Molecular Diagnosis of Gastrointestinal Tumors

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EEA and Norwegian Financial Mechanisms in Hungary, Development of joint Hungarian and Norwegian strategy for cancer treatment by molecular methods. (Prevention, early diagnosis and therapy)
Colorectal cancers
Patients and Methods

• Totally 634 surgically resected colorectal cancer samples.
• Database construction.
• Isolation of DNA form FFPE tumor samples by the assistance of MagNa Pure Compact machine.
• PCR-based microsatellite instability test according to Dietmaier and Hofstadter (Lab. Invest., 81:1453-1456, 2001).
• Real-time PCR amplification and melting point analysis of KRAS exon 2 and BRAF exon 15 gene regions.
• DNA sequence analysis of amplicons.
Five-year Survival Rate of Gastic and Colorectal Cancer Based on the National Cancer Registry

Genetic classification of colorectal cancers

MMR negative phenotype
- Sporadic CrC develops via "polyp-cancer" sequence
  - Microsatellite stability (MSS)
  - Chromosomal instability

MMR positive phenotype
- Hereditary non-polyposis CrC (HNPCC)
  - Chromosomal instability
  - Microsatellite instability (MSI-H)
- Sporadic CRC develops due to the CpG methylation of hMLH1 (CIMP+)
  - Chromosomal stability
Kaplan-Meier probability survival of CrC according to the TNM-based clinical staging

\[ p = 0.0000 \]
Correlation of TNM-based clinical stage and the genotype of CrC at the time of diagnosis

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>HNPCC MSI-H</th>
<th>CIMP+ MSI-H</th>
<th>Polyp-Cancer MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 + 2 (%)</td>
<td>69.8</td>
<td>66.7</td>
<td>38.4</td>
</tr>
<tr>
<td>Stage 3 + 4 (%)</td>
<td>30.2</td>
<td>33.3</td>
<td>61.6</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Kaplan-Meier probability survive of CrC according to the genotype

- HNPCC
- Sporadic MLH1+, MSI-H
- Sporadic MSS

P = 0.0002
Localization of genetically different CRCs

<table>
<thead>
<tr>
<th>Colorectal cancer</th>
<th>Localization %</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right half-colon</td>
<td>Left half-colon &amp; rectum</td>
</tr>
<tr>
<td>Sporadic CIMP+ MSI-H</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>HNPCC MSI-H</td>
<td>65.1</td>
<td>34.9</td>
</tr>
<tr>
<td>Sporadic MSS</td>
<td>21.9</td>
<td>78.1</td>
</tr>
</tbody>
</table>

P = 0.000
Significant genetic alterations influencing the therapy response of colorectal cancers

1. KRAS or BRAF mutation inhibits the therapeutic effects of anti-EGFR antibodies.
2. The 5-fluorouracil-based chemotherapy has no any effects on microsatellite instable (MSI-H) carcinoma.
Enhanced EGFR sensitivity

Ligand-dependent gene activation

Enhanced EGF signal
Autocrine receptor stimulation

The receptor function is blocked
Anti-EGFR mab

There is no EGFR signal

PI3K
PTEN

PI3K
mTOR
AKT

angiogenesis, cell proliferation, survive

RAS → BRAF → MEK → ERK

2. exon 12/13 codon or 15. exon V600E (GTG → GAG)

mutation

uncontrolled cell proliferation

TGFβ → p21 → cyclin D → CDK 4/6

unregulated cell cycle

MSI

There is no EGFR signal

Enhanced EGFR signal
Disease-process:
In spite of the anti-EGFR treatment liver metastasis developed.
The patient died within two years.
Frequency distribution of gain-of-function mutations in codon 12 and codon 13 of KRAS gene (184 tumor samples)
# Localization of KRAS and BRAF positive CrCs

<table>
<thead>
<tr>
<th>Genetical state</th>
<th>Incidence %</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right half-colon</td>
<td>Left half colon &amp; rectum</td>
</tr>
<tr>
<td>KRAS mut</td>
<td>24.2</td>
<td>75.8</td>
</tr>
<tr>
<td>BRAF mut</td>
<td>66.7</td>
<td>32.3</td>
</tr>
</tbody>
</table>

P = 0.000
Frequency distribution of KRAS and BRAF mutations in hereditary and sporadic CrC

<table>
<thead>
<tr>
<th>CrC types</th>
<th>KRAS mut %</th>
<th>BRAF mut %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNPCC  MSI-H</td>
<td>39.5</td>
<td>-</td>
</tr>
<tr>
<td>Sporadic CIMP+, MSI-H</td>
<td>8.4</td>
<td>52.8</td>
</tr>
<tr>
<td>Sporadic MSS</td>
<td>45.9</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Kaplan-Meier survival function of CrCs according to the KRAS mutation state

P = 0.17
Kaplan-Meier survival function of CrCs according to the BRAF mutation state

- BRAF wt
- BRAF mutant

p = 0.0000
Gastrointestinal stromal tumor (GIST)
Study Population

- No. of tumor samples investigated: 72
- Sex: Male 38, Female 34
- Patients follow up period: between 17 years and 6 mounts
- Age distribution:
Methods

• Collection of formalin-fixed and paraffin-embedded tumor samples and database construction.
• CD117 and CD34 immunohistochemistry and pathological assessment of the tumor risk categories.
• Isolation of DNA form FFPE tumor samples by the assistance of MagNa Pure Compact machine.
• Real-time PCR amplification and melting point analysis of c-kit exon 9, exon 11, and PDGFRA exon 12 and exon 18 gene regions.
• High resolution capillary gel electrophoresis analysis of amplicons.
• High resolution melting analysis of c-kit exon 11 using LightCycler 480 PCR system to analyze genetic variations in PCR amplicons.
• DNA sequence analysis of amplicons.
High Resolution Melting

Deletion (others)
Point mut (red)
Wt (blue)
C-kit exon 11
Comparison of the Mutation Analysis Methods

<table>
<thead>
<tr>
<th>Real-time PCR amplification and melting point analysis &amp; sequencing</th>
<th>High Resolution Melting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt</td>
</tr>
<tr>
<td>Wt</td>
<td>**********</td>
</tr>
<tr>
<td>Point mut</td>
<td>**********</td>
</tr>
<tr>
<td>Deletion</td>
<td>**********</td>
</tr>
</tbody>
</table>

Asterisks represent tumor samples
Localization of GIST
(No = 72)

<table>
<thead>
<tr>
<th>Localization</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>2.8</td>
</tr>
<tr>
<td>Stomach</td>
<td>48.6</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>26.4</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>11.1</td>
</tr>
<tr>
<td>Extra-intestinal</td>
<td>6.9</td>
</tr>
<tr>
<td>Metastasis</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Kaplan-Meier probability survival function

p = 0.0076
Pathological Risk Grouping of GIST (No = 72)

<table>
<thead>
<tr>
<th>RISK GROUPS</th>
<th>FREQUENCY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk GIST</td>
<td>40.3</td>
</tr>
<tr>
<td>High risk GIST</td>
<td>59.7</td>
</tr>
</tbody>
</table>

Kaplan-Meier probability survival function

p = 0.0052
Summary of Significant Genetik Alterations in GIST

GIST, AML

GIST

Kit

Extracellular domain (exon 9)

Juxtmembrane domain (exon 11)

Ligand-binding domain

PDGFRA

Juxtmembrane domain (exon 12)

Tyrosine kinase II domain (exon 18)

Imatinib (Gleevec) sensitive mutations

Gleevec resistant mutations

Imatinib (Gleevec) sensitive mutations
## Significant mutations in GIST 72 tumors

<table>
<thead>
<tr>
<th>Gleevec sensitive mutations, c-kit</th>
<th>Gleevec resistance mutation, PDGFRA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 9 point m. &amp; dupl</td>
<td>Exon 11 del</td>
<td></td>
</tr>
<tr>
<td>5 (6.9%)</td>
<td>31 (43.1%)</td>
<td></td>
</tr>
<tr>
<td>Exon 11 point mut</td>
<td>15 (20.8%)</td>
<td></td>
</tr>
<tr>
<td>Exon 18 point mut &amp; delins</td>
<td>8 (11.1%)</td>
<td></td>
</tr>
<tr>
<td>59 (81.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C-Kit exon 11
Highly Gleevec sensitive deletion mutations

Types of deletion in 14 tumor samples

cDNA

1650
ACCCATGTATGAAGTACAGTGGAAGGTGTTGTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
A---A
ACCCATGTATGAAGTACA------------------GAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----TGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC
ACCCATGTATGAAGTACAG-----GTTGTTGAGGAGATAATGGAAACAAATTATGTTTACATAGACCCACACAACCTTCC

1730
GG

C.1735-1737
C-Kit exon 11
Highly Gleevec sensitive point mutations

Mutation pattern (14 tumors)

c.1669T  c.1669T  
1650  c.1666C  c.1676T  c.1727T

> T
  > C
  > C
  > A
  > A

> A
  > G
  > C

> A
  > A
  > A

> C
> C
C-Kit exon 9
Moderately Gleevec sensitive point mutations and SNPs

9 tumors (5 mutations, 4 SNPs)

c.1357T >C
1354
c.1383A >G snp
c.1391C >G snp
c.1414C >T snp
c.1445C >T
1431
c.1486G >A snp
c.1497G >A snp
c.1504-1509 dup GCCTAT
## Detection and Frequency of Gene Alterations in exon 18 of PDGFRA

<table>
<thead>
<tr>
<th>GENE ALTERATION</th>
<th>FREQUENCY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2525A&gt;T (p.D842V) Delins</td>
<td>9.7 1.4</td>
</tr>
<tr>
<td>c.2472C&gt;T snp</td>
<td>11.1</td>
</tr>
<tr>
<td>c.2442T&gt;C snp</td>
<td>2.8</td>
</tr>
<tr>
<td>c.2526C&gt;T snp</td>
<td>1.4</td>
</tr>
<tr>
<td>c.2538T&gt;C snp</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Detection of p.D842V mutation by real-time PCR and melting point analysis.

![Graph showing fluorescence vs temperature](image_url)
Correlation Between the Mutation Status of c-kit exon 9 & 11 and the Metastatic Potential of GIST

<table>
<thead>
<tr>
<th>C-kit</th>
<th>Metastasis</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Wild type</td>
<td>76.2</td>
<td>23.8</td>
</tr>
<tr>
<td>Exon 9 &amp; 11 point mut</td>
<td>60.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Exon 11 deletion</td>
<td>25.8</td>
<td>74.2</td>
</tr>
</tbody>
</table>

Pearson chi-square \[ P = 0.0010 \]

The c-kit mutations promote the metastatic tendency of GIST
MP487/08, 55-year-old male with a 42x35x31 mm abdominal mass and a 22 mm in diameter nodule in the 5th segment of liver. Core biopsy from the abdominal mass and liver nodule.
cKIT exon 11: 35 bp deletion

Mutant allele

Wt allele

Heterodimers

High resolution capillary gel electrophoresis
55 year-old male, at the time of diagnosis

8 months after Gleevec treatment
Kaplan-Meier Probability Function of Gleevec Sensitive Mutation Bearing GIST Versus other Subtype of GIST

- C-kit exon 9 & 11 mutant GIST (51 tumors)
- C-kit wt and PDGFRA resistance mutant GIST (21 tumors)