Characterisation of genes involved in development and progression of mesenchymal tumours

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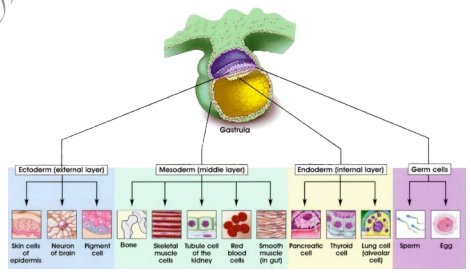
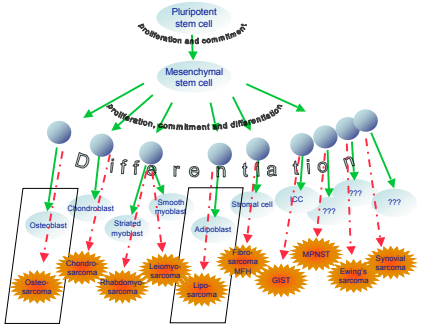
Mesenchymal developement and differentiation



Mesenchymal cell types and cancers



Adapted from a figure by Paul S Meltzer

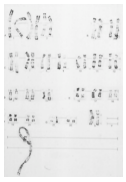
Main lines of research

- Characterisation of patient samples to identifiy recurrent aberrations that may
  - ✓ give clues about oncogenesis
  - ✓ be used for diagnosis
  - ✓ be targets for therapy
- Experimental studies of mesenchymal biology
  - ✓ Functional studies of selected proteins
  - ✓ Therapeutic experiments in human cell lines and tumours transplanted to immunodeficient mice
- Stem cell biology
- Microarray technology
  - ✓ Genomic microarrays
  - ✓ microRNA microarrays

Identification and characterisation of amplified genes

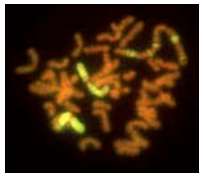
Possible targets for therapy

Well diff. liposarcomas contain markers with amplified sequences



Liposarcoma with giant marker chromosome, Probed with chromosome 1

Chromosome 12 probe gives similar pattern



Florence Pedeutour, Nice

Gene amplification

Chromosome segment

Double minutes, homogeneously staining regions

Over expression of target genes

Further re-amplification of target gene(s) and loss of irrelevant ones

Competitive genomic hybridization (CGH)

Normal metaphase chromosomes

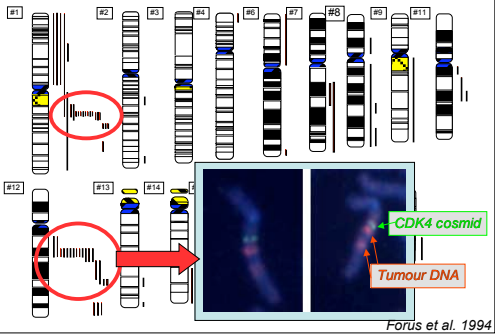
Tumor DNA + Normal DNA

Fluorescently labeled DNA

Competitive hybridization

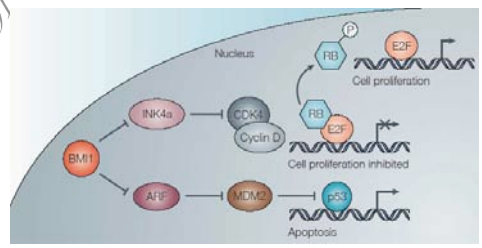
Microscopy, digital image analysis

CGH analysis of soft tissue sarcomas



Forus et al. 1994

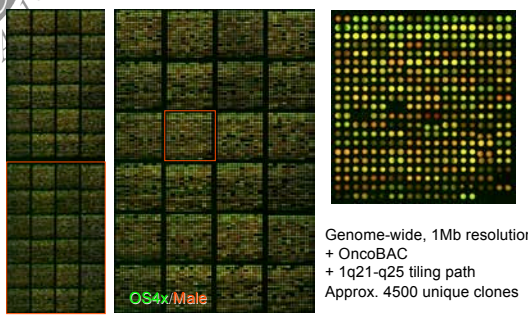
CDK4 and MDM2 pathways



Pathways deranged in sarcomas are critical in controlling growth, differentiation, and stem cell self-renewal

Pardal et al. Nature Reviews Cancer 2003

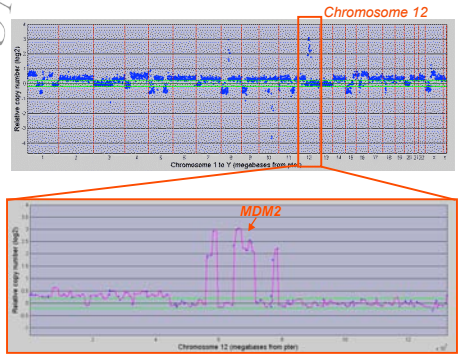
Genome-wide genomic array



Genome-wide, 1Mb resolution
+ OncoBAC
+ 1q21-q25 tiling path
Approx. 4500 unique clones

Meza-Zepeda, Kresse, Myklebost et al

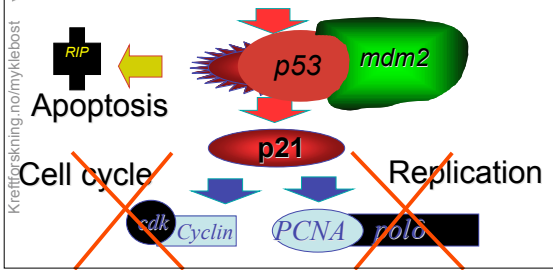
OS11x at 1Mb resolution



Meza-Zepeda, Kresse, Myklebost *et al*

mdm2 inhibits p53 and induces degradation through ubiquitination

DNA damage / ”Oncogenic activation”



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Targets for therapy

- Ampl. of MDM2 is not associated with survival
- However, this specific aberration of the p53-pathway may be a target for therapy because this pathway is central in oncogenesis and response to therapy
- Many groups search for inhibitors of the p53-mdm2 interaction, because this may stabilise p53 and give therapeutic response

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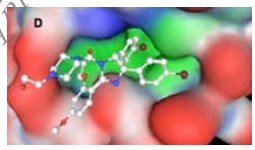
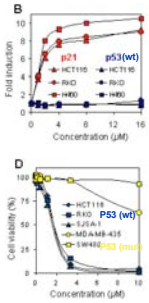
Small-molecule antagonist of MDM2





- Binds in the p53-binding pocket of mdm2
- Prevents inactivation of p53
- May activates downstream genes through release of 53
  - ✓ NB if the rest of the pathway is intact

Vassiliev et al.(Roche, Nutley NJ) Science 2004

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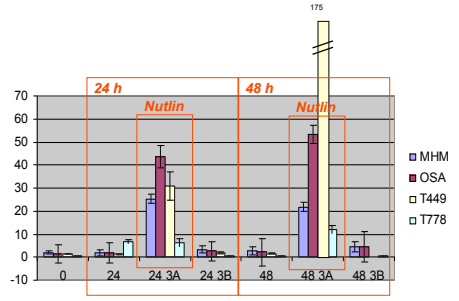
Active p53 induces p21



Activation of p53 by Nutlin treatment of four sarcoma cell lines

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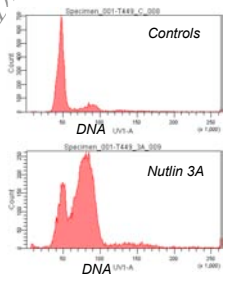
Cell cycle response



- Liposarcoma T449 with amplified MDM2
- Treatment with Nutlin induces G2 arrest

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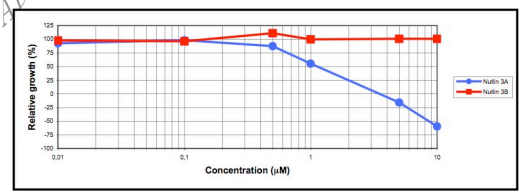
Dose response and specificity



- T778 liposarcoma cells with amplified MDM2, treated with Nutlin 3A and the enantiomer 3B

Expt by Paul Noordhuis

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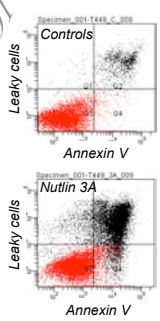
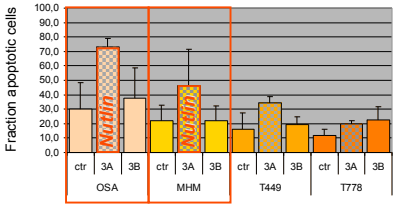
Induction of apoptosis



- Liposarcoma cells with amplified MDM2
- Treatment with Nutlin 3A induces apoptosis

Expt by Christoph Müller

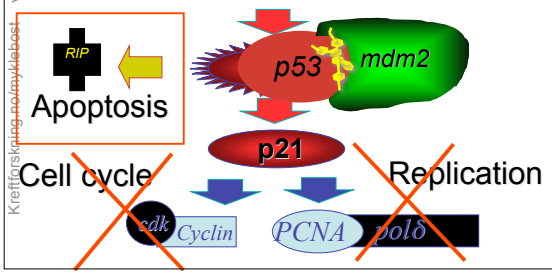
Apoptosis is induced in all of our cell lines with amplified MDM2



Two osteosarcomas (OSA, MHM) treated for 24 h with Nutlin 3A

Expt by Christoph Müller

p53 is released from mdm2 by Nutlin



Conclusions

- Nutlin activates p53 in cell lines with mdm2 amplification
- The mdm2 anatgonist nutlin selectively kills sarcoma cells depending on their mdm2 status
- Nutlin has potential as an efficient therapeutic agent for tumours with amplified MDM2
- Nutlin may become an orphan drug due to the small patient population

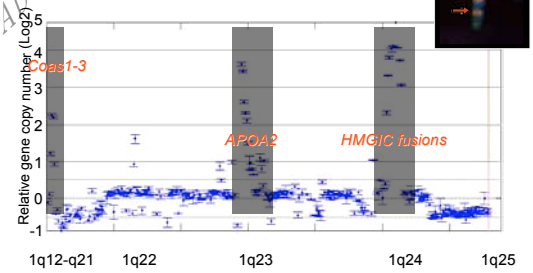
Further plans

- Currently investigating av panel of cell lines *without* amplification of MDM2
  - ✓ In most cases these only arrest, but do not go into apoptosis
    - The p53 pathway most likely is defective
    - Identifying the defects and the exceptions may give new knowledge about this pathway
- Testing combination treatment
  - We find that Nutlin can be synergistic with chemotherapy, probably by rescuing an insufficient p53 response, thus contributing to apoptosis
- Elucidate mechanisms and selection criteria for possible clinical trial

Mapping amplified regions using genomic microarrays

Example of results using a complete tiling path array covering 50 Mb of chromosome 1

1q12-q25 tiling-path array



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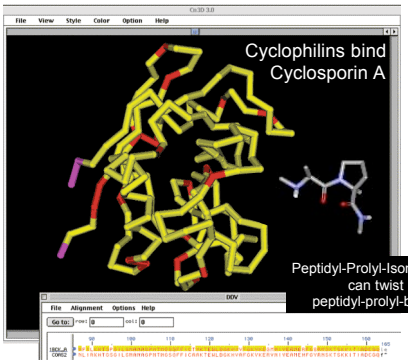
Cyclophilin A - Coas2 homology vs. structure



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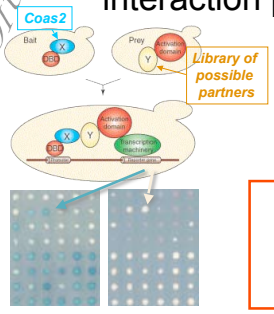
Yeast 2-hybrid screening for interaction partners



- Library from human bone marrow
- Screen for yeast colonies that contain prey that interacts with bait

- Initial screen needs validation
- 8 of 11 validated clones coded for a filamin

Birgitte Lygren and Øyvind Dahle

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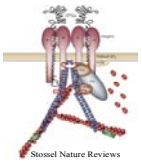
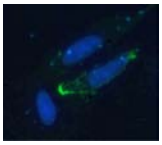
EGFP-Coas2 in osteosarcoma cells





- Coas2 interacts with filamin
  - ✓Filamin is an actin-binding protein. Associated with the cell surface attachments
- In osteosarcoma cells Coas2 is associated with the cell perimeter and ruffling edges

Expt by Magne Skårn

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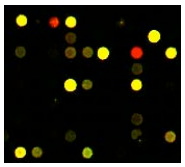
Transcriptional response to COAS2 overexpression. *Preliminary results*

- Several different cell clones
  - ✓ 2 clones with inducible expression of COAS2
  - ✓ 1 clone with constitutive expression.
  - ✓ Time course
  - ✓ 15k cDNA arrays
- The transcription factors L-SOX5 and SOX6 are found upregulated
  - ✓ -These proteins are known to regulate activation of COL2A1 in the early phase of chondrocyte development



Expts by Erik Braarød PAulsen

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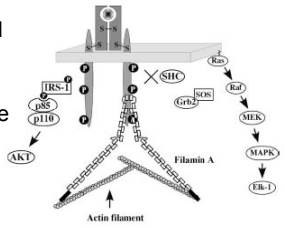
Interaction between filamin & INSR signalling

- Reported by Michel Bernier (NIH, Baltimore)
- Collaboration to see if coas2 +/- affects INSR signalling in our cell system



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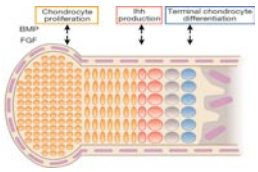
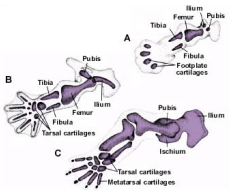
Bone development

Chondrocytes make cartilage, that is ossified by osteoblasts





HENRY M. KRONENBERG, Nature 423 , 332 - 336 (15 May 2003)

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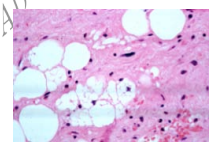
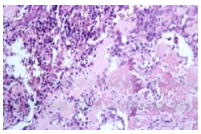
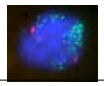
Case study







- Patient with liposarcoma
  - ✓ Primary tumour
  - ✓ Lung metastasis
- Lung metastases had
  - ✓ Osteogenic phenotype
  - ✓ Amplified YAC789f2 (COAS2)
  - ✓ Amplifiied CDK4

Forus et al.

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Developement of in vitro model systems for studies of mesenchymal oncogenesis and stem cell biology

Needed for functional studies of mesenchymal protooncogenes

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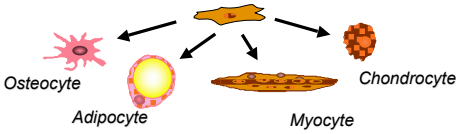
Choice of cell line is not trivial

- What cell types to use to study the normal function of these genes?
  - ✓ Ease of handling (transfection, growth, confluence level, size etc)
  - ✓ Do they respond properly to mesenchymal cues?
  - ✓ Sarcoma cell lines, - too malignant?
  - ✓ Fibroblasts, kidney cells
  - ✓ Mesenchymal stroma-derived cells (MSC)



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Bone Marrow-derived mesenchymal cells

- Characterisation of BM-MSC in culture
  - ✓Differentiation experiments
  - ✓Gene expression profiling
  - ✓Passage and growth conditions
- These cells are not convenient
  - ✓Grow slowly
  - ✓Change over time and with donor
  - ✓Senesce in culture

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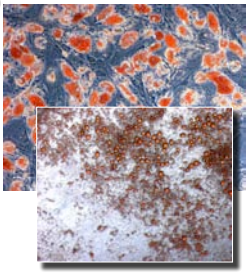
Adipocytic differentiation of bone marrow stroma cells



- MSC 21 days w. Rosglitazone
- Works also with a telomerase-transfected stroma-derived cell line
  - ✓ *Simonsen et al. 2002*

Paul Noordhuis

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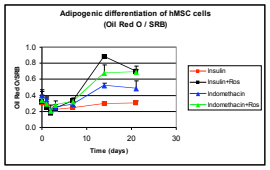
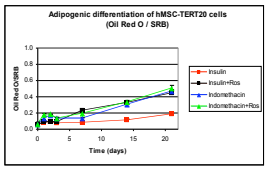
Quantitation of Adipogenesis



hMSC



hMSC-TERT20

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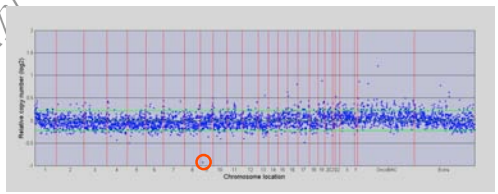
Array CGH of hMSC-TERT20



○ **= CDKN2A 7 p16 deletion**

Leonardo Meza-Zepeda & Stine Kresse

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Efficient Stem Cell-like Recipient Cell Lines

- To study the effect of candidate protooncogenes on mesenchymal stem cell biology we need efficient model systems
  - ✓Representative
  - ✓Regeneratable
  - ✓Non-cancerous
  - ✓Efficient recipients of gene constructs

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New bone marrow-derived cell lines

- Have produced telomerised bone marrow-derived ”MSC” cell line(s)
  - ✓Retroviral transduction
- Will add tet-regulatable expression
- and FRT site for efficient monogenic transfection
- May also include EGFP or Luciferase gene
  - ✓ In collaboration with Gunnar Kvalheim, Guttorm Haraldsen and Marjan Veuger

Work by Paul Noordhuis

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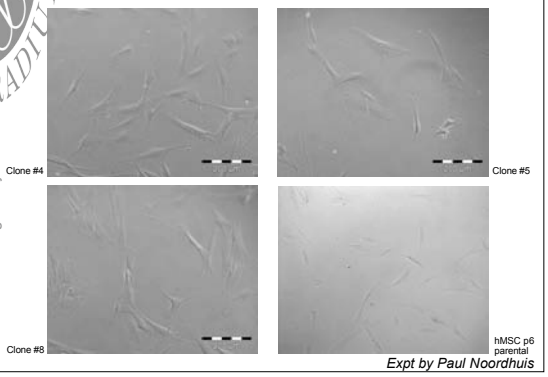
Telomerised MSC Clones



Expt by Paul Noordhuis

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Characterisation of proteins important in mesenchymal oncogenesis

2: HMGA2 (HMGIC)

High mobility group A2

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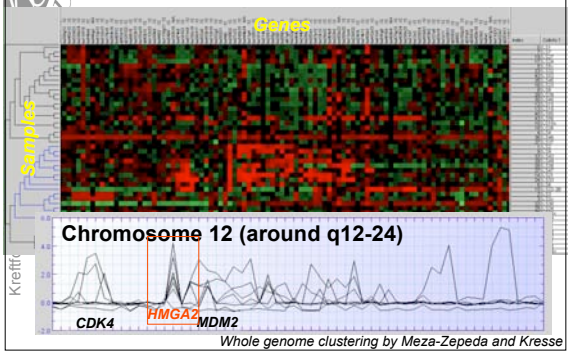
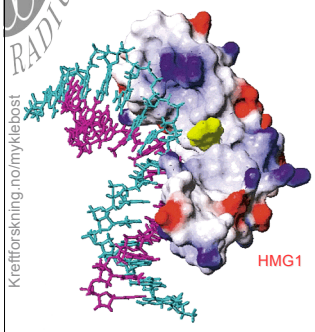
Amplification of the CDK4-MDM2 region in liposarcomas

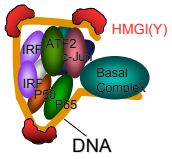


Whole genome clustering by Meza-Zepeda and Kresse

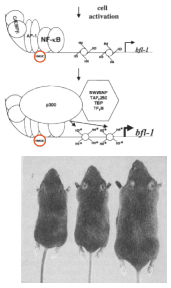
Hmgic (HmgA2) is an HMG-family architectural transcription factor



The bending of specific DNA-segments probably facilitates the recruitment of components of the transcription complex to adjacent sites



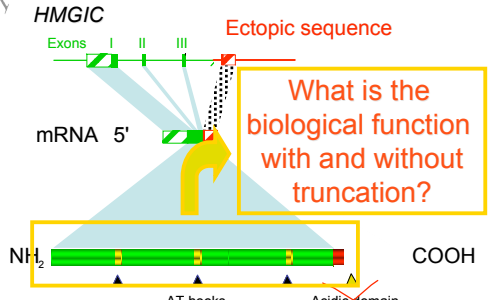
Hmgic in mesenchymal embryogenesis



- The predominant expression of hmgic is during embryogenesis
- *Pygmy* mice have mutated HMGIC
  - ✓ Reduced growth
  - ✓ Hardly any adipose tissue
- Overexpression causes lipoblastosis

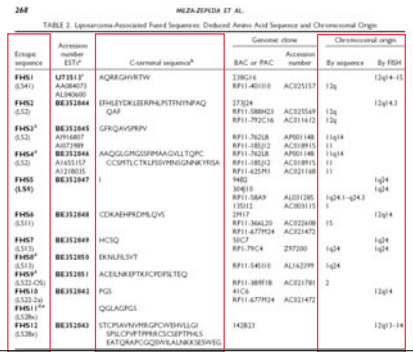
Xiang et al. 1990 Science 247:967
Zhou et.al. 1995 Nature 376:771

Amplification of HMGIC removes the 3' end



Berner et al. Oncogene 1997, Meza-Zepeda et al. Genes Chrom Cancer 2001

Characterisation of ectopic sequences

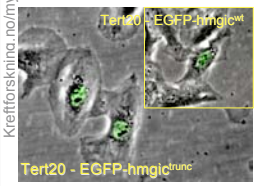


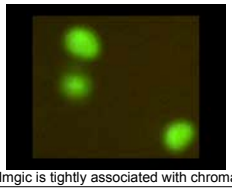
Nuclear localisation of EGFP-hmgic

- Expression of wild type and truncated hmgic in liposarcoma (LiSa) and MSC-derived (Tert20) cell lines

Jørn Henriksen

Next step is microarray analysis (in progress)





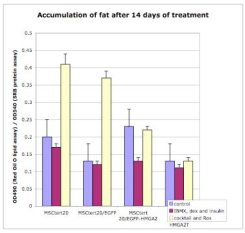
Hmgic is tightly associated with chromatin

Expression profile of $HMGA2_{wt}$ transfectants

- Transient transfection of MSC-tert20 cells
- Up: GRO1, IL-24, COX-2, POP3, BMP6
- Down: XP5, lipocalin 2, S100A9, MMP3
- NF-kappaB and HMGA1 regulate GRO1 and COX-2.
- TNF and IL-24 regulate NF-kappaB and the MMP3 promoter has a repressor-element for NF-kappaB.
- Experiments to be extended with inducible cells

Expt by Jørn Henriksen

Effect of HMGA2 on mesenchymal differentiation



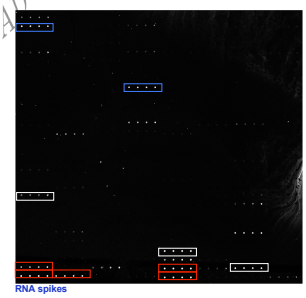
- Differentiation expt. with ectopic HMGA2

Expt by Jørn Henriksen

microRNA

- Small, about 22 b, antisense RNAs
- Can regulate many target genes by blocking of translation and degradation of mRNAs
- Each miRNA may regulate many targets
- Have developed miRNA array for expression profiling
  - ✓Sense arrays for labelling of cDNA
  - ✓Alternative: Antisense arrays for direct labelling of miRNAs

Analysis of miRNA expression in MSC-tert20 cells



Sense miRNA arrays designed and produced at DNR

miR-125b; expressed in human BC-1 cells, but no known function?

Let-7 family (red) and miR-21 (white); up-regulated during TCA-induced differentiation (Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells, Kasashima et al, 2004)

miR-21; up-regulated during RA-induced differentiation (also let-7a and miR-26b) (Human embryonic stem cells express a unique set of microRNAs, Suh et al, 2004)

miR-21 might play a general role in regulation of development and differentiation by targeting certain crucial factors

Expt by Magne Skårn

Group members