CLINICAL PROTEOMIC TUMOR ANALYSIS CONSORTIUM

Proteomic analysis of colon cancer phenotypes

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Financial Interest Disclosure

CCD Week 2016
Robbert JC Slebos, PhD

I have the following financial relationships to disclose:

Co-Founder and Stockholder:
Protypia, Inc

I will not discuss off label use and/or investigational use in my presentation

CanMEDS Roles Covered

	Medical Expert (as *Medical Experts*, physicians integrate all of the CanMEDS Roles, applying medical knowledge, clinical skills, and professional values in their provision of high-quality and safe patient-centered care. *Medical Expert* is the central physician Role in the CanMEDS Framework and defines the physician's clinical scope of practice.)
	Communicator (as Communicators, physicians form relationships with patients and their families that facilitate the gathering and sharing of essential information for effective health care.)
	Collaborator (as *Collaborators*, physicians work effectively with other health care professionals to provide safe, high-quality, patient-centred care.)
	Leader (as *Leaders*, physicians engage with others to contribute to a vision of a high-quality health care system and take responsibility for the delivery of excellent patient care through their activities as clinicians, administrators, scholars, or teachers.)
	Health Advocate (as *Health Advocates*, physicians contribute their expertise and influence as they work with communities or patient populations to improve health. They work with those they serve to determine and understand needs, speak on behalf of others when required, and support the mobilization of resources to effect change.)
X	**Scholar** (as *Scholars*, physicians demonstrate a lifelong commitment to excellence in practice through continuous learning and by teaching others, evaluating evidence, and contributing to scholarship.)
	Professional (as *Professionals,* physicians are committed to the health and well-being of individual patients and society through ethical practice, high personal standards of behaviour, accountability to the profession and society, physician-led regulation, and maintenance of personal health.)

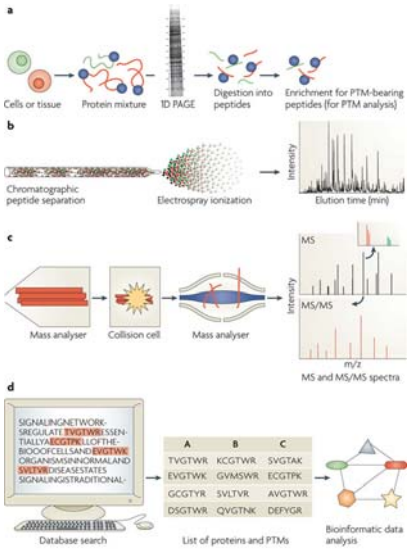
Outline

- **Proteomic techniques:**
  - Global 'shotgun' and Targeted Proteomics
- **Colon carcinoma cell line models:**
  - Proteomic analysis of a panel of 10 colorectal cell lines by MMR status

    Cancer Res (2014) 74:387-97
- **Proteogenomic analysis of TCGA colorectal carcinomas:**
  - Integrative analysis of 90 TCGA samples

    Nature (2014) 513:382-287

Proteomics by Mass Spectrometry



Protein isolation

(Biochemical separation)

Digestion (trypsin)

Biochemical separation

Ionization

Mass selection

Peptide fragmentation

Fragment detection

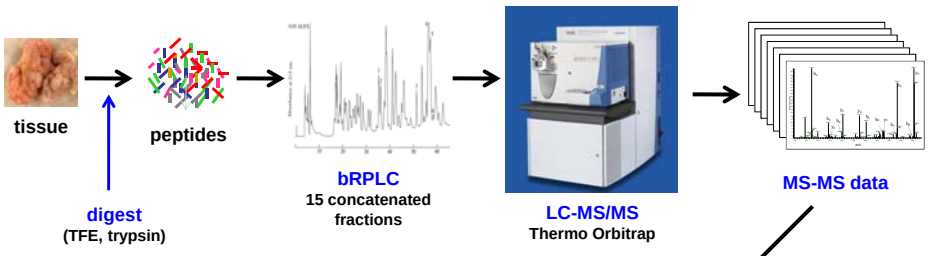
Spectrum identification

Protein assembly

Protein quantitation

Nat Rev Mol Cell Biol (2010) 11:427-39

VU Shotgun Proteomics Platform



Preprocessing
- MSConvert

Identification
- Myrimatch
- Pepitome
- MS-GF+

Peptide assembly
- IDPicker

Two-group comparison
- QuasiTel

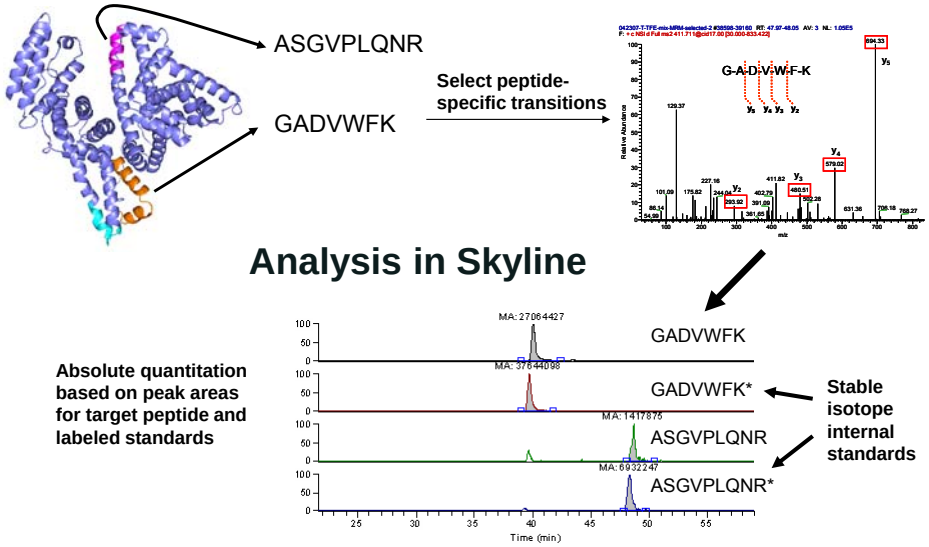
Protein	Ratio	Quasi.fdr	Counts	Spec1 Rep1	Spec1 Rep2	Spec1 Rep3	Spec2 Rep1	Spec2 Rep2	Spec2 Rep3
MX2	-37.59	0.0000	47	0	0	0	16	15	16
IGF2BP3	-36.52	0.0010	61	0	0	0	18	22	21
ACSS1	-36.40	0.0004	56	0	0	0	20	17	19
HSD17B4	-36.17	0.0012	48	0	0	0	17	14	17
AGPS	-35.50	0.0009	30	0	0	0	10	9	11
MMP1	-35.50	0.0009	30	0	0	0	10	11	9
IFIT2	-35.11	0.0037	23	0	0	0	9	7	7
COL6A3	-7.090	0.0040	549	2	1	1	186	206	153
MVP	-5.864	0.0047	237	1	2	1	81	87	65
OAS3	-5.554	0.0039	96	1	0	1	32	33	29
KRT6B	4.115	0.0042	110	36	36	32	1	2	3
MSH2	4.432	0.0018	113	38	37	33	2	1	2
POGZ	34.19	0.0040	33	12	12	9	0	0	0
WDR70	34.76	0.0047	18	7	6	5	0	0	0
RFC4	34.98	0.0031	21	6	8	7	0	0	0
UGT8	36.73	0.0002	26	9	8	9	0	0	0
LSP1	37.22	0.0004	99	31	36	32	0	0	0

Cluster Analysis
- Tumor classification
- Protein expression analyses (Webgestalt)

Associations
- Genomic features
- Molecular subtypes
- Clinical features

Co-expression networks
- Protein expression patterns (Netgestalt)

LC-MRM-MS targeted protein quantitation



Zhang *et al.* MCP 2011:M110.006593

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Nature (2014) 513:382-287

Analysis of 10 colorectal cell lines

- Are known DNA alterations detectable as proteomic changes?
- How do the proteomes compare based on MMR status?

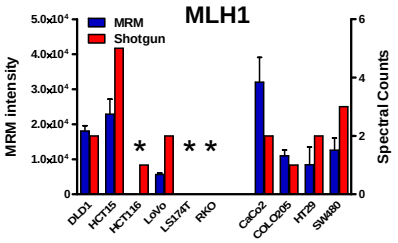
- 10 colorectal cancer cell lines: RKO, HCT116, HCT15, DLD1, LOVO, LS174T, CACO2, COLO205, HT29, SW480
- Selected based on DNA mismatch repair capacity
- Analyzed by shotgun proteomics

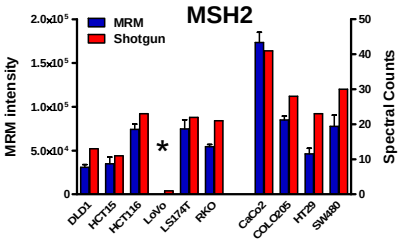
Proteomics:

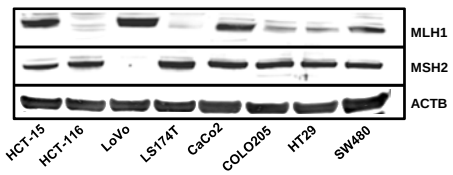
1) Shotgun proteomics by IEF peptide separation, LC-MS/MS on Thermo-LTQ
2) Targeted proteomics for selected proteins using MRM on LTQ-vantage

Shotgun protein inventory of 5,284 protein groups at a protein FDR of 4.6%

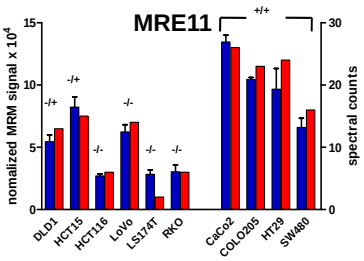
Proteomics of known genetic changes





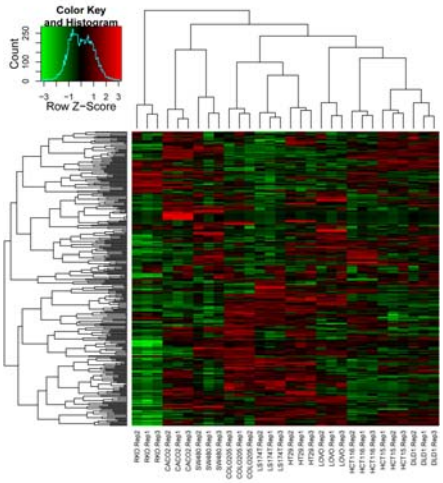


Proteomics confirms known genetic changes



Intron shift

Unsupervised protein expression analysis



Observations:

- **Biological replicates co-cluster**
- **HCT15 and DLD1 are related**
- **No separation by MMR status**
- **RKO has many unique features**

Enrichment analysis of RKO proteins

Transcription Factor 3 (E2A) Complex

CAGGTG binding site

A4GALT
AAK1
ABCA12
ABCA2
ABCB7
ABHD4
ABHD7
ABL2
ABLIM3
ABR
ABTB2
ACAA2
ACADSB
ACCN1
ACLY
ACOX3
ACSL4
ACTA1
ACVR1B
……etc

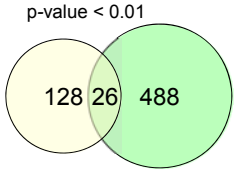
2571 genes in the human genome having **conserved** TCF3 promoter of which 514 are detectable as proteins by LC-MS/MS

compare

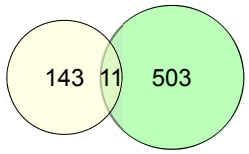
JUP
KRT8
ADAM10
ITGA6
OCLN
PKP3
CDH1
LLGL2
DLG1
DLG2
CTNND1
ITGB4
CLDN3
PKP3
…….etc.

154 proteins down-regulated in RKO

Enrichment ratio: 2.30
p-value < 0.01



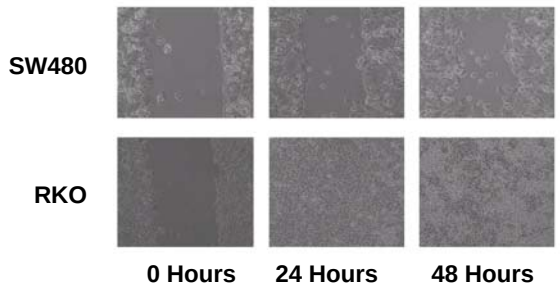
Observed



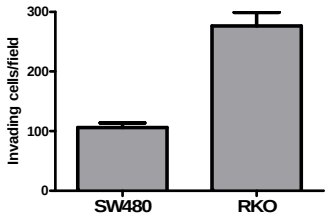
Expected

Conclusion:
Absence of tight-junction and cellular adhesion molecules due to reduced **TCF3 activity** is evidence for **Epithelial-to-Mesenchymal Transition (EMT)**

EMT phenotype of RKO cell line



Wound-healing assay



Invasion Assay

RKO prior results on EMT phenotype

- EMT phenotype not discovered by gene expression studies

  Cancer Res (2014) 74:3438

- Observed in study on sensitivity to EGFR-inhibition
  - RKO is resistant to EGFR-inhibition
  - Loss of epithelial markers E-cadherin and β-catenin
  - High ZEB1 expression by RT-PCR
  - No gain of mesenchymal markers vimentin or fibronectin
  - No biological tests for EMT

Mol Cancer Ther (2007) 6:532

Novel proteomic evidence for EMT in RKO by global protein analysis

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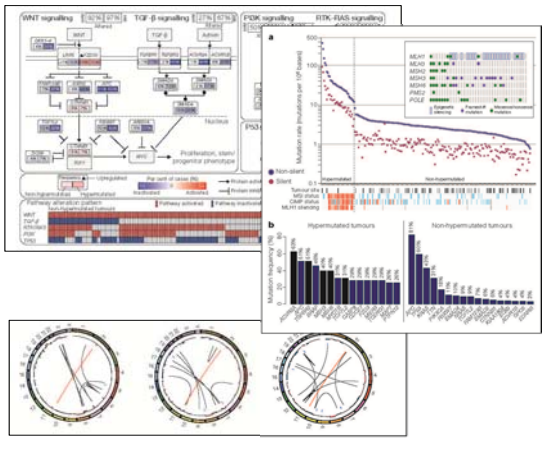
  Nature (2014) 513:382-287

TCGA colon genomic analyses

19 JULY 2012 | VOL 487 | NATURE | 333

Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network*

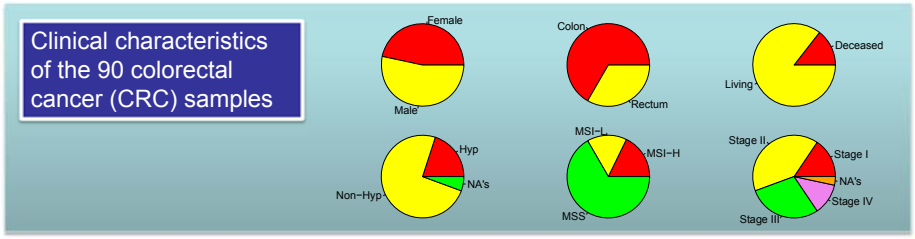


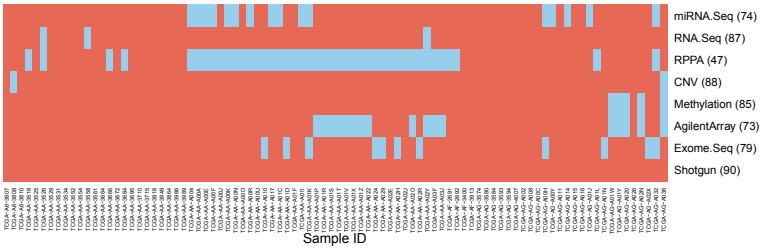
TCGA colorectal dataset
276 carcinomas
- Exon sequence
- DNA copy number
- Promoter methylation
- mRNA/miRNA expression

- 90 tumors available for proteomic analyses

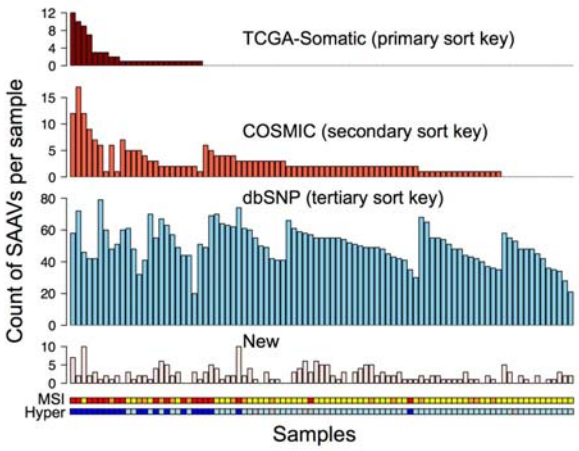
Tumor sample and data information

Clinical characteristics of the 90 colorectal cancer (CRC) samples





Analysis of Single Amino Acid Variants



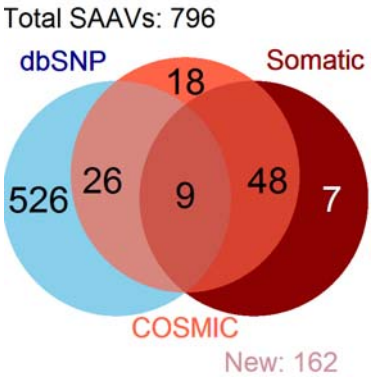
Reported by TCGA as somatic variant based on DNA

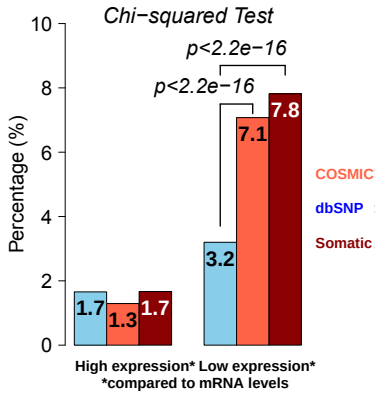
Present in COSMIC database of cancer-associated mutations

Present in dbSNP database of reported variants in the genome

'New' variants for this study

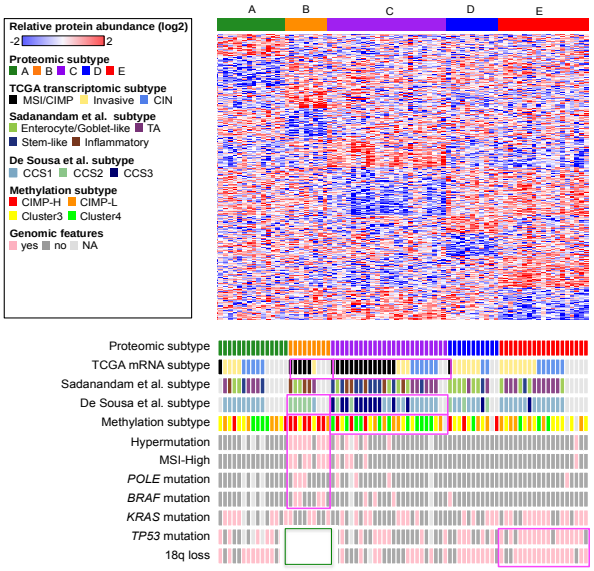
Prevalence of Single Amino Acid Variants



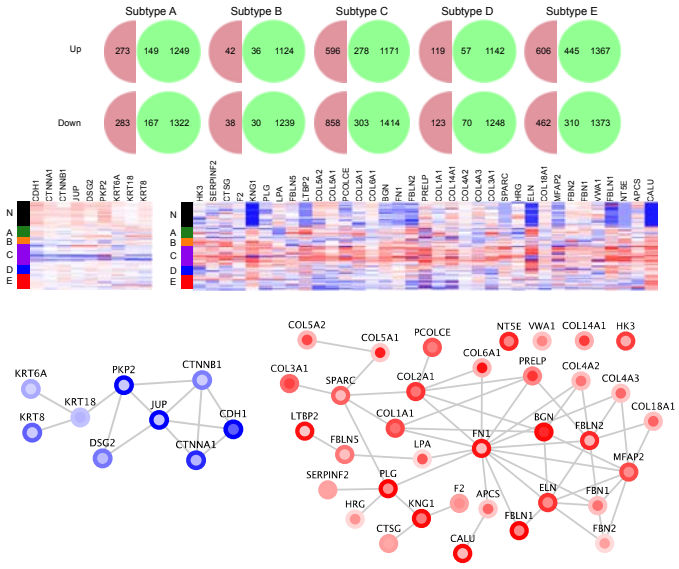


Majority of identified SAAVs represent SNPs, which are more likely to be expressed in the proteome than somatic SAAVs

Proteomic subtypes of colorectal carcinoma



Co-expression analysis of Subtype C



What does proteomics teach us about colon cancer that genetics does not?

- Proteomic profiling identifies a functionally relevant phenotype (EMT) that cannot be inferred from genetic features of RKO cells
- Proteomics identifies DNA variants that are translated to proteins and shows that many have reduced expression
- Proteomics identifies distinct colorectal cancer subtypes that are not detected by transcriptome profiles

Proteomics bridges the inference gap between genetic features and phenotypic characteristics in colon cancer

Acknowledgements

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