The Molecular Cytogenetics of Multiple Myeloma: Risk Stratification and Clonal Evolution

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Learning Objectives

• Define the different risk stratification factors in myeloma

• Describe the different chromosome aberrations associated with different risk groups

• Understand the basic mechanisms of clonal evolution in myeloma
Cancer Hallmarks

- Sustaining proliferative signaling
- Evading growth suppressors
- Deregulating cellular energetics
- Avoiding immune destruction
- Resisting cell death
- Enabling replicative immortality
- Genome instability & mutation
- Tumor-promoting inflammation
- Inducing angiogenesis
- Activating invasion & metastasis

Hanahan D and Weinberg RA, Cell 144, 2011
Clonal Evolution of Cancer Genomes

• Myeloma as a model for cancer chromosome aberrations

• Novel therapies (personalized medicine) have increased survival in most cancers

• Tumor cells evade therapeutic interventions by evolving along different trajectories, leading to aggressive chromosomally unstable cancers

• Chromosomal instability is a major cause of treatment failure and disease relapse
Copy Number Alteration (CNAs) in Human Cancer

- Single nucleotide polymorphisms (SNPs) array data from 3,131 cancers, 26 histological types

- Three general types of copy number alterations (CNAs)
  1. whole chromosome gains or losses
  2. whole-arm gains or losses - in a typical cancer 25% of genome affected
  3. very short (focal) amplifications or deletions - 10% of genome affected

- MCL1 is one of nine genes in an amplification peak in cytogenetic band 1q21 with focal amplifications observed in 10.9% of cancers.

Beroukin R et al., *Nature* 2010, 463:899-905
Multiple myeloma (MM) is a plasma cell disorder of the bone marrow. Characterized by complex numerical and structural chromosome aberrations.

Interaction between genetic aberrations and factors in the bone marrow microenvironment drive the disease. Average age at diagnosis is 70, focal lesions (plasmacytomas) can occur at multiple sites.

Patients are stratified for treatment based on clinical and cytogenetic findings.

Increasing evidence indicates that clonal evolution involving the accumulation of adverse cytogenetic aberrations modulates the outcome of patients.
Whole Body Magnetic Resonance Imaging (MRIs) of Myeloma

- Focal lesions in spine and left femur
- Multiple focal lesions
MRI of Spine at Diagnosis, Remission, and Relapse

White arrows indicate focal lesions in the spine
Multi-stage Progression to Multiple Myeloma (MM)

- **Monoclonal gammopathy of undetermined significance (MGUS):** plasma cell content less than 10% in the bone marrow, monoclonal protein spike, no end organ damage, progresses to MM at a rate of ~1% per year

- **Smoldering myeloma (SM) or asymptomatic myeloma:** is an intermediate entity between MGUS and active MM. 10-30% tumor cells in marrow, no bone lesions, anemia or other secondary findings

- **Symptomatic myeloma:** end organ damage, highlighted by the acronym **CRAB**
  - Calcium elevation
  - Renal insufficiency
  - Anemia
  - Bone disease
Disease Progression in Myeloma

Initiation

MGUS → Smouldering myeloma → Myeloma → Relapse → Extramedullary myeloma

Primary genetic events
- IgH translocations
- Hyperdiploidy

Progression

Secondary aberrations
- Copy number abnormalities

Accumulation of adverse cytogenetic lesions

Tumor cell diversity

DNA hypomethylation
Myeloma Clonal Evolution to High-Risk Disease

COMPETITION AND SELECTIVE PRESSURE

TUMOUR CELL DIVERSITY

Chromosome instability

Extramedullary myeloma or plasma cell leukemia

Myeloma progenitor cell

Clonal advantage

Secondary genetic events
Cytogenetic and Molecular Methods

Conventional Cytogenetics
  Giemsa-Banding (G-banding)

Molecular Cytogenetic Techniques
  Fluorescence In Situ Hybridization (FISH)
  Multicolor Spectral Karyotyping (SKY)

Molecular Techniques
  array Comparative Genomic Hybridization (aCGH)
  Gene Expression Profiling (GEP)
Chromosome 1
FISH probes
1q12 (red) pericentromeric region
1q21 (green)

Normal male G-band karyotype
46,XY

Background Cytogenetics
Hyperdiploid clones have 48-75 chromosomes with trisomies 3,5,7,9,11,15,19,21 fewer IgH translocations (~10%) 

Hypodiploid clones have <48 and /or >75 chromosomes with loss or deletion 13,14,16,22 prevalent IgH translocations (~70%) near tetraploid cells (81~ 103) extra medullary tumors and cell lines 

Novel Numerical Subgroup Hyperhaploidy - clones have 24-34 chromosomes 
Monosomies 1,2,4,6,8,10,12,13,14,16,17,22 Disomies 3,5,7,9,11,15,18,19
Chromosome Aberrations In Multiple Myeloma

Average 11 Events / Karyotype

p arm

q arm

Gains

Losses
Hyperdiploid karyotype, trisomies of 3, 5, 7, 9, 11, 15, 19, and 21
Structural Aberrations

- Karyotypes are very complex with both balanced and unbalanced structural aberrations

**Primary chromosome aberrations**
- Found early and involve IgH translocations
  - t(11;14)(q13;q32) ~15-20% cyclin D1
  - t(4;14)(p16;q32) ~10-12% FGFR3 and MMSET
  - t(14;16)(q32;q23) ~5% c-MAF

**Secondary chromosome aberrations**
- Found later in tumor progression in addition to the primary changes, many are copy number aberrations (CNAs) and associated with resistance to therapy
  - gain of 1q21
  - del 17p
  - MYC translocations

**Chromosome instability**
- Jumping translocations 1q12
  - amplification of 1q12-23 and collateral CNAs
Structural Aberrations in MM

- **A**: t(11;14)(q13;q32)
- **B**: t(4;14)(p16;q32)
- **C**: t(8;14)(q24;q32)
- **D**: del(1p12~21)
- **E**: del(13)(q14)
- **F**: del(17)(p11)
Jumping translocations of 1q12 to 16q and 22q

G-band pseudo-diploid karyotype, copy number of 1q21 = 4
Spectral Karyotype of Myeloma

SKY  1q21  MYC
FISH  FISH
Cytogenetic Risk Stratification in MM

- Risk stratification relies on a number of different cytogenetic markers including, t(4;14), t(14;16), t(14;20), del(17p), and gain of 1q21

- Used to assess disease aggressiveness and therapeutic decision-making

- Newly diagnosed patients are stratified into
  - Standard risk
  - Intermediate risk
  - High risk

Mayo Clin Proc. 88:360-376; 2013
Levels of Risk Stratification

<table>
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<tr>
<th>Risk Level</th>
<th>Incidence</th>
<th>Median OS</th>
<th>Incidence Genes</th>
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<tr>
<td>Standard Risk</td>
<td>60%</td>
<td>8-10 years</td>
<td>t(11;14), t(6;14), Hyperdiploid</td>
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<tr>
<td>Intermediate</td>
<td>20%</td>
<td>4-5 years</td>
<td>t(4;14)</td>
</tr>
<tr>
<td>High Risk</td>
<td>20%</td>
<td>3 years</td>
<td>del17p, t(14;16), t(14;20), +1q21</td>
</tr>
<tr>
<td>All others</td>
<td></td>
<td></td>
<td>GEP High Risk Signature</td>
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Modified from Bergsagel L., Hematol Oncol 31; 2013
Inter-relationship of adverse Cytogenetic lesions
Adverse lesions occur together

Implications
i. In order to understand the prognosis of any lesion need to know if other lesions are present.

ii. Lesions may collaborate to mediate prognosis.

Collaboration Between Oncogenes and Tumour Suppressors

Adverse lesions collaborate to define risk

The number of adverse markers has an additive effect on overall survival.

Prognostic Significance of Amp 1q21 by Fluorescence in-situ Hybridization (FISH)

- Frequency of Amp1q21 increases from 30% at diagnosis to 72% at relapse by iFISH

- Amp1q21 is concurrent with dysregulated expression of c-MAF, MMSET/FGFR3, or del13 and is associated with a more aggressive clinical course

- Gain of more than 4 copies of 1q is associated with a drug-resistant phenotype

- Amp1q21 is an independent adverse prognostic factor

Survival of Patients According to Amp1q21 and Copy Number of 1q21 at Relapse

Amplification of 1q by array comparative genomic hybridization (aCGH)

- aCGH defines distinct genomic subtypes of MM
- Recurrent and highly focal CN alterations
- High priority minimal common regions (MCRs) harbor candidate genes of biological and clinical relevance
- MCR identified on 1q21~23 with overexpressed genes spanning 143-158 Mb region

(Carrasco D. et al. Cancer Cell, 2006)
Distribution of genes differentially expressed on 1q

1q12 pericentromeric heterochromatin

10-15 Mb 1q12-23 amplicon

PDZK1  (1q21)  drug resistance
BCL9   (1q21.1) oncogene, unknown function
MCL1   (1q21.2) myeloid cell leukemia 1
PSMD4  (1q21.2) antisecretory factor protein
IL6Ra  (1q21.2) interleukin 6 receptor
CKS1B  (1q21.2) protein, regulates p27Kip1
UBAP2L (1q21.3) ubiquitin associated protein
UBE2Q1 (1q21.3) ubiquitin-conjugating enzy

(Modified from Carrasco D. et al. Cancer Cell, 2006)
Myeloma Associated Copy Number Aberrations

SNPs microarray

(Walker B. et al., Blood Vol.116, 2010)
Prognostic Significance of AMP 1q by Gene Expression Profiling (GEP)

• GEP identifies a high-risk gene expression signature of genes mapping to chromosome 1

• Over-representation of chromosome 1 genes in a group of 70 genes whose expression was linked to poor outcome

• Genes on 1p are under expressed, genes on 1q are overexpressed

• CKS1B and a number of other genes important in myeloma biology map to the amplicon at 1q21

(Shaughnessy J, et al., Blood, 2007)
Global gene expression profiling

Red: over expression (1q, 3, 5, 11, 15, 19, 21)
Green: under expression (13)

(Shaughnessy J, et al., Blood, 2007)
Overall survival based on 70 highly overexpressed or underexpressed genes distinguished 3 groups of patients: good, intermediate or poor prognosis (CD138+ purified plasma cells)
What are the Mechanisms for Gain of 1q21?

High-risk disease associated with the accumulation of copy number aberrations (CNAs) of genes on 1q21 --- multiple mechanisms

- Direct or Inverted Duplications
- Jumping Translocations of 1q (JT1q12)
- Breakage-Fusion-Bridge (BFB) Cycles
1q12 Pericentromeric Heterochromatin

- Gene poor, repetitive DNA sequences (satellite II and III), A+T rich, tandemly repeated ~171 bp monomers

- Late replicating, high levels of DNA methylation and “repressive” histone codes

- Epigenetic changes in gene expression and chromosome structure can be mediated by mechanisms other than alterations in the primary nucleotide sequence
  - methylation of DNA
  - modification of core histones
  - gene silencing through noncoding microRNA

- Associated with chromosome instability
Association of Metaphase Chromosome Structure and 1q12 Chromosome Instability

Hypomethylation affects DNA and acetylation of histone proteins
Direct and Inverted Duplications of 1q12-23

A

1q21 = 3

del(1p)
dup 1q

B

1q21 = 5

del(1p)
inv dup 1q

direct duplication inverted duplication
Jumping Translocations 1q12

- Jumping 1q12 translocations (JT1q12) occur when the whole 1q arm acts as the donor chromosome (DC) to different receptor chromosomes (RC).

- Two most common types of JT1q12:
  - JT1q12 translocates to the telomere of a receptor chromosome (RC)
  - JT1q12 translocates to the pericentromeric region of RC

- JT1q12 begins morphologically with a triradial structure of 1q where by 1q undergoes partial endoreduplication

- Novel type of JT1q12 which duplicates and translocates a segment of a RC, thus increasing the copy number (CN) of the receptor chromosome
1q12 Pericentromeric Decondensation and Triradial Formation

Increasing CNs of 1q21
Whole-arm 1q12 aberrations

A
1q21 gain and loss of 16q

B
1q21 gain and loss of 19q
Model for Jumping Whole-Arm 1q12

- **1q12**
- **1q21**

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### Chromosome Breaks and Translocations

**Pericentromeric Disruption and Undercondensation**

**Donor 1q**

**Receptor Chromosome 16**

**Whole-arm 1q Unbalanced Translocation**

1q21 (green)
1q12 (red)
16q11 (aqua)
Gene amplification via BFB cycles

Triggering mechanism is damaged or shortened telomeres resulting in end-to-end fusion of chromosomes

- results in a dicentric chromosome that is pulled apart during anaphase, causing double strand break (DSB) distal to the gene under selection

- sister chromatid fusion (SCF) occurs at site of DSB

- new isodicentric undergoes a DSB distal to gene under selection in next anaphase resulting in an inverted repeat organization of an amplicon
High Level Focal 1q12~23 amplification

1 copy CKS1B

18 copies CKS1B

18 copies of BCL9, IL6Ra, CKS1B

Pericentromeric Heterochromatin

Sister Chromatid Fusion at pericentromeric heterochromatin
1q21 Gene Amplification by the BFB Cycles

4 copies > 8 copies

8 copies > 16 copies
Model for BFB cycles of the 1q12~23 amplicon

A

1p  
break

1q  
loss of 1q

B

1p  

1p

C

1p 
SCF

D

1p 
break

amp 
loss of 1p

E

1p 
amp

amp

amp

SCF

F

1p 
break

loss of 1p
Possible Model for Clonal Selection by Bortezomib in High-Risk MM
ICF Syndrome
(Imunodeficiency, Centromeric instability, Facial anomalies)

Pediatric chromosome instability syndrome

Clinical features
Immunodeficiency, facial anomalies, developmental delay, mental retardation

Mendelian Disorder
with cytogenetic effects

AR inheritance
biallelic mutations to *DNMT3B*, causes hypomethylation of 1q12

Cytogenetic aberrations
1q12 instability and multiradials of chromosome 1q and exchanges with 16q

ICF patient at 22 months
Chromosomes 1 in ICF Syndrome

Chromosome 1 multiradials

1q x 4
1q x 8
1q x 10
Evidence of an Epigenetic Origin for 1q21 Copy Number Aberrations

• Hypothesis: hypomethylation of 1q12 pericentromeric heterochromatin plays a role in 1q21 amplification.

  Peripheral blood cultures of 5 patients with balanced constitutional rearrangements of 1q12 and 5 normal controls were treated with 5-azacytidine.

• Findings: Structural aberrations and copy number gains of 1q21 in the treated cells were similar to those found in patients with cytogenetically defined high-risk disease.

  All 5 patients showed amplifications on the derivative chromosomes distal to the inverted or translocated 1q12 region, including MYCN in 1 case.

  These findings provide evidence that the hypomethylation of the 1q12 region can amplify any genomic region juxtaposed to it, and mimic CNAs found in the bone marrow of patients with high-risk disease.

Blood. 2015;125(24):3756-3759
Epigenetic Effects of 5-azacytidine on 1q12

• 5-azacytidine is a chemotherapy drug approved for the treatment of AML and MDS
  – 5-azacytidine is a methyltransferase inhibitor which induces hypomethylation of the 1q12 pericentromeric DNA
  – Results in pericentromeric decondensation, somatic associations and multibranched chromosomes 1
  – a 5-azacytidine fragile site (FRA1J) has been mapped to 1q12, and is a possible candidate for a fragile site in MM
### 5-azacytidine treated cells in control subjects

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<tr>
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<tbody>
<tr>
<td>A</td>
<td>dir dup 1q12-23</td>
</tr>
<tr>
<td>B</td>
<td>Triradial 1q</td>
</tr>
<tr>
<td>C</td>
<td>Trisomy 1q</td>
</tr>
<tr>
<td>D</td>
<td>Triradial 1q</td>
</tr>
<tr>
<td>E</td>
<td>Multiradial 1q</td>
</tr>
<tr>
<td>F</td>
<td>Allocyclic triradial 1q</td>
</tr>
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Balanced constitutional t(1;2)(q12;p11.2)

G-bands

FISH (left) and SKY (right) of constitutional t(1;2)(q12;p11.2)
5-aza induced amplification of N-MYC

A

1q12
1q21

triradial 2p
der(2)(1;2)

B

1p12(J19)

N-MYC (2p24)

Genes amplified because of being juxtaposed distal to 1q12
• Treatment should be targeted to the molecular/cytogenetic subtype (personalized medicine)

• Genetic and cytogenetic events accumulate and collaborate to determine clinical behavior and introduce intraclonal and subclonal genetic heterogeneity

• JT1q12 provides a mechanism for amplification and deletions in cytogenetically defined high-risk myeloma

• Hypomethylation of the 1q12 pericentromeric region plays a role in the amplification and deletion of diverse chromosome regions resulting in chromosome instability
## Acknowledgements

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<th>Myeloma Institute</th>
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