BRAF mutant colorectal cancer: A different entity

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ESMO 16th World Congress on Gastrointestinal Cancer
Colorectal cancer (CRC) is a heterogeneous disease but patients are not yet selected for individualized treatments

- Colorectal cancer is the second leading cause of cancer death
- Although several treatments exist, we do not have a good way to select patients for individualized treatments
- Only RAS status has been established as a predictor of anti-EGFR treatment activity
- New technologies that allow genetic definition of different types of colon cancer based on the expression, methylation, mutation rate of the genes might help in better understanding CRC biology
- The better understanding of CRC biology might drive personalized treatments
Colon carcinogenesis is a multi step process

The chromosomal instability (CIN) pathway

Adapted from Ahnen DJ: The American College of Gastroenterology Emily Couric Lecture—The Adenoma–Carcinoma Sequence Revisited: Has the Era of Genetic Tailoring Finally Arrived? *The American Journal of Gastroenterology* 106, 190-198 (February 2011)
Colon carcinogenesis is a multi step process

**The chromosomal instability (CIN) pathway**

**The microsatellite instability (MIN) pathway**

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Colon carcinogenesis is a multi step process

The chromosomal instability (CIN) pathway

The microsatellite instability (MIN) pathway

The epigenetic (CIN) pathway

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CRC is a heterogeneous disease at …

...gene expression level

Adapted from Tejpar et al, PETACC 3 trial, ASCO 2010

...methylation level

Adapted from Hinoue T et al: Genome-scale analysis of aberrant DNA methylation in colorectal cancer. Genome Res. 2011 Jun 9
CRC is a heterogeneous disease at mutational rate level

The biology of CRC tells us that CRC is a heterogeneous disease at different levels but we still treat our patients by only considering the TNM classification.
The BRAF - signature identifies:

1) Tumors that carry the BRAF V600E gene mutation with 96% sensitivity and 86% specificity

2) 30% of KRAS mutant CC
13% of double wild type CC
which carry the same gene expression profile as BRAF V600E
BRAF V600E and BRAF like CC tumors have poor outcome as compared to non BRAF like tumours

Adapted from Popovici et al, JCO March 2012

Hypermutation (MSI)
Hypermethylation (CIMP)
BRAF status (BRAF –like)
Serrated pathway
Activation of MAPK pathway

Poor SAR
Non responsive to chemotherapy
Identification of synthetic lethal interactions with the BRAF oncogene in CC

- to provide additional drug targets for therapeutic exploration

- to shed new light on BRAF mechanisms of action
Which are the genes synthetically lethal with BRAF V600E in CC?
BRAF signature up-regulated genes are most likely to be candidates for synthetic lethality with BRAFV600E in CC.

\[ N=399 \text{ patients} \]

Set up experiment

**In vitro models**

**BRAF V600E and WT2 CC cell lines**

Upregulated genes in BRAF V600E CC as compared with WT2

PETACC3 data set

Agendia data set

163

+best 100

+best 100

Infect with the BRAF library

Lim1215, Vaco432, Widr

pool n.1, 2, 3, 4
MOI= 0.5
Coverage 400X
Criteria for selecting hits:

Depletion of BRAF mut cells over time must be at least 50% as compared to Time 0

\[ \log_2 \left( \frac{\text{Mut T13/T0}}{T0} \right) \leq -1 \]

Depletion of BRAF mut cells must be at least 1.5 fold higher than LIM1215

\[ \frac{\text{LIM1215 T13/T0 fold change}}{\text{BRAF Mut T13/T0 fold change}} \geq 1.5 \]

multiple hairpins
RANBP2 is selectively synthetic lethal with BRAF V600E in CC

WT2  Lim1215  
   MSI  

WiDr  MSS  

BRAF V600E  

VaCo432  MSI  

RANBP2 mRNA relative expression
RANBP2 is selectively synthetic lethal with BRAF V600E in CRC
RANBP2 is also synthetic lethal with BRAF likeness in CRC

**KRAS mut non BRAF like**

- HCT15
  - MSI
- Lim1863
  - MSI

**KRAS mut BRAF like**

- pLKO
  - #2
  - #4
- SW620
  - MSS
- SKCO-1
  - MSS
- LoVo
  - MSI
**RANBP2 (Nup358)**

Schematic representation of RANBP2 domain organization and binding partners

Nucleus 3:2, 162–171; March/April 2012; G 2012 Landes Bioscience

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**Specific armadillo repeat sequences facilitate β-catenin nuclear transport in live cells via direct binding to nucleoporins Nup62, Nup153, and RanBP2/Nup358.**

Sharma M, Jamieson C, Johnson M, Molloy MP, Henderson BR

Westmead Institute for Cancer Research, The University of Sydney, Westmead, New South Wales 2145, Australia. manisha.sharma@sydney.edu.au

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**Regulation of Wnt signaling by the nuclear pore complex.**

Shitashige M, Satow R, Honda K, Ono M, Hirohashi S, Yamada T.

Chemotherapy Division and Cancer Proteomics Project, National Cancer Center Research Institute, Tokyo, Japan.

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**Sumoylation of Mdm2 by Protein Inhibitor of Activated STAT (PIAS) and RanBP2 Enzymes**

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RANBP2 depletion induces abnormal chromosomal segregation
Conclusions

- The BRAF-like signature identifies BRAF V600E tumors with 96% sensitivity and 86% specificity and also a group of BRAF wild type tumors which share the same gene expression profile.

- The BRAF-like tumors share a common poor prognosis.

- The BRAF-like tumors belong to a specific CC subtype: hypermutated (MSI), hypermethylated, right sided location, serrated and MAPK pathway activated tumors.

- **RANBP2** is selectively synthetic lethal with BRAF V600E and BRAF likeness in CC.

- Ongoing research is focused on better defining the role of RANBP2 and its depletion in BRAF V600E tumors and on identifying treatments which could mimic RANBP2 KD.
Acknowledgments

NKI/AvL
Valentina Gambino
Andreas Schlicker
René Bernards
Lodewyk Wessels
Bernards’ group
Beijersbergen’s group

Agenda
Iris Simon
Sun Tian
Paul Roepman

SIB
Giovanni d’ ario
Mauro Delorenzi

Clinical Pharmacology
Jan Schellens

KUL
Sabine Tejpar

The work has been granted by ESMO (fellowship for translational research) and FP7 - Coltheres