Integrated genomic analysis of human osteosarcomas

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Outline

- Osteosarcomas
- Project strategy
- Technologies
- Results
- Gene networks and pathways
- Summary and further work
Mesenchymal differentiation

Adapted from a figure by Paul S Meltzer
Osteosarcomas

- Most common primary malignant tumours of bone
- Children/adolescents and older people
- Long bones (arm and leg)
- High grade tumours
- Highly aggressive
Complex karyotype
Aim

Identify transcriptional networks in osteosarcomas

Integration of different levels of genome-wide data

- DNA copy number
- DNA methylation
- mRNA expression
- miRNA expression
EuroBoNeT

EU funded European network of excellence for research on bone tumours

- 24 labs
- 11 countries
- Technology platforms
- Collection of tumours
- Preclinical models

eurobonet.eu
Tumor panel

- 20 OS cell lines (EuroBoNeT panel)
  - Well characterised preclinical model
- Normal samples
  - Immortalized mesenchymal stem cells (2)
  - Osteoblasts primary cultures (2)
  - Long bones (4)
Strategy

- Genome-wide information
- Identify differences and similarities
- Integrate different levels of data
- Genes, networks and pathways
- Biomarkers and potential targets for therapy
Genome-wide data sets

- mRNA expression
  - Illumina HumanWG-6 v2.0 (Leiden)

- DNA methylation
  - Illumina Infinium HumanMethylation27 (Oslo)

- DNA copy number
  - Affymetrix SNP6 array (Oslo)

- miRNA expression
  - Agilent miRNA array (Oslo)
mRNA expression

- Illumina HumanWG-6 v2

Osteosarcoma vs. Bone
- 2,834 over expressed genes
- 1,748 under expressed genes
CpG Island Methylation

Gene silencing

Genome-wide methylation maps
Infinium Methylation

27,578 CpG sites - 14,000 genes
Hierarchical cluster methylation

Gene expression heatmap showing methylation levels for OB, Bone, MSC, and OS.
Differential methylation

Osteosarcomas vs. Bone

- 1,954 genes hypermethylated
- 200 genes hypomethylated
Methylation and gene expression

**HSPA2 methylation**

- Average methylation
- Osteosarcomas vs Bone

**HSPA2 methylation and expression**

- Expression (intensity)
- Methylation

\[ r^2 = 0.91 \]
DNA copy number changes

Stine H. Kresse

Gains
Losses

Stine H. Kresse
DNA copy number

Higher number of gains than losses

Recurrent changes (≥ 35%)

• 2,881 genes increase copy number
• 2,491 genes decrease copy number
miRNAs

- Small non-coding RNAs
  - 18-25 nucleotides
  - Approx. 1000 human miRNAs
  - Highly conserved
  - Involved in development

- Regulate gene expression
  - mRNA degradation
  - Translational inhibition

Caldas & Brenton, Nat Med, 2005
miRNA expression

- 799 miRNA
miRNAs in OS vs. bone

- Identify miRNAs different expressed between OS and bone
- 799 miRNAs, preprocessed and filtered
  - T-test $p > 0.05$ and $FC > 2$
  
  $\Rightarrow$ 174 miRNAs separating OS and bone
- Identified subclusters with interesting miRNAs

Heidi M. Namløs
Integrative approach

- DNA copy Number
- CpG methylation
- Gene expression

Compared to bone
Recurrent genes across osteosarcomas

336 genes (in at least 4 cell lines)

<table>
<thead>
<tr>
<th>Gene expression</th>
<th>Methylation</th>
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<tbody>
<tr>
<td>Over &amp; Under</td>
<td>Hypo &amp; Hyper</td>
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DNA copy number
Gain & Loss

336
Biological functions

- Cellular growth and proliferation
- Antigen presentation
- Cellular development
- Cell death
- Cell-to-cell signalling and interaction
- Cellular movement
- Cellular compromise
- Cell cycle
- Cellular function and maintenance
- Cell morphology
Summary

- Identified known and novel target genes for
  - DNA copy number changes
  - CpG island methylation (vs. Bone)
  - mRNA differentially expression (vs. Bone)
  - miRNA differentially expression (vs. Bone)

- Integrative analysis identified gene networks and pathways in osteosarcomas
Further work

- Further analysis of networks and pathways
- Integrative analysis versus osteoblasts and MSC
- Validate and confirm target genes and pathways in osteosarcoma clinical samples and xenografts (EuroBoNeT)
Cancer is the result of proliferation and differentiation getting out of balance.

Understanding how this balance is maintained is central both in oncology and stem cell research.
Nuclear programs of mesenchymal differentiation

Osteogenic differentiation

iMSC

Differentiated state

Levels of information

DNA Methylation

Chromatin remodelling (H3K4, H3K9, H3K27)

miRNA expression

mRNA expression

Second Generation Sequencing

Transcriptional and regulatory networks