Integrated genomic analysis of human osteosarcomas

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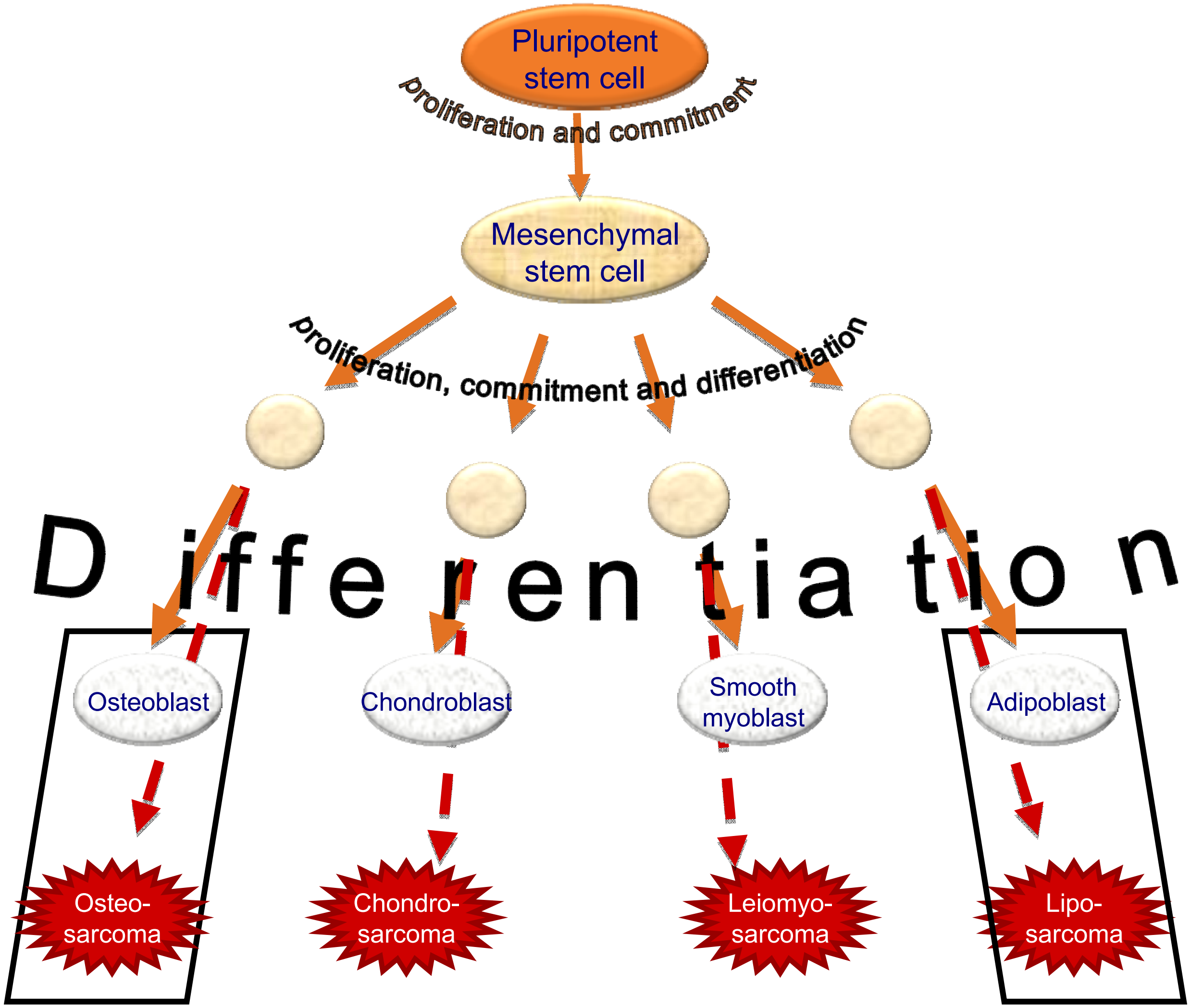
Outline

- 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

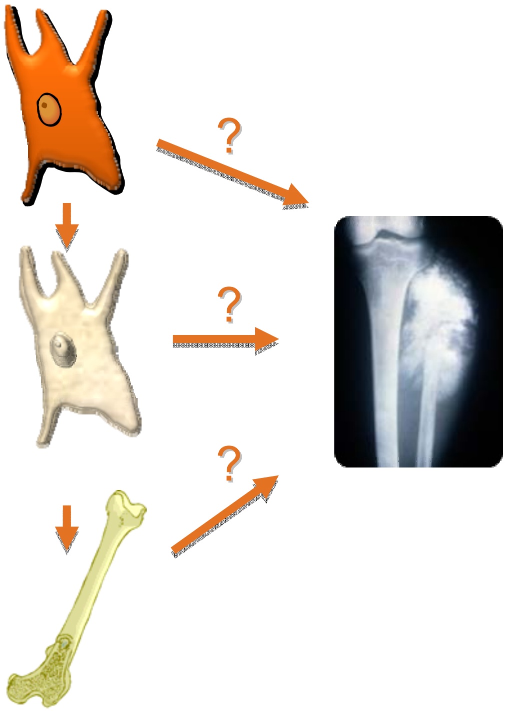
EU funded European network of excellence for research on bone tumours

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

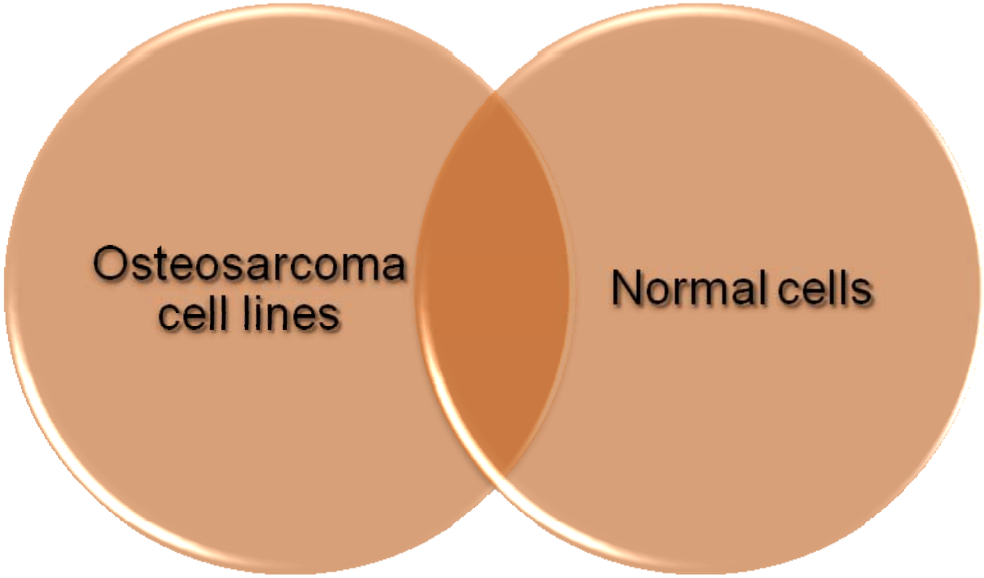
eurobonet.eu

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


Osteosarcoma cell lines

Normal cells

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

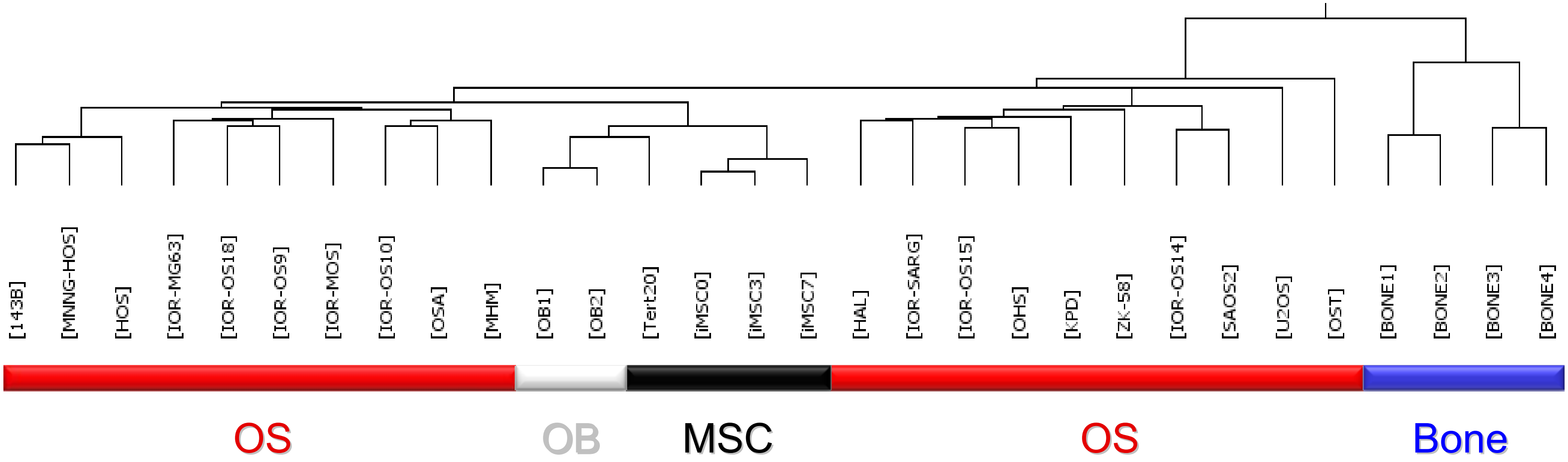
Genome-wide data sets



- 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

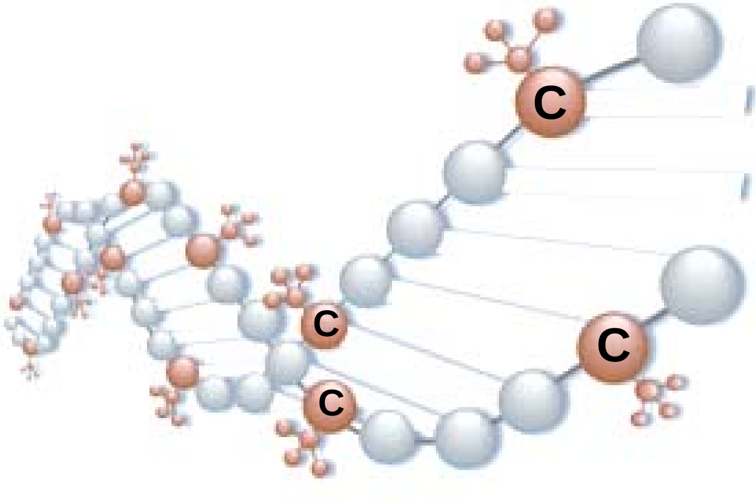
- Illumina HumanWG-6 v2



Osteosarcoma vs. Bone

- 2,834 over expressed genes
- 1,748 under expressed genes

CpG Island Methylation



Gene silencing

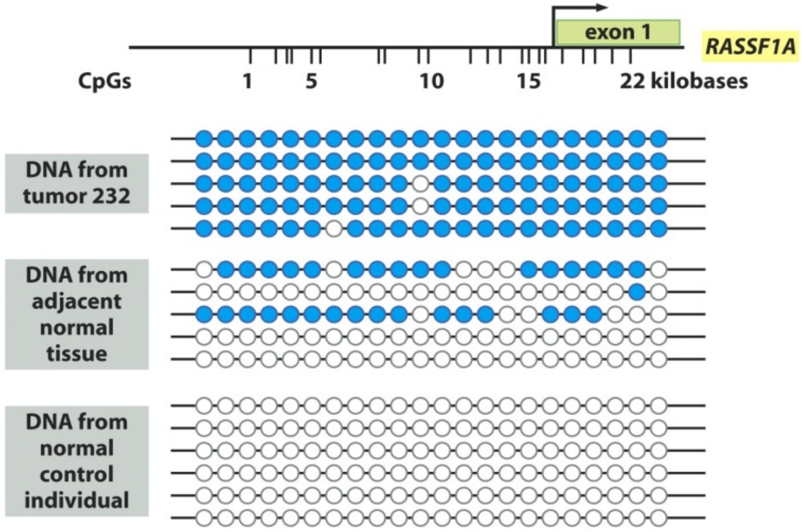
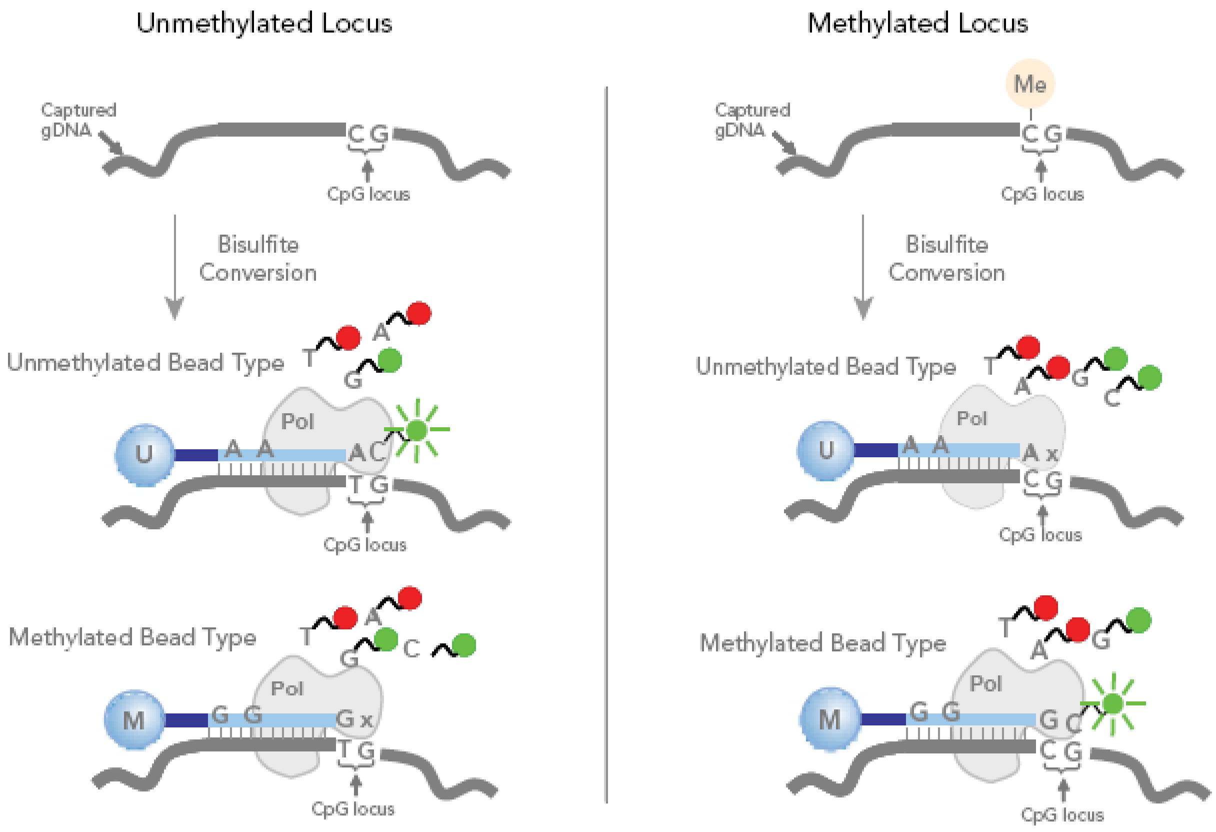


Figure 7-17 The Biology of Cancer (© Garland Science 2007)

● Methylated
○ Unmethylated

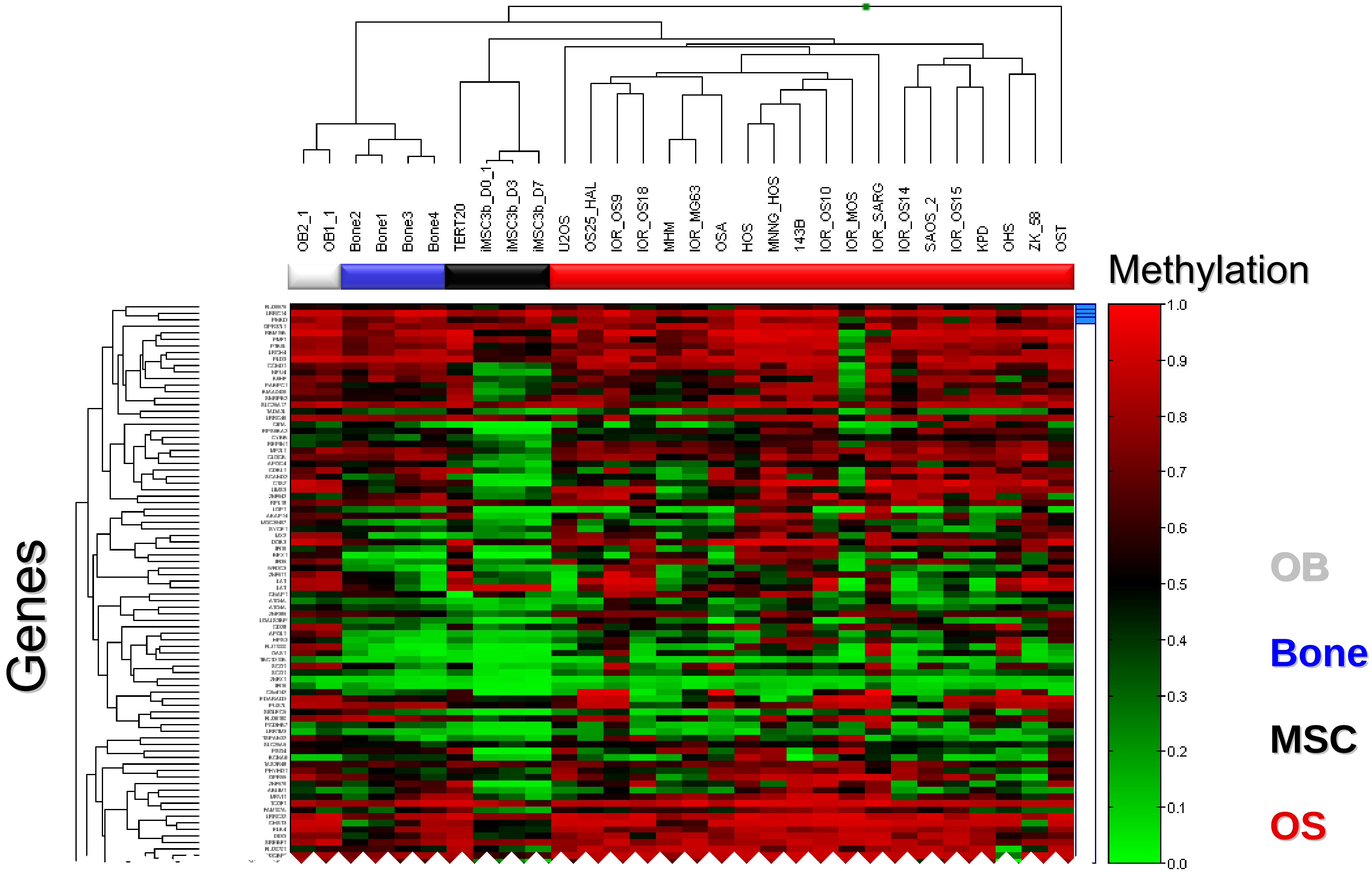
Genome-wide methylation maps

Infinium Methylation



27,578 CpG sites - 14,000 genes

Hierarchical cluster methylation



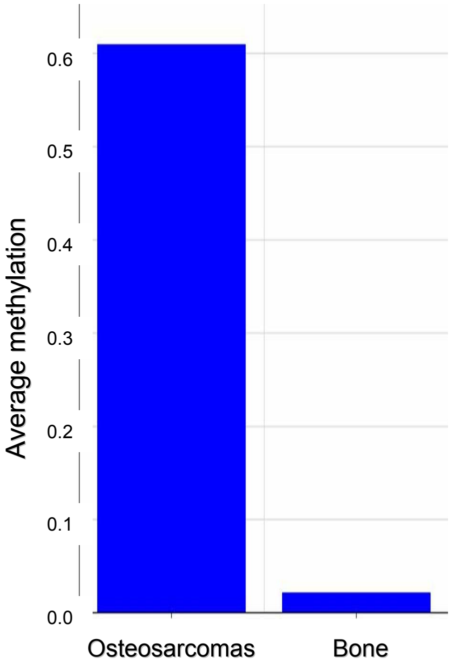
Differential methylation

Osteosarcomas vs. Bone

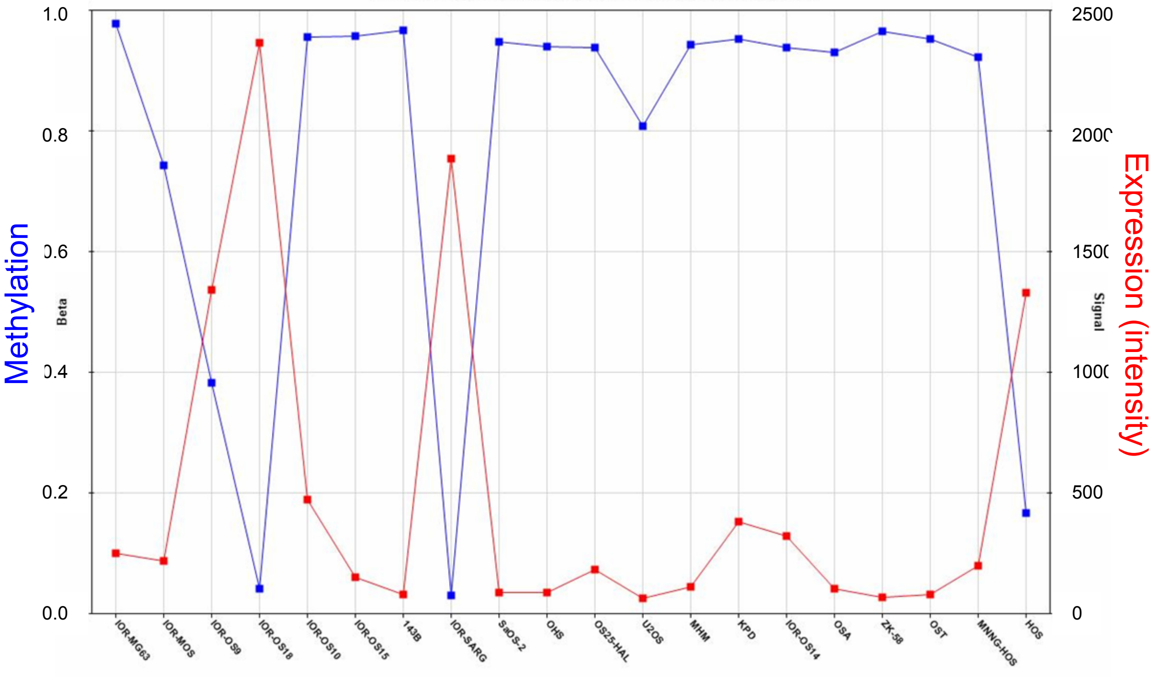
- 1,954 genes hypermethylated
- 200 genes hypomethylated

Methylation and gene expression

HSPA2 methylation

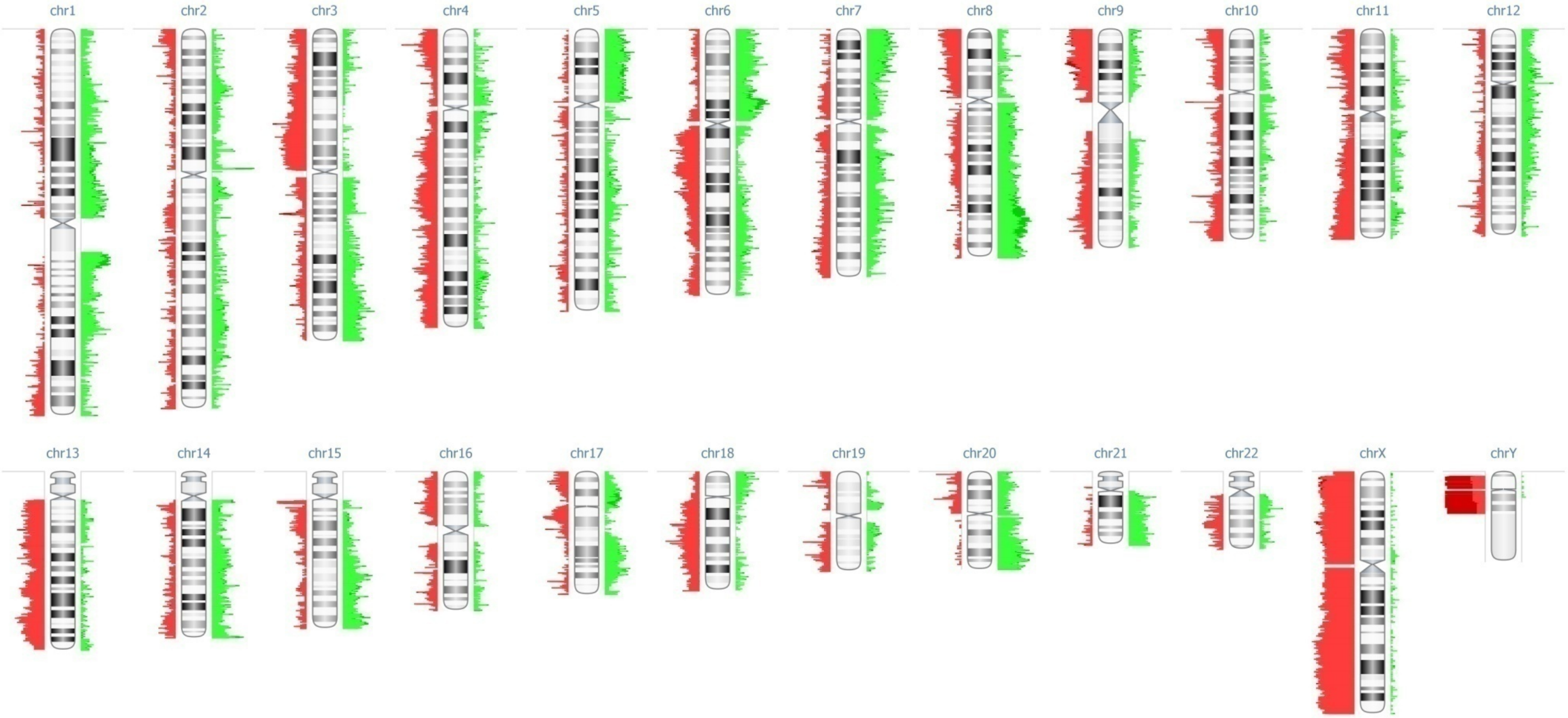


HSPA2 methylation and expression



$r^2 = 0.91$

DNA copy number changes



Gains
Losses

Stine H. Kresse

DNA copy number

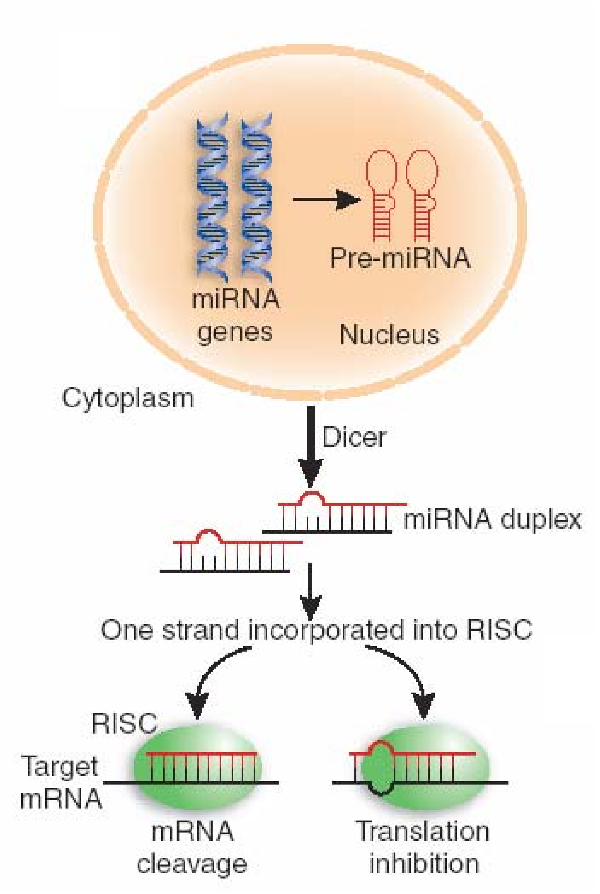
Higher number of gains than losses

Recurrent changes (≥ 35%)

- 2,881 genes increase copy number
- 2,491 genes decrease copy number

miRNAs

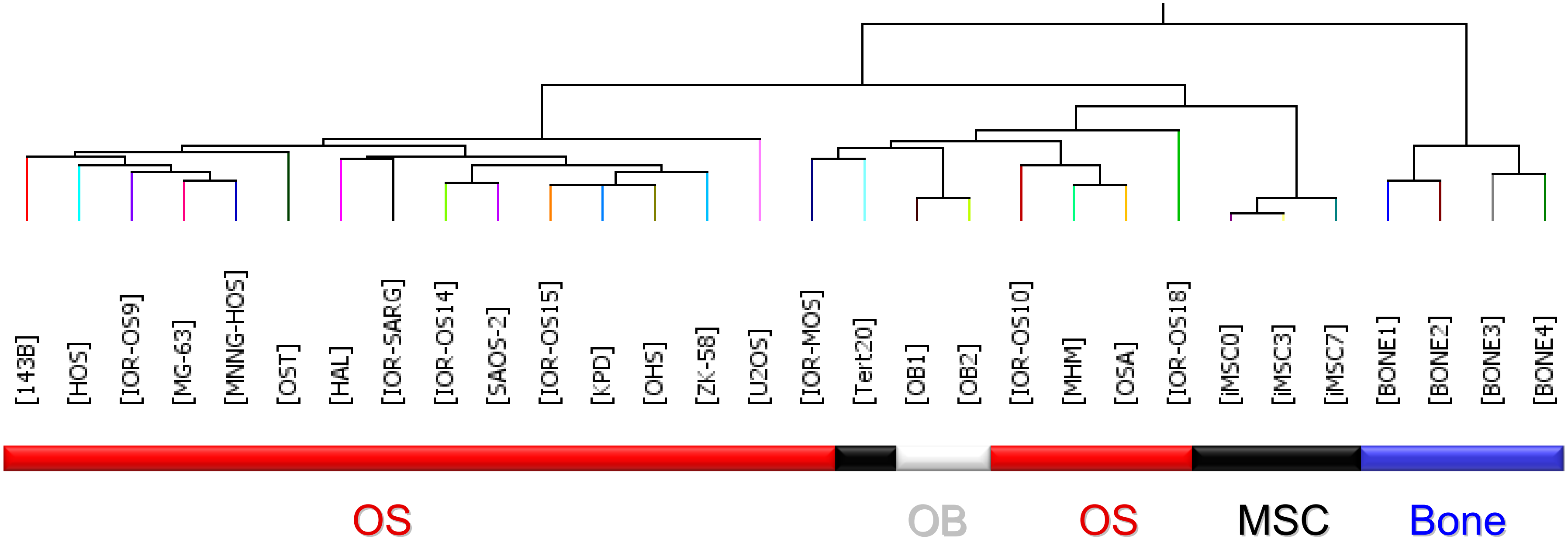
- Small non-coding RNAs
  - 18-25 nucleotides
  - Approx. 1000 human miRNAs
  - Highly conserved
  - Involved in development
- Regulate gene expression
  - mRNA degradation
  - Translational inhibition



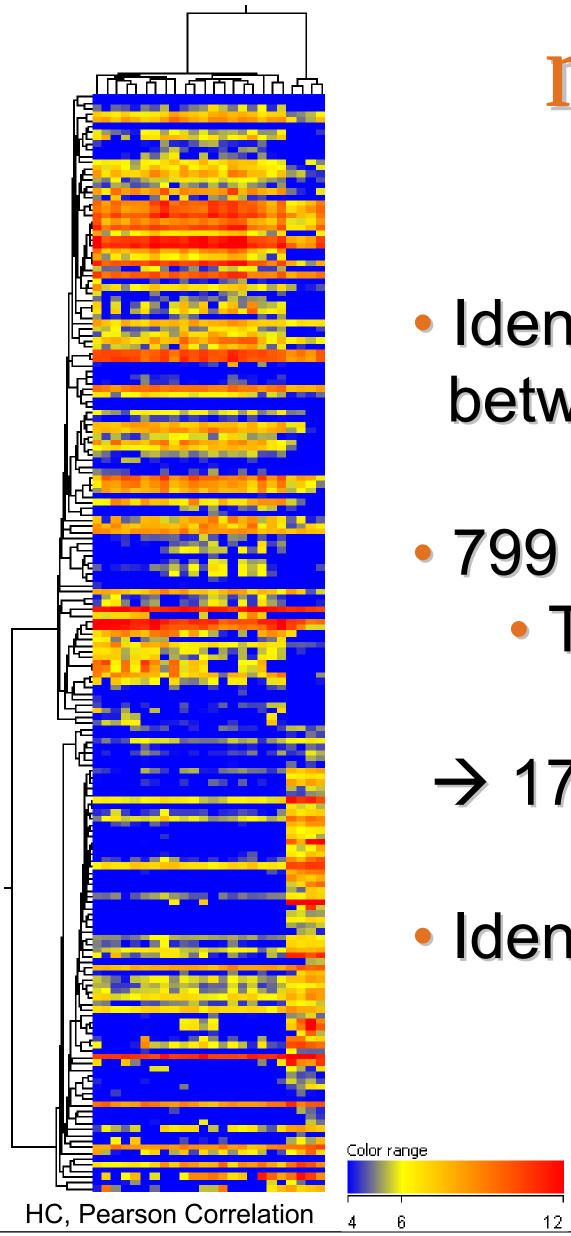
Caldas & Brenton, Nat Med, 2005

miRNA expression

- 799 miRNA



miRNAs in OS vs. bone



HC, Pearson Correlation

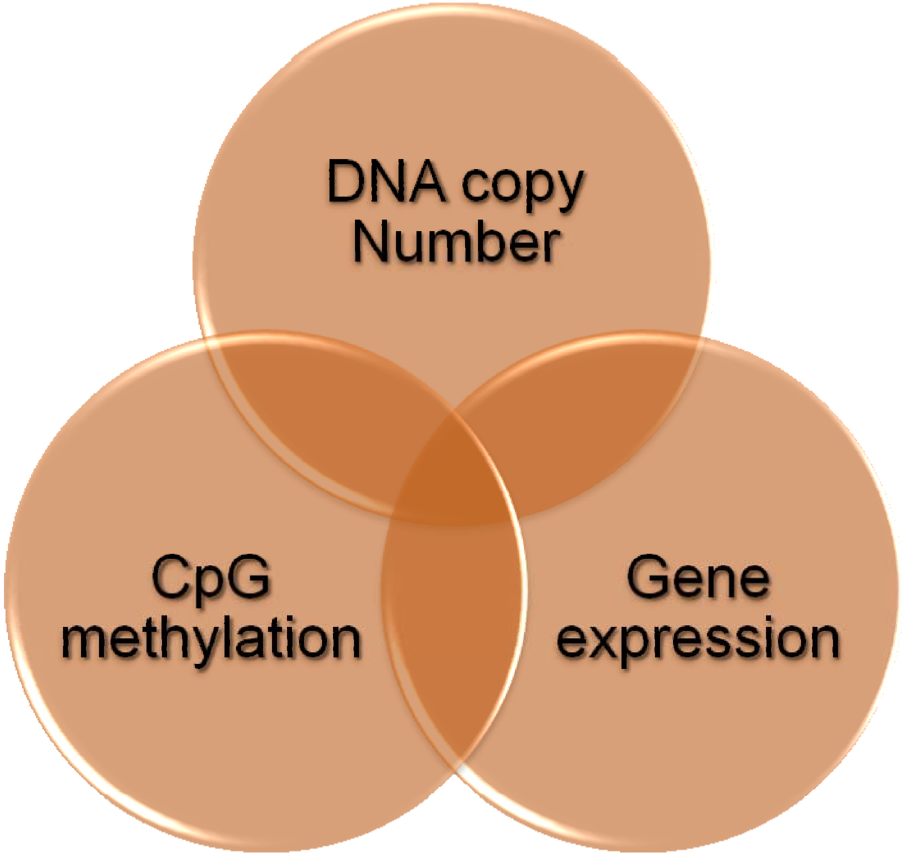
- Identify miRNAs different expressed between OS and bone

- 799 miRNAs, preprosessed and filtered
  - T-test $p > 0.05$ and $\text{FC} > 2$

→ 174 miRNAs separating OS and bone

- Identified subclusters with interesting miRNAs

Integrative approach

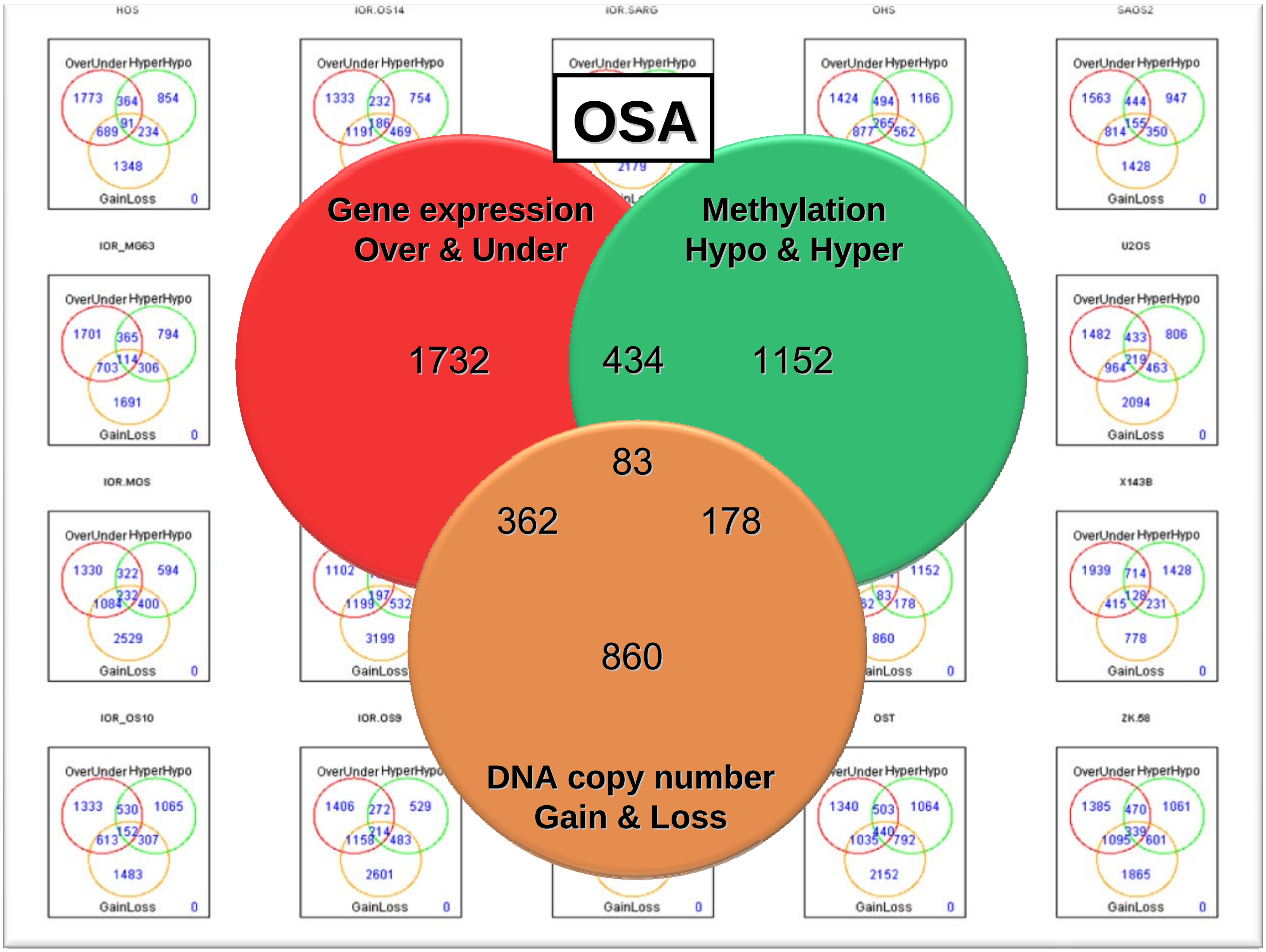


DNA copy Number

CpG methylation

Gene expression

Compared to bone



OSA

**Gene expression
Over & Under**

**Methylation
Hypo & Hyper**

**DNA copy number
Gain & Loss**

1732

434

1152

83

362

178

860

Recurrent genes accross osteosarcomas

Gene expression
Over & Under

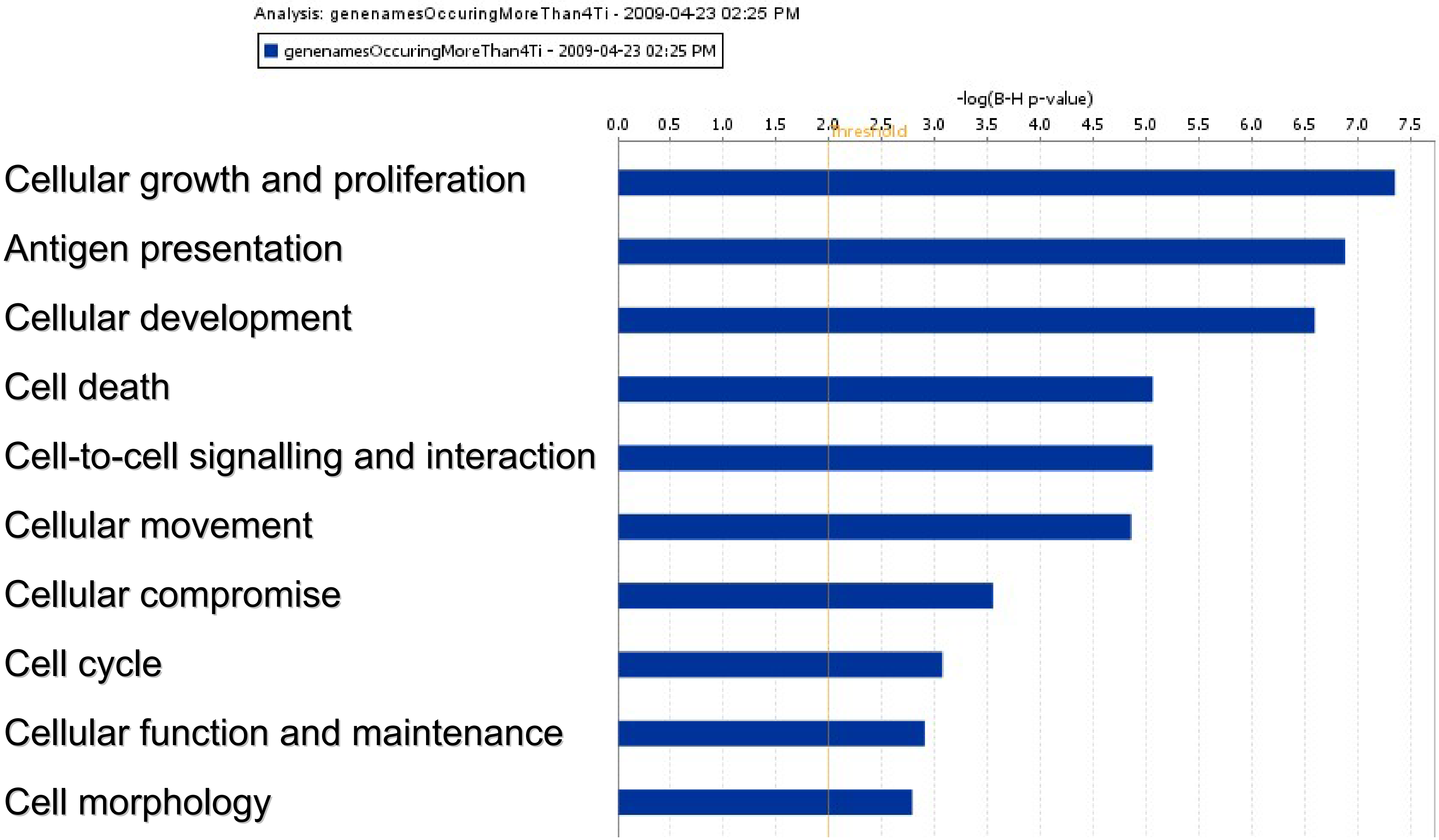
Methylation
Hypo & Hyper

336

DNA copy number
Gain & Loss

336 genes (in at least 4 cell lines)

Biological functions



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)

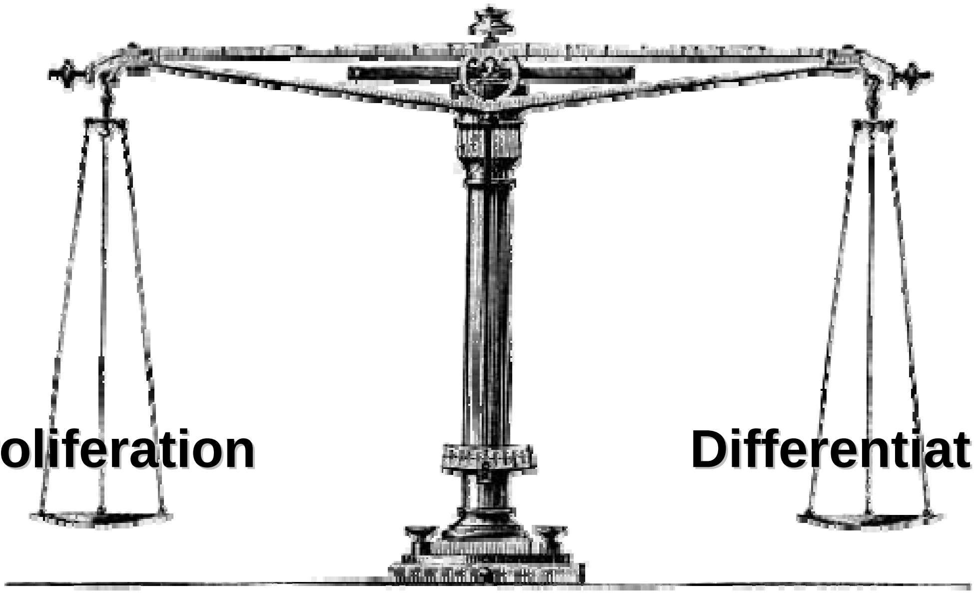
- Integrative analysis identified gene networks and pathways in osteosarcomas

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)

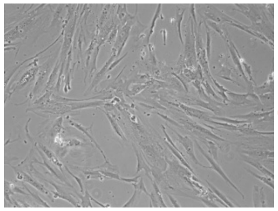
Cancer is the result of proliferation and differentiation getting out of balance

Understanding how this balance is maintained is central both in oncology and stem cell research

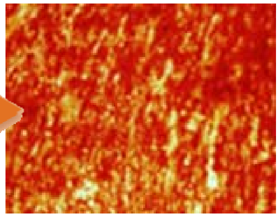


Nuclear programs of mesenchymal differentiation

Osteogenic differentiation

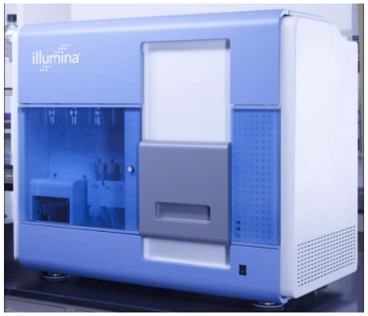


iMSC



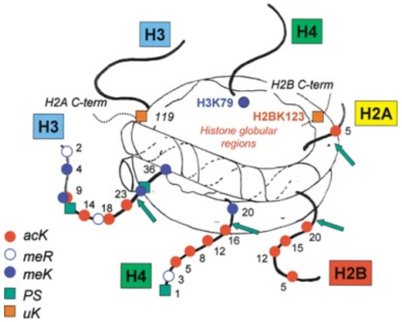
Differentiated state

Levels of information



Second Generation Sequencing

- DNA Methylation
- Chromatin remodelling (H3K4, H3K9, H3K27)
- miRNA expression
- mRNA expression



Transcriptional and regulatory networks

Dept. of Tumor Biology

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