

AP-1 function in bone development and disease

Dissertation zur Erlangung des akademischen Grades
des Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von

Dipl.-Biol. Vice Mandic
geb. am 23. November 1975 in Berlin

April, 2007

1. Gutachter: Prof. Dr. Wolfgang Schuster
2. Gutachter: Prof. Dr. Petra Knaus

Disputation am 10. Juli 2007

Table of contents

Table of contents	4
Abstract	7
Zusammenfassung	8
1. Introduction	11
1. 1. Bone: a complex dynamic organ	11
1. 2. Bone development: 2 systems and 4 cell types.	11
1. 2. 1. The 2 ways of bone development.....	12
1. 2. 2. The cells	14
Chondrocytes and chondrogenesis	14
1. 2. 3. Osteoblast, osteocytes and Osteogenesis	19
1. 2. 4. Osteoclast differentiation	22
1. 3. The AP-1 transcription factor	24
1. 3. 1. Structure and regulation of AP-1	24
1. 3. 2. Biological functions of AP-1	27
1. 3. 3. AP-1 in bone cell biology	30
1. 3. 4. AP-1 in tumorigenesis	35
1. 4. Aim of the work	37
2. Material and methods	39
2. 1. Mouse strains and housing of animals	39
2. 2. Plasmids	39
2. 3. Molecular biology and biochemistry	39
2. 3. 1. Isolation of genomic mouse DNA	39
2. 3. 2. Genotyping	40
2. 3. 3. RNA isolation and cDNA synthesis.....	41
2. 3. 4. Conventional RT-PCR and Light Cycler PCR.....	42
2. 3. 5. Protein extraction and Western Blot	45
2. 3. 6. Antibodies:.....	46
2. 4. Cell biology	46
2. 4. 1. Isolation of primary mesenchymal cells from mouse calvariae and osteoblast differentiation.....	46
2. 4. 2. Isolation of primary tumour cells from osteosarcomas.....	47
2. 4. 3. Preparation of viral supernatants with Phoenix cells and viral infection.	47
2. 4. 4. FACS analysis and sorting	48
2. 4. 5. TUNEL assay	49
2. 5. Histology	49
2. 5. 1. Paraffin sections and immunofluorescence	49
2. 5. 2. Bone Histomorphometry	50
2. 6. Animal experiments.....	51
2. 6. 1. Tumor cell transplantation	51
2. 6. 2. Ovariectomy:	51
3. Results.....	52
3. 1. Analysis of c-Jun and JunD expression in bone cells and bone tumors.....	52
3. 2. c-Jun function in bone development.....	53
3. 2. 1. Conditional deletion of <i>c-jun</i> by crossing <i>c-jun</i> ^{f/f} mice with RUNX2-cre mice.....	53
3. 2. 2. Conditional deletion of <i>c-jun</i> in <i>in vitro</i> cultures of primary osteoblasts by means of infection with Cre-encoding virus	55
3. 3. c-Jun function in c-Fos induced tumour formation.....	56
3. 3. 1. Conditional deletion of <i>c-jun</i> <i>in vitro</i> in <i>c-fos</i> tg cell lines by means of viral infection, impact of c-Jun in cell growth properties	56

3. 4. JunD function in bone development.....	59
3. 4. 1. JunD function in bone development <i>in vivo</i>	59
3. 4. 2. JunD function in primary osteoblasts.....	63
3. 5. Impact of JunD in Fra-1 induced osteosclerosis	65
3. 6. Role of JunD in c-Fos induced osteosarcoma formation.....	65
3. 6. 1. Expression analysis of genes related to tumor formation, cell cycle progression and apoptosis in tumor samples from <i>c-fos</i> tg and <i>c-fos</i> tg/ <i>junD</i> KO mice.....	73
3. 6. 2. Expression analysis of genes related to vascularization and senescence	74
3. 6. 3. Expression analysis of genes related to chondrocytes, osteoblasts and osteocytes	75
3. 6. 4. Expression analysis of genes related to osteoclasts	76
3. 7. Expression analysis of osteoblast, osteoclast markers and p27 in calvaria samples from <i>junD</i> ^{-/-} mice	78
4. Discussion	80
4. 1. c-Jun function in bone development.....	80
4. 2. c-Jun function in c-Fos induced osteosarcoma formation	81
4. 3. JunD in bone development	82
4. 4. JunD function in Fra-1 induced osteosclerosis.....	84
4. 5. JunD function in c-Fos induced osteosarcoma formation.....	85
5. References	90
Acknowledgement	109