Genomic Heterogeneity in Gastric Cancer: Therapeutic Implications and Challenges

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Disclosure slide

• No disclosures
Gastric Cancer Pathogenesis: Interplay Between Environmental and Host Factors

Yeoh and Tan (2015) Gastroenterology
Can Genomics Improve Gastric Cancer Patient Outcomes?

1) How many GC subtypes exist? What are their driver alterations and pathologic associations?

2) How can driver alterations reveals therapeutic opportunities and clinical responses?

3) How similar is GC across different countries?
There are ~3-4 Major GC Genomic Subtypes

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

USA TCGA (2014) Nature
GC Genomic Subtypes Show Distinct Molecular and Pathological Characteristics

Chromosomal Instability (CIN) (50%)
- Intestinal-type GCs
- TP53 mutations
- Focal somatic gene amplifications in RTK/RAS genes

Microsatellite Instability (MSI) (20%)
- Intestinal-type GC ARID1A, CIMP
- TGFBR2, HLA-B mutations

Genome Stable (GS) (20%)
- Diffuse-type GC
- CDH1, RHOA** mutations

Epstein-Barr Virus (EBV) (10%)
- Global hypermethylation
- PDL-1/2 Gene Amplification**

Wang et al (2011) Nat Genetics
Yoon et al (2013) Genome Res
USA TCGA (2014) Nature
Mutations in GC (9/110)

Associated with MSI and EBV-positive GC

ARID1A (Wild-type)  ARID1A (Mutated)

Wang et al., 2011 *Nature Genetics*
Zang et al., 2012 *Nature Genetics*
Can Genomics Improve Gastric Cancer Patient Outcomes?

1) How many GC subtypes exist? What are their driver alterations and pathologic associations?

2) How can driver alterations reveals therapeutic opportunities and clinical responses?

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Targeted Therapies in Gastric Cancer

ERBB2/HER2 Amplification

OS in IHC 2+ / FISH+ or IHC 3+
(exploratory analysis)

<table>
<thead>
<tr>
<th>Events</th>
<th>OS</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP/FP + T</td>
<td>120</td>
<td>16.0</td>
<td>0.65</td>
</tr>
<tr>
<td>XP/FP</td>
<td>136</td>
<td>11.8</td>
<td>0.51, 0.83</td>
</tr>
</tbody>
</table>

Bang et al (2011) *Lancet*

The TOGA Trial

ERBB2 Positive (8-10%)

Gastric Cancer

??????
Focal Genomic Alterations Highlight Therapeutic Opportunities in GC

RTK/KRAS Amplifications

Deng et al., 2012 Gut
Dovitinib (TKI258) is a Subtype-Specific Therapy for FGFR2-Amplified GCs (Collaboration with Novartis)
Relationships Between RTK Drivers?

Deng et al., 2012 *Gut*

Dulak et al., 2012 *Cancer Research*

USA TCGA, 2014 *Nature*
Intra-Tumoral RTK Heterogeneity in GC

Kilgour et al., 2014 BJC

Das et al., 2015 Cancer Letters

MET-therapy resistant GC

Kwak et al., 2015 Cancer Discovery
Transcription factors **KLF5, GATA4** and **GATA6** are amplified in GC samples

- **Singapore Cohort** (193 patients)
- **TCGA Cohort** (254 patients)
Genome-wide Binding Profiles of KLF5, GATA4 and GATA6 (ChIP-Sequencing)

<table>
<thead>
<tr>
<th>Transcription Factors</th>
<th>KLF5</th>
<th>GATA4</th>
<th>GATA6</th>
</tr>
</thead>
<tbody>
<tr>
<td>YCC3</td>
<td>GGG</td>
<td>AGATA</td>
<td>GATAA</td>
</tr>
<tr>
<td>AGS</td>
<td>GGG</td>
<td>AGATA</td>
<td>GATAA</td>
</tr>
<tr>
<td>KATO-III</td>
<td>GGG</td>
<td>AGATA</td>
<td>GATAA</td>
</tr>
<tr>
<td>JASPAR</td>
<td>KLF4</td>
<td>EVI1</td>
<td>EVI1</td>
</tr>
</tbody>
</table>

EVI1 = GATA-motif Binding Factor
KLF5, GATA4 and GATA6 target common downstream pathways and genes

P<0.01
*HNF4α* is a Common Downstream Target of KLF5 and GATA Factors
The Anti-diabetic Drug Metformin is a Potential HNF4α Therapeutic

1. Treatment of type 2 diabetes
2. Poly Cystic Ovarian syndrome.

HNF4α is a potential predictor of Metformin response in GC
Summary

- **KLF5** and **GATA Factors** Exhibit **Lineage-Specific Amplification** in GI Tract Cancers

- KLF5 and GATA Factors **Interact and Collaborate** to Regulate a Common Pro-oncogenic Expression Program

- **HNF4α** is a common target of KLF5 and GATA factors in GC

- KLF5/GATA-amplified tumors might be treated with Metformin, via HNF4α downregulation

*Chia et al., 2015 Gut*
Can Genomics Improve Gastric Cancer Patient Outcomes?

1) How many GC subtypes exist? What are their driver alterations and pathologic associations?

2) How can driver alterations reveal therapeutic opportunities and clinical responses?

3) How similar is GC across different countries?
Phase III GC Clinical Trials Reveal an Association between Geography and Clinical Outcome

**AVAGAST**
- **AVAGAST (Asian)**: HR = 0.97, 95% CI: [0.75 - 1.25]
- **AVAGAST (Europe)**: HR = 0.85, 95% CI: [0.63 - 1.14]
- **AVAGAST (America)**: HR = 0.63, 95% CI: [0.43 - 0.94]
- **AVAGAST Overall**: HR = 0.87, 95% CI: [0.73 - 1.03]

**RAINBOW**
- **RAINBOW (Asia)**: HR = 0.99, 95% CI: [0.73 - 1.34]
- **RAINBOW (Europe/ Americas/ Australia)**: HR = 0.73, 95% CI: [0.59 - 0.91]
- **RAINBOW Overall**: HR = 0.81, 95% CI: [0.68 - 0.96]

**LOGIC**
- **LOGIC (Asia)**: HR = 0.68, 95% CI: [0.48 - 0.96]
- **LOGIC (N.America)**: HR = 1.61, 95% CI: [0.53 - 4.83]
- **LOGIC (Rest of World)**: HR = 1.04, 95% CI: [0.79 - 1.37]
- **LOGIC Overall**: HR = 0.91, 95% CI: [0.73 - 1.12]
Comparing Asian and Non-Asian GCs
Analysis of 1,600 Gastric Tumors

9 expression profiling studies (n=1,016)
- 6 studies of Asian origin (n = 890)
- 3 studies of non-Asian origin (n = 126)

Stage 1

4 Affymetrix platform studies
- 2 studies of Asian origin (n = 207)
- 2 studies of non-Asian origin (n = 92)

Stage 2

5 non Affymetrix platform studies
- 4 studies of Asian origin (n = 683)
- 1 study of non-Asian origin (n = 34)

Validation analyses

Immunohistochemistry assessment in tissue array studies
- 1 study of Asian origin (n = 219); Japanese cohort
- 1 study of non-Asian origin (n = 446); Caucasian cohort

IHC Profiles (>600)

Collaboration: Johann A. Gagnon-Bartsch
Terry Speed, UC Berkeley
RUV algorithm: Nature Biotechnology (2014)
GC Expression Cohorts Recapitulate Well Known Geographic Differences in 5-yr Overall Survival

Kim et al (2010) *Annals of Oncology*
Stage I: Non-Asian GCs are Enriched in T-cell Gene Signatures Relative to Asian GCs

Gene Signatures:
- Vasculature-related
- Tumorigenesis-related
- Methylated-related
- Metastasis-related
- Immune-related
- Estrogen regulation
- Development/differentiation-related
- Adipocyte-related

Asian:
- MsigDB C2 genesets

Non-Asian:
- MsigDB C7 genesets

- Thymic stromal cell signature
- T-cell signature
- Thymic progenitor signature
- Monocyte signature
- Dysregulation of innate and adaptive immune signature
- Dendritic cell signature
- B-cell signature
Stage 2 (Non-Affymetrix): Validation of T-cell Immune Signatures in non-Asian GCs

Normalized enrichment score = -1.46
FWER p-value < 0.05
GC Tissue Microarray Cohorts

• **JUST cohort**
  – Japanese high-volume cancer centre (Kanagawa Cancer Centre, Yokohama, Japan)
  – Part of ACTS-GC and SAMIT Phase III trials
  – 253 total cohort (110 5FU-related-chemotherapy)
  – **219** total immunohistochemistry (IHC) cohort

• **Leeds cohort**
  – St James’s University Hospital, Leeds, United Kingdom
  – 906 total cohort (62 5FU-related-chemotherapy)
  – **446** total IHC cohort
Immunohistochemistry Validation of T-cell Signatures in Non-Asian GCs
Asian and Non-Asian GCs
Differences in Other Immune Markers

Macrophages
(> Caucasian)

Neutrophils
(> Japanese)

Pan-Leucocyte
(> Japanese)
Adjusting for T-cell Signatures Impacts Geographic Differences in Overall Survival

<table>
<thead>
<tr>
<th>Variable used for adjustment</th>
<th>HR</th>
<th>95%-CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic locality (Asian vs Non-Asian)</td>
<td>0.79</td>
<td>[0.67 - 0.93]</td>
<td>5 x 10^{-3}</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>0.61</td>
<td>[0.50 - 0.75]</td>
<td>1 x 10^{-6}</td>
</tr>
<tr>
<td>AJCC7 Staging</td>
<td>0.59</td>
<td>[0.50 - 0.71]</td>
<td>2 x 10^{-9}</td>
</tr>
<tr>
<td>Age</td>
<td>0.87</td>
<td>[0.74 - 1.02]</td>
<td>0.09</td>
</tr>
<tr>
<td>CD3</td>
<td>0.80</td>
<td>[0.63 - 1.02]</td>
<td>0.07</td>
</tr>
<tr>
<td>CD68</td>
<td>0.84</td>
<td>[0.66 - 1.06]</td>
<td>0.14</td>
</tr>
<tr>
<td>CD66b</td>
<td>0.87</td>
<td>[0.69 - 1.10]</td>
<td>0.25</td>
</tr>
<tr>
<td>CD8</td>
<td>1.17</td>
<td>[0.92 - 1.47]</td>
<td>0.20</td>
</tr>
<tr>
<td>CD45RO</td>
<td>0.86</td>
<td>[0.69 - 1.07]</td>
<td>0.17</td>
</tr>
<tr>
<td>FOXP3</td>
<td>0.94</td>
<td>[0.72 - 1.23]</td>
<td>0.67</td>
</tr>
</tbody>
</table>
East vs West GCs May Differ in Combined MSI/EBV Frequency

- **EBV (+)**
  - 12/216 (5.48%)

- **MSI (+)**
  - 21/216 (9.59%)

Chi-sq test of proportion p-value = 2.8 x10^{-07}

- **EBV (+)**
  - 15/437 (3.4%)

- **MSI (+)**
  - 13/175 (7.42%)
Conclusions and Discussion

• Large-scale expression analysis reveals differences in the immune microenvironment between Asian vs non-Asian GCs

• Non-Asian GCs appear enriched in T-cell pathways (eg CTLA-4) and other immune cells (eg macrophages)

• Tumor immunity differences may be due to differences in combined MSI/EBV frequency

• Adjusting for immune differences (esp CD68/CD3) impacts region-specific survival

• Tumor immunity differences may influence GC immunotherapy trials?

Lin et al 2015 Gut
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