year-old male</td>
<td>Colon rectum</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ D</td>
<td>Primary tumor HT-29,</td>
<td>McBain et al.,\textsuperscript{74}</td>
</tr>
<tr>
<td>WiDr</td>
<td></td>
<td>Colon</td>
<td>Colorectal adenocarcinoma</td>
<td>Dukes’ D</td>
<td>misclassified</td>
<td>Fogh\textsuperscript{67}</td>
</tr>
</tbody>
</table>

Abbreviation: HNPCC, hereditary non-polyposis colorectal cancer. All data on cell line origins was retrieved from the original papers describing the cell lines.
Table 2. Colon cancer cell lines classified by the molecular pathways CIN, MSI and CIMP, and mutation status of cancer critical genes

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MSI status</th>
<th>CIMP panel 1</th>
<th>CIMP panel 2</th>
<th>CIN</th>
<th>KRAS</th>
<th>BRAF</th>
<th>PIK3CA</th>
<th>PTEN</th>
<th>TP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO-115</td>
<td>MSI</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>wt</td>
<td>V600E</td>
<td>wt</td>
<td>E157fs;R233X</td>
<td>wt</td>
</tr>
<tr>
<td>DLD-1</td>
<td>MSI</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>G13D</td>
<td>wt</td>
<td>E545K;D549N</td>
<td>wt</td>
<td>S241F</td>
</tr>
<tr>
<td>HCT-116</td>
<td>MSI</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>G13D</td>
<td>wt</td>
<td>H1047R</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>HCT-15</td>
<td>MSI</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>G13D</td>
<td>wt</td>
<td>E545K;D549N</td>
<td>wt</td>
<td>S241F</td>
</tr>
<tr>
<td>LoVo</td>
<td>MSI</td>
<td>–</td>
<td>–</td>
<td>G13D;A14V</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>LS-174T</td>
<td>MSI</td>
<td>–</td>
<td>–</td>
<td>G12D</td>
<td>wt</td>
<td>H1047R</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>RKO</td>
<td>MSI</td>
<td>+</td>
<td>–</td>
<td>wt</td>
<td>G12D</td>
<td>wt</td>
<td>V600E</td>
<td>H1047R</td>
<td>wt</td>
</tr>
<tr>
<td>SW48</td>
<td>MSI</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>TC71</td>
<td>MSI</td>
<td>+</td>
<td>–</td>
<td>G12D</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>R233X</td>
<td>C176Y;R213X</td>
</tr>
<tr>
<td>Caco-2</td>
<td>MSS</td>
<td>+</td>
<td>–</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>E204X</td>
</tr>
<tr>
<td>COLO 320</td>
<td>MSS</td>
<td>–</td>
<td>+</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>R248W</td>
</tr>
<tr>
<td>EB</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>FRI</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>HT-29</td>
<td>MSS</td>
<td>+</td>
<td>+</td>
<td>wt</td>
<td>G12D</td>
<td>wt</td>
<td>V600E</td>
<td>P449T&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R273H</td>
</tr>
<tr>
<td>IS1</td>
<td>MSS</td>
<td>–</td>
<td>+</td>
<td>G12D</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>R273H</td>
<td>Y163H</td>
</tr>
<tr>
<td>IS3</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>G12D</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>Y163H</td>
</tr>
<tr>
<td>LS1034</td>
<td>MSS</td>
<td>–</td>
<td>+</td>
<td>A146T&lt;sup&gt;b&lt;/sup&gt;</td>
<td>wt</td>
<td>wt</td>
<td>G245S</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>NCI-H508</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>wt</td>
<td>wt</td>
<td>G596R</td>
<td>E545K</td>
<td>R273H</td>
</tr>
<tr>
<td>SW1116</td>
<td>MSS</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>G12A</td>
<td>wt</td>
<td>wt</td>
<td>A159D</td>
<td>wt</td>
</tr>
<tr>
<td>SW480</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>G12V</td>
<td>wt</td>
<td>wt</td>
<td>R273H;P309S</td>
<td>wt</td>
</tr>
<tr>
<td>SW620</td>
<td>MSS</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>G12V</td>
<td>wt</td>
<td>wt</td>
<td>R273H;P309S</td>
<td>wt</td>
</tr>
<tr>
<td>SW948</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Q61L</td>
<td>wt</td>
<td>E542K</td>
<td>wt</td>
<td>G117fs</td>
</tr>
<tr>
<td>V9P</td>
<td>MSS</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>G245D</td>
</tr>
<tr>
<td>WiDr</td>
<td>MSS</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>wt</td>
<td>V600E</td>
<td>P449T&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R273H</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CIN, chromosomal instability pathway; MSI, microsatellite instability; MSS, microsatellite stable; CIMP, CpG island methylator phenotype; X, stop codon; fs, frame shift; wt, wild type. Mutations are annotated at the protein level as described by den Dunnen et al. (standard one-letter amino acid abbreviations. X and fs). For further details, see Supplementary Table 1. <sup>a</sup>No publication on WiDr karyotype was found; however, WiDr and HT-29 are identical cell lines. <sup>b</sup>Previously reported mutations not covered by our assays.
Rationale for adding chemotherapy to radiation

Spatial cooperation
- Radiation: local control (in-field)
- Chemotherapy: distal control (out-of-field)
- No interaction—modalities work independently

In-field cooperation
- Molecular level
- Cellular level
- Tissue level

Locoregional control

Independent toxicities
- Radiation toxicities
- Chemotherapy toxicities

Supra-additivity (‘synergism’)

Additivity

Infra-additivity (antagonism)\(^a\)

‘Radiosensitization’

Synergistic toxicities

www.nature.com/clinical practice/onc
Isobologram depicting the combination of radiation and a systemic agent.
Dose-response curves for tumor and normal tissue damage with radiation/chemoradiation

- **Normal tissue**
- **Tumor**

**Therapeutic range (IR)**

**Chemoradiotherapy**

**Therapeutic range (IR + Chemo)**

Response/toxicity rate (%) vs. Radiation dose (Gy)
• Fluoropyrimidines act as radiosensitizers

• Fluoropyrimidines increase effectiveness of radiation chiefly when given before and during radiation
Cytotoxicity in colon cancer cell lines: radiation alone and chemoradiation

RT – radiation therapy
CRT – chemoradiation (5-FU 10 µM + radiation)

Mechanisms of radiosensitization

• Killing of S phase cells

• 5-FU pre-incubation is hypothesized to radiosensitize tumor cells through changes in the S-phase cell-cycle checkpoint – inappropriate progression out of the S phase into the G2 phase

• Modification of thymidylate synthase levels
Strongest correlation established between 5-fluorouracil response and replication error status (RER, mismatch repair deficiency)

Bracht, et al, 5-Fluorouracil response in a large panel of colorectal cancer cell lines is associated with mismatch repair deficiency, BJC, 2010
Genotype effects on treatment \textit{(in vitro)}

- Effect of MLH1 mutation and mismatch repair defect on cytotoxicity and radiosensitization of HCT-116 human colon cancer cells by Gemcitabine (5-FU prodrug)

HCT116 cells containing chromosome 3 (mismatch repair competent)
HCT116 cells containing chromosome 2 (mismatch repair defective)

Lawrence et al, The mechanism of action of radiosensitization of conventional chemotherapeutic agents, Seminars in Radiation Oncology, 2003
Colorectal cancer treatment

- Adjuvant treatment of colon cancer is more focused on preventing distant metastases
- Stage II/III Rectal cancer relatively high risk of locoregional recurrence
- Mainstay of rectal cancer treatment is surgery
- Radiation therapy performed preoperatively or postoperatively
  - Short-course or long-course
  - With or without chemotherapy
  - 45 or 50 Gy in 25-28 fractions
Preoperative chemoradiotherapy (PCRT) *versus* postoperative chemoradiotherapy (CRT)

PCRT yielded significantly lower local failure rates and toxicity rates than those receiving CRT in rectal cancer.
• Tumoral thymidylate synthase activity was found to be important for 5-FU responsiveness, and p53 mutations as biological predictors of survival

• Patients with microsatellite unstable neoplasms have a better overall survival rate and a modified response to conventional chemotherapy

○ Colon cancer patients with a predicted functional p53 had a better prognosis than patients with non functional p53


Etienne, et al, Prognostic Value of Tumoral Thymidylate Synthase and p53 in Metastatic Colorectal Cancer Patients Receiving Fluorouracil-Based Chemotherapy: Phenotypic and Genotypic analyses
Conclusions

• Colorectal cancer treatment requires personalized approach

• Combinations of radiotherapy and chemotherapy have to be rationally designed and optimized
Hydroxyurea – inhibitor of ribonucleotide reductase (ask Desmond)
Germcitabine
Cisplatin

Ask Daohai for ER stressing compound
Autophagy markers, ER stress antibodies/apoptosis markers
Immunogenicity of cancer
Calnexin calreticulin endogenous ‘danger signals’