A Novel Approach for Unique MRD Markers Identification in Acute Leukemia Patients

Tereza Jančušková

synlab genetics s.r.o.
Evropska 176/16, Prague, Czech Republic
Czech Republic, Prague

synlab genetics, Laboratory for Molecular Diagnostics

Departments:
- Cytogenetics
- Molecular hematooncology
- Molecular detection of pathogens
- Molecular detection of rare genetic syndromes
Introduction – Acute Leukemia

• Acute leukemias (AL) – acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL)
• Different prognosis depending on many factors
• Sensitive minimal residual disease (MRD) monitoring:
  – strong prognostic factor
  – assessment of the quality of treatment response
  – prediction of individual risk of relapse
• Real-time PCR technique
  – sensitivity $\sim 10^{-5}$
  – molecular marker is necessary
Introduction – Molecular Markers

• adult ALL patients – in majority cases suitable marker is identified
  – IgH/TCR gene rearrangements
  – cytogenetic abnormalities = fusion transcripts (BCR-ABL, MLL-AF4...)

• adult AML patients – suitable molecular marker in **50 % only**
  – cytogenetic abnormalities = fusion transcripts (PML-RARA, AML1-ETO...)
  – gene mutation (*NPM1, CEBPA, WT1, c-KIT...*)
Our Aim

• To develop a flexible strategy for identification of unique molecular targets for sensitive MRD assessment in AL patients
  - mapping of cytogenetic abnormalities down to the single nucleotide level
  - design a specific real-time PCR assay
Study Design

- Pilot study - cell line K562
- Patients with acute leukemia
Our strategy – K562 cell line

G-banding, mFISH a mBAND: der(10)t(3;10)(p21;q23)

Our strategy – K562 cell line

DOP-PCR

Sanger sequencing

Chromosome microdissection

Long-range PCR

Next-generation sequencing and data analysis

Chromosome 10 reference

Chromosome 3 reference

CDC25A chromosome 3

GRID1 chromosome 10

Real-time PCR assay
Acute Leukemia Patients
Patient 1

- Fusion transcript MLL-AF4 = comparison of standardized target and newly characterized targets
- Dissection of der(4)
- Dissection of der(11) – with fusion gene MLL-AF4
- DNA sequences of chromosomal breakpoints

46,XX,t(X;4;11)(q25;q21;q23)[20]

Gene AF4 chromosome 4
LOC392539 chromosome X

Gene MLL chromosome 11
Gene AF4 chromosome 4
Patient 1 – Quantification Graphs

**Relative quantity of derivative chromosome 4**
(breakpoint 4q21;Xq25)

**Relative quantity of derivative chromosome 11**
(breakpoint 11q23;4q21)

**Relative quantification of MLL/AF4**
Patient 2

- Fusion transcript MLL-AF10 = low expression
- Need to quantify on DNA level
- Dissection of der(10)
- DNA sequence of MLL-AF10 fusion

46,XY,ins(10;11)(p12;q13q23),t(11;14)(q13;q11)[20]
Patient 2 – Quantification Graph

Relative quantity of derivative chromosome 10
(breakpoint 10p12; 11q23)

Day
0 200 400 600

Relative quantity of der(10)
1x10^-1 1x10^-2 1x10^-3 1x10^-4

1. BM

1 27.09.2012
2 15.10.2012
3 25.10.2012
4 30.10.2012
6 23.01.2013
7 26.03.2013
8 21.05.2013
9 23.07.2013
10 21.10.2013
11 06.01.2014
12 14.04.2014

allo Tx
below PCR detection limit
Patient 3

- Screening for MRD targets = negative
- Dissection of der(8)
- DNA sequence of der(8) breakpoint

46,XY,der(7)del(7)(p21)del(7)(q21),t(7;8)(q21;q24)[20]
Patient 3 – Quantification Graph

Relative quantity of derivative chromosome 8 (breakpoint 7q21;8q24)

Relative quantity der(8)

Day

Below PCR detection limit

04.01.2013
05.02.2013
11.03.2013
10.04.2013
06.05.2013
... and

- Beside characterization of unique markers for MRD monitoring
- Identification of unreported partner genes
- *MECOM* gene
MECOM gene

- MDS1 and EVI1 complex locus (*MECOM*)
- 3q26.2 region
- Fusion partners: 3q21 (*RPN1*), 7q21 (*CDK6*), 7q34 (*TCRB*), 12p13 (*ETV6*), 21q22 (*RUNX1*)...
- In healthy individuals - low EVI1 expression in PB and BM
- In AML patients - overexpression in BM/PB because of 3q26 rearrangements
- EVI1 overexpression - MRD target (low sensitivity = ~10^{-2}!)
Patient 4

46,XX,t(3;10)(q26;q21)[20]/46,XX[2]

- MECOM locus involved in both translocation
- Dissection of der(10) and der(3)
- Identification of MRD targets and MECOM’s fusion partners

Patient 5

46,XX,t(3;6)(q26;q25)[20]
DNA sequences of breakpoints

Patient 4

C10orf107 chromosome 10

Patient 5

MECOM chromosome 3

LOC101928923 chromosome 6
Patient 4

Relative quantity of derivative chromosome 10
(breakpoint 3q26; 10q21)

Day

0 50 100 150 200

1 x 10^-1 1 x 10^-2 1 x 10^-3 1 x 10^-4

Below PCR detection limit

Patient 5

Relative quantity of derivative chromosome 3
(breakpoint 3q26; 6q25)

Day

0 50 100 150 200

1 x 10^-1 1 x 10^-2 1 x 10^-3 1 x 10^-4

Below PCR detection limit
Conclusions

• Techniques combination – from chromosomal level to single nucleotide level
• Identification of unique clone-specific marker of leukemic blasts
• Design of patient-specific molecular real-time PCR assay for MRD assessment
• New fusion partners of *MECOM*
  → *C10orf107* chromosome 10q21
  → *LOC101928923* chromosome 6q25
• Personalized medicine – „tailor-made“
• Also suitable for characterizing unique chromosomal translocations in other fields (e.g. human genetics)
Thank you for your attention