Molecular Classification of Gastric and Esophageal Cancers

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Outline of Presentation

1) Classification of Gastric and Esophageal CA
   - Geographical Differences
   - Molecular Profiles

2) Examining the Tumor Microenvironment
   - Driver roles for the Tumor Stroma

3) Modulation of Tumor Immunity
   - Evasion of host immune surveillance
Gastric and Esophageal Cancers: Leading Causes of Cancer Incidence and Death

Worldwide Cancer Incidence

An estimated 14.1 million adults in the world were diagnosed with cancer in 2012. These cases were not spread evenly across the globe and the reliability of cancer statistics available for each country varies.

Most Common Cancers Worldwide

Cancer Incidence by Region

Most Common Causes of Cancer Death

Deaths per year (thousands)

Total: 8.2 million

1. Lung
2. Liver
3. Stomach
4. Bowel (inc. anus)
5. Breast
6. Oesophagus
7. Pancreas
8. Prostate
9. Cervix
10. Leukaemia
11. Lip, oral cavity
12. Kaposi sarcoma
13. Other cancers

3rd
6th
Gastric Cancer Pathogenesis: Interplay Between Environmental and Host Factors

Yeoh and Tan (2015) Gastroenterology
Chia and Tan (2016) Annals of Oncology
Esophageal CAs are Divided into Adenocarcinomas (EACs) and Squamous Cell Carcinomas (ESCC)

Rubenstein and Shaheen (2015) Gastroenterology
Esophageal Adenocarcinomas (EACs) and Gastric Cancers – Similarities and Differences

• Premalignant Lesions
  - Barrett’s Esophagus and Intestinal Metaplasia

• Treatment Regimes
  - Often grouped in trials (esp GEJ tumors)
  - Similar targets (eg HER2)

• Carcinogens and Exposures
  - *H. pylori* and Acid/Bile reflux

• Histological Subtypes
  - Intestinal type GC
  - Diffuse/Schirrous Type GC
Gastric Cancer – Four Genomic Subtypes (USA TCGA)

- A) Chromosomal Instability (CIN)
- B) Microsatellite Instability (MSI)
- C) Genome Stable (GS)
- D) Epstein-Barr Virus (EBV)

USA TCGA (2014) Nature
ACRG Classification Based on Gene Expression

EAC: Pre-Malignant Barrett’s Esophagus Exhibits a Surprising Number of Somatic Mutations

Esophageal Adenocarcinomas (EACs) Exhibit Increased Copy Number Alterations

Discovery of Therapeutically Relevant EAC Subtypes Based on Mutation Signatures

Secrier, Li et al., (2016) Nature Genetics
Remarkable Similarity of Esophageal Adenocarcinoma to CIN-type GC

USA TCGA (2017) Nature
ESCCs - a Distinct Molecular Entity

"ESCC Specific":
NFE2L2/NRF2, FAT genes, NOTCH1, SOX2 and TP63 sCNAs and others...

Epigenetic Heterogeneity in ESCC

Song et al., (2014) Nature
Hao et al., (2016) Nature Genetics
USA TCGA (2017) Nature
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Molecular and Clinical Heterogeneity in Gastric Cancer (GC)

Tay et al., Cancer Research (2003)
Human Cancers as Cellular Ecosystems

Am J Physiol Cell Physiol (2011)
Cellular Heterogeneity in Gastric Cancer (GC)

**Intestinal**
- Glandular Morphology
- Gastric Atrophy
- Well Differentiated
- Better Prognosis
- Several targetable genetic alterations (eg HER2)

**Diffuse / Scirrhouous**
- Little cell adhesion
- No atrophy
- Poorly Differentiated
- Worse Prognosis
- Few targetable genetic alterations (“GS tumors”)
GC Molecular Heterogeneity Reflects Distinct Cell Programs and Populations
Tumor Stromal Proportion Predicts Prognosis
(Gene Expression/Digital Pathology)

Gene Expression

Survival Probability (Overall Survival)

Follow-up (months)

Low stromal module expression

High stromal module expression

$p = 0.004$

Histopathology

Survival Probability (Overall Survival)

Follow-up (months)

ITS Low

ITS High

$p < 0.001$

Wu et al., 2013 Gut
Diffuse-Type Gastric Tumors Exhibit Elevated CAF/Cancer Cell Interactions

Takatsugu Ishimoto
Hideo Baba
Kumamoto University
Isolation of Paired Normal Fibroblasts (NFs) and Cancer Associated Fibroblasts (CAFs) from DGC Patients

Surgically Resected Tissue

Gastric Ca

Exome-seq Confirms Almost No Somatic Mutations (ie Not “EMT-Cancer Cells”)
GC Movement is Enhanced by CAF Co-culture

OCUM12 (GC)  OCUM12+NFs  OCUM-12+CAF

Experiments Done on ECM
CAF Co-culture Enhances GC Lymph Node Metastasis *in vivo* (PDX)
RNA-Sequencing of Paired NFs and CAFs Reveals Consistent Activation of TGFβ-Signaling
*RHBDF2/iRHOM2* is upregulated in CAFs and required for CAF motility.
RHBDL2/IRHOM2 Encodes a Pseudoprotease that Mediates Activation of TACE/ADAM17

Tumor Necrosis Factor Signaling Requires iRhom2 to Promote Trafficking and Activation of TACE
Colin Adrain et al.
Science 335, 225 (2012);
DOI: 10.1126/science.1214400

TRAF6 ubiquitinates TGFβ type I receptor to promote its cleavage and nuclear translocation in cancer
Yabing Mu1,2, Reshma Sundar1,2, Noopur Thakur1,2, Maria Ekman1, Shyam Kumar Gudey1,2, Mariya Yakymovych1, Annika Hermansson1, Helen Dimitriou1, Maria Teresa Bengoechea-Alonso1, Johan Ericsson1, Carl-Henrik Heldin2 & Marene Landstrom1

Canonical TGFβ Pathway

Huang and Chen, Cell and Bioscience, 2012
TACE/ADAM17 Regulates Cleavage and Nuclear Accumulation of TGFBR1 ICD in CAFs
**RHBDL2/iRHOM2 Silencing Reduces TGFβR1 Cleavage, Nuclear Accumulation, and TGFβ Target Gene Expression**

<table>
<thead>
<tr>
<th>TGF-β1</th>
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<th>(+)</th>
<th>(+)</th>
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<tr>
<td>siRNA kDa</td>
<td>Control</td>
<td>Control</td>
<td>RHBDL2 #1</td>
<td>RHBDL2 #2</td>
<td>TβR1</td>
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<tr>
<td>50</td>
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<td>100</td>
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<tr>
<td>Phospho-Smad2</td>
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<tr>
<td>Total Smad2/3</td>
<td></td>
<td></td>
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<tr>
<td>β-actin</td>
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### Western Blot
- **Pro**, **Mature**, and **TACE**
- **TβR1 ICD**

### Immunofluorescence
- **Nuclear**, **TβR1 ICD**, **Merge**

### mRNA Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>(+)</th>
<th>(+)</th>
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<tbody>
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<td>CDKN1A (p21)</td>
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<td>✔️</td>
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<tr>
<td>CDKN2B (p15)</td>
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<tr>
<td>SERPINE1 (PAI1)</td>
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<td>✔️</td>
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<tr>
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<tr>
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<td>RHOB</td>
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<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

*Significance: *p < 0.05, **p < 0.01*
Summary

- Spatial Analysis Highlights Intimate CAF/Cancer Cell Interactions in Diffuse-Type GC

- CAFs Promote Cancer Cell Migration and Metastasis in vitro and in vivo (“Tugboat Mechanism”)

- Genomic Analysis of CAFs Highlights RHBDF2 as a Regulator of the TGFβ-driven Metastatic Program

- RHBDF2 Stimulates TGFBR1 Cleavage and ICD Nuclear Import (Non-canonical TGFβ Signaling)

- A Potential Role for TACE/ADAM17 Inhibitors in Treatment of Diffuse-Type GC

Ishimoto et al., (2017) Gastroenterology
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Cancer Gene Expression is Controlled by Epigenomic Changes

Regulatory Elements can be Identified by Histone Modifications

<table>
<thead>
<tr>
<th>Modification</th>
<th>Regulatory Elements</th>
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<tbody>
<tr>
<td>H3K4Me1</td>
<td>Enhancers</td>
</tr>
<tr>
<td>H3K4Me3</td>
<td>Promoters</td>
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<tr>
<td>H3K4Me2</td>
<td>Active Enhancers/Promoters</td>
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<tr>
<td>H2A.Z</td>
<td>Inactive Enhancers/Promoters</td>
</tr>
<tr>
<td>H3K27Ac</td>
<td>Active Enhancers/Promoters</td>
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<tr>
<td>H3K27Me3</td>
<td>Inactive Enhancers/Promoters</td>
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<tr>
<td>H3K36Me3</td>
<td>Transcribed Regions</td>
</tr>
<tr>
<td>H3K9Me3</td>
<td>Constitutively Repressed Genes</td>
</tr>
</tbody>
</table>

Example:
- Active Promoter
- Poised Promoter
- Inactive Promoter

绿色标记：H3K4Me3
橙色标记：H3K27Me3
Gene Promoters: Critical Integrators of Regulatory Inputs

Valen and Sandelin (2011) *Trends in Genetics*
>50% of Human Genes have Multiple Promoters

Epigenomic Promoter Profiling Localizes Somatic Promoters

19 N/T pairs + 12 cell lines (H3K4me3, H3K27ac, H3K4me1)

23,000 Promoter Elements (H3K4me3 High, H3K4me1 Low)

Unaltered

Tumor-Specific Gain (Somatic Gain)

Tumor-Specific Loss (Somatic Loss)

Unaltered

Tumor-Specific Gain

Tumor-Specific Loss

90% ~1,500 ~500
Somatic Promoters are Correlated with H3K27ac Activity and Frequently Map to Multi-Isoform Genes

- **H3K4me3 - Altered Promoters**
  - Gain (75%)
  - Loss
  - Normal
  - GC

- **Activity Mark (Log2(H3K27ac))**
  - Somatic Promoters
  - Unaltered Promoters
  - $r=0.91, P<0.001$

- **Percentage of Genes**
  - # Isoforms/
  - Somatic promoter associated gene
18% of GC Somatic Promoters Comprise Alternative Promoters

Adapted from Amit et al (2015)
**MET** – Altered Signaling Shown by N-terminal Promoter Variants

Muratani et al., 2014 *Nat Comm*

TCGA (Papillary RCC) 2016 *NEJM*
Recurrent Alternative Promoters are Predicted to Cause Loss of N-terminal Immunogenic Peptides

MHC Class I Binding (HLA A, B, C)
Recurrent N-terminal Peptides Stimulate Immune Responses in Experimental Systems
GCs with High Alternative Promoter Usage Exhibit Decreased T-Cell Cytolytic Activity (GZMA, PRF1)*

* Adjusted for mutation count and tumor purity
Model - Somatic Promoter Alterations in GC May Reflect the Impact of Tumor Immunoediting
Summary

• Largest catalogue of epigenome-guided promoter elements in gastric cancer

• Tumor-specific promoter alterations are widespread and frequently map to multi-isoform genes

• Alternative promoters can drive expression of novel pro-oncogenic isoforms (eg RASA3, MET)

• Alternative promoter usage may decrease tumor antigenicity (immunoediting)

• Somatic promoters exhibit heightened sensitivity to EZH2 perturbation

Qamra et al., (2017) Cancer Discovery
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