# Integrative analysis of osteosarcoma cell lines

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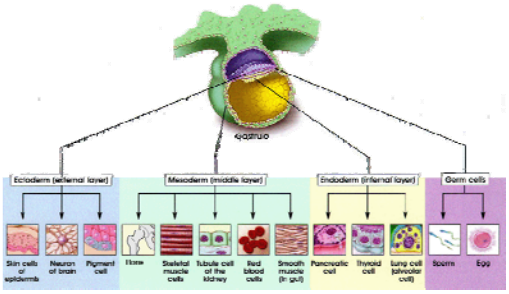
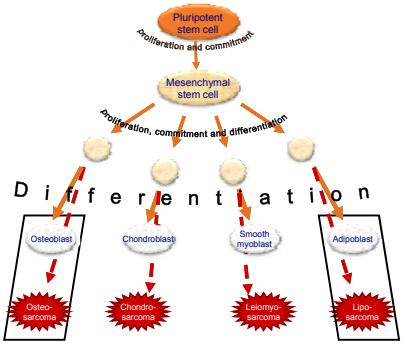
## Development and differentiation



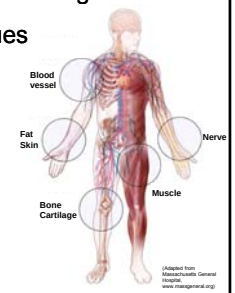

## Mesenchymal differentiation




*Adapted from a figure by Paul S Meltzer*

## Sarcomas

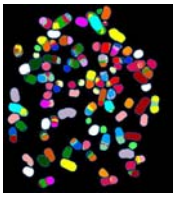
- Malignant tumours of mesenchymal origin
- Supportive and connective tissues
  - Soft tissue and bone
- ~ 1 % of human malignancies
  - ~ 10 % in children
- Model system

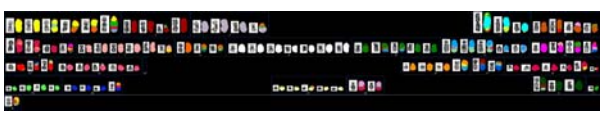



## Osteosarcomas

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

## Complex karyotype





*Karl-Ludwig Schäfer, Germany*

## Aim

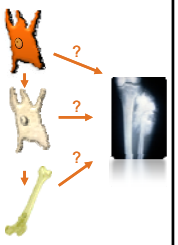
Identify transcriptional networks
in osteosarcomas and how they are regulated

Integration of different levels of genome-wide data

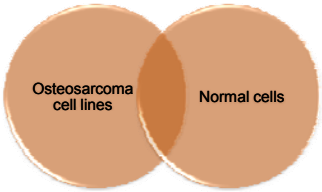
- DNA copy number
- DNA methylation
- mRNA expression
- miRNA expression

## 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

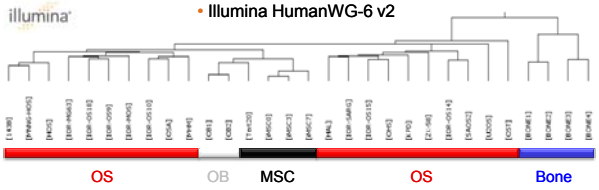
- mRNA expression
  - Illumina HumanWG-6 v2.0 (Leiden)
- DNA methylation
  - Illumina Infinium HumanMethylation27 (Oslo)

- DNA copy number
  - Affymetrix SNP6 array (Oslo)

- miRNA expression
  - Agilent miRNA array (Oslo)

## mRNA expression

- Illumina HumanWG-6 v2



OS OB MSC OS Bone

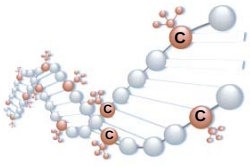
**Over and under expressed genes (log2 ratio)**

**Osteosarcoma vs. Bone**

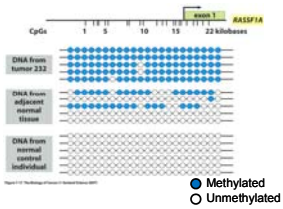
- 2,834 over expressed genes
- 1,748 under expressed genes

*Pearson Corr., Absolute average*

## CpG Island Methylation

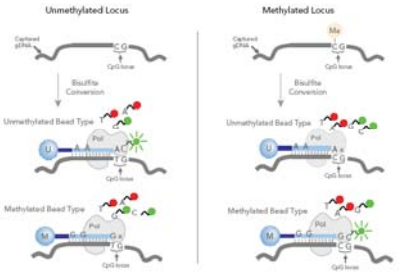



Gene silencing



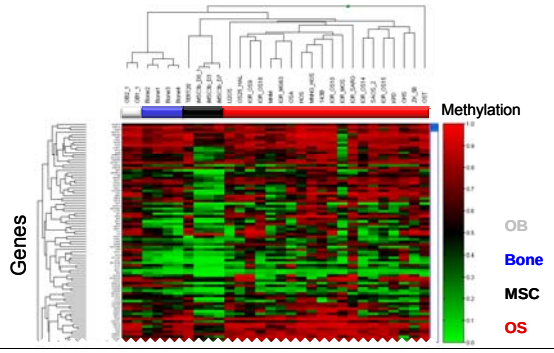

Genome-wide methylation maps

## Infinium Methylation




**27,578 CpG sites - 14,000 genes**

## Hierarchical cluster methylation



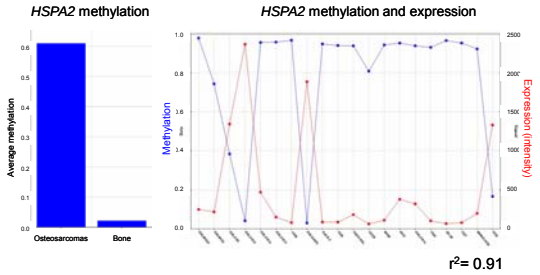

## Differential methylation

Hyper and hypo (delta beta)
Compared to Bone or Osteoblasts

Osteosarcomas vs. Bone

- 1,954 genes hypermethylated
- 200 genes hypomethylated

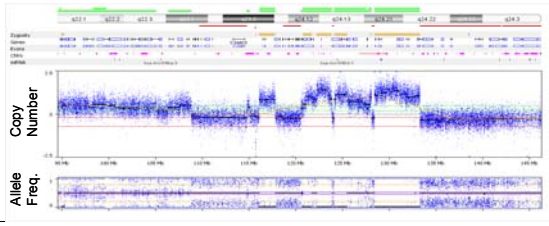
## Methylation and gene expression



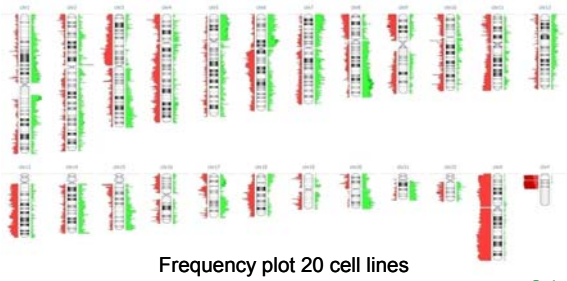

$r^2$= 0.91

## DNA copy number changes

- Affymetrix Genome Wide SNP array 6.0
  - 906k SNP probes, 946k copy number probes
  - Gain and loss (SNPRank segmentation, log2 ratio)




## DNA copy number changes



Frequency plot 20 cell lines

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

## Gene lists compared to bone

| | Expression | | Methylation | | Copy number | |
|---|---|---|---|---|---|---|
| | Over | Under | Hyper | Hypo | Gain | Loss |
| HOS | 962 | 1442 | 1243 | 309 | 1993 | 369 |
| IOR_MG63 | 921 | 1431 | 1250 | 338 | 1117 | 1697 |
| IOR.MOS | 993 | 1445 | 1380 | 175 | 2122 | 2123 |
| IOR_OS10 | 884 | 1244 | 1944 | 115 | 1491 | 1064 |
| IOR.OS14 | 971 | 1422 | 660 | 993 | 2335 | 1895 |
| IOR.OS15 | 964 | 1420 | 1427 | 504 | 2720 | 1474 |
| IOR.OS18 | 911 | 1308 | 709 | 791 | 3247 | 1880 |
| IOR.OS9 | 1023 | 1459 | 1270 | 937 | 2570 | 1886 |
| IOR.SARG | 1149 | 1647 | 2211 | 220 | 1698 | 2561 |
| KPD | 1030 | 1511 | 1268 | 476 | 862 | 2895 |
| MHM | 843 | 1282 | 888 | 522 | 1865 | 1147 |
| MNN.HOS | 1064 | 1478 | 1715 | 254 | 1553 | 1665 |
| OHS | 983 | 1517 | 1810 | 693 | 2257 | 1140 |
| OS25_HAL | 1078 | 1478 | 1187 | 351 | 2443 | 888 |
| OSA | 871 | 1245 | 1412 | 448 | 776 | 707 |
| OST | 1160 | 1596 | 2287 | 533 | 1671 | 2748 |
| SAOS2 | 975 | 1423 | 1189 | 718 | 2079 | 668 |
| U2OS | 1065 | 1517 | 1532 | 403 | 1724 | 2016 |
| X143B | 1055 | 1547 | 2384 | 130 | 1256 | 296 |
| ZK.58 | 1150 | 1591 | 1687 | 809 | 2877 | 1023 |

## Combinations

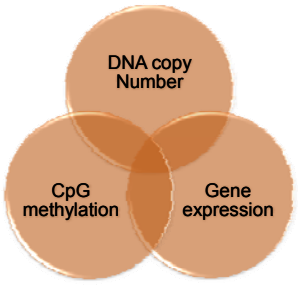
- 2-way comparison: 12 combinations

| | | |
|---|---|---|
| Gain/over | Gain/hyper | Gain/under |
| Loss/under | Gain/hypo | Loss/over |
| Hypo/over | Loss/hyper | Hypo/under |
| Hyper/under | Loss/hypo | Hyper/over |

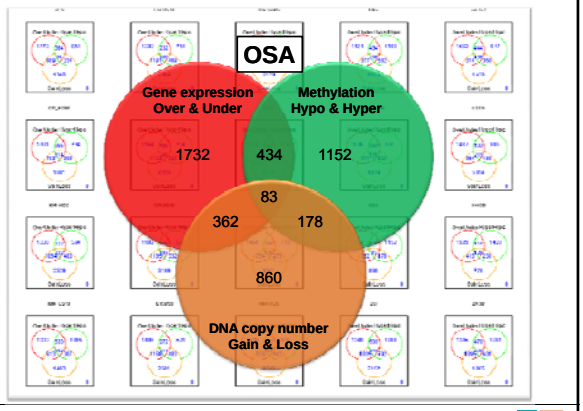
- 3-way comparison: 8 combinations

| | |
|---|---|
| Gain/hypo/over | Gain/hypo/under |
| Loss/hyper/under | Loss/hyper/over |
| Gain/hyper/over | Loss/hypo/under |
| Gain/hyper/under | Loss/hypo/over |

## 3-way comparison

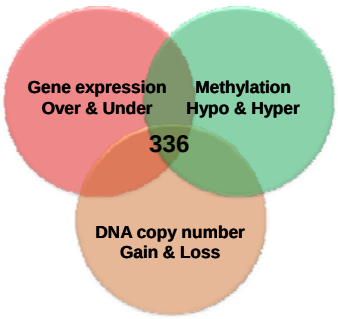



Compared to bone




*Halfdan Rydbeck*

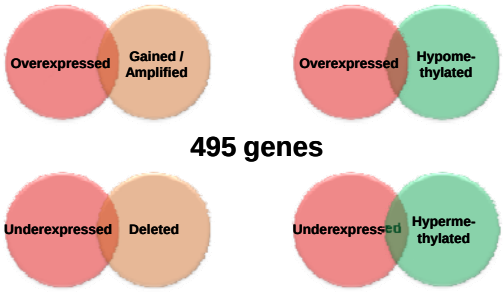
## Recurrent genes accross osteosarcomas




336 genes (in at least 4 cell lines) compared to bone

*Halfdan Rydbeck*

## Pairwise comparison (Bone)

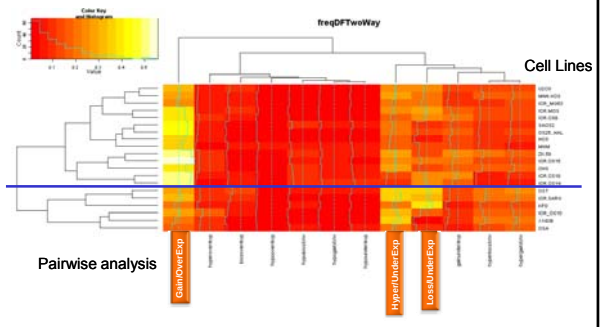



Recurrent in at least 8 of the 20 cell lines

## Recurrent genes

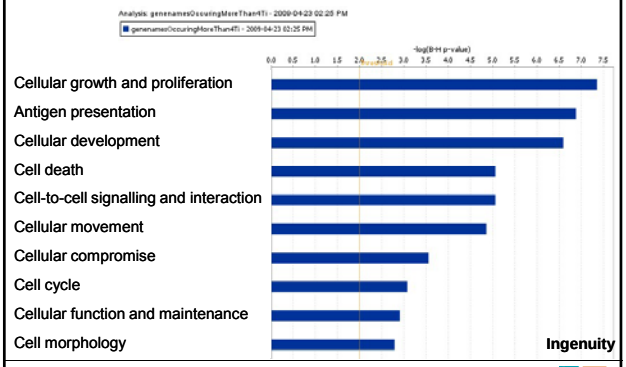
- ≥ 8 (40 %) cell lines
- Four 2-way combinations
  - Bone: 495 — Gain/over
  - Osteoblasts: 393 — Loss/under
  - Hypo/over
  - In common: 307 — Hyper/under

## Pairwise analysis

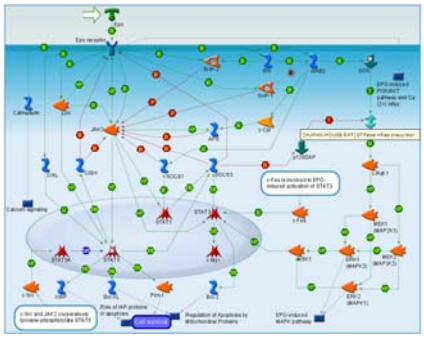


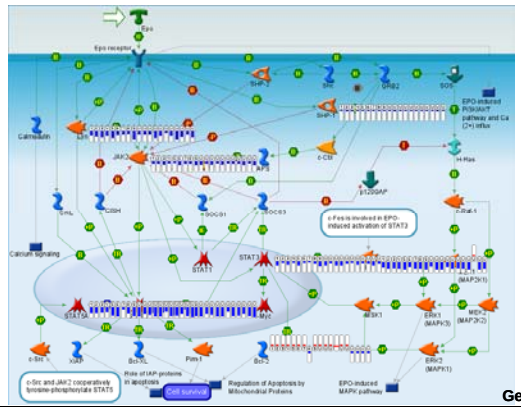

## Gene Ontology

## Biological functions

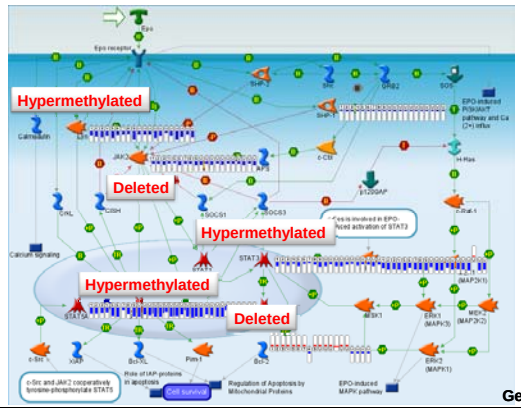



## JAK-STAT pathway



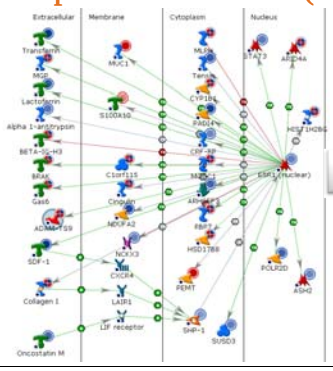


GeneGO




GeneGO

## Transcriptional network (ESR1)




GeneGO

## Summary

- Identified known and novel target genes for
  - DNA copy number changes
  - CpG island methylation (vs. Bone & osteoblasts)
  - mRNA differentially expression (vs. & osteoblasts)
  - miRNA differentially expression (vs. Bone & osteoblasts)
- Integrative analysis identified gene networks and pathways in osteosarcomas

## Further work

- Further analysis of networks and pathways
- Validate and confirm target genes and pathways in osteosarcoma clinical samples and xenografts (EuroBoNeT)
- Identify subgroups of cell lines that resemble specifc tumours subgroups (Model systems)
- Integrate osteogenic differentiation data for molecular staging

**Dept. of Tumor Biology**
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- Ola Myklebost

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