

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Use of Mass Spectrometry in Characterization of Bone Morphogenetic Proteins from Biological Samples

Genadij Razdorov and Slobodan Vukicevic

*Laboratory of Mineralized Tissues, Center for Translational and Clinical Research,
School of Medicine, University of Zagreb,
Zagreb
Croatia*

1. Introduction

1.1 Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are family of growth factors. (Chen et al., 2004; Vukicevic & Sampath, 2008) Discovered in context of bone biology, today they are recognized as important signaling molecules in wide range of biological processes, such as vertebrate embryonic development (Hogan, 1996), mesenchymal stem cell differentiation (Vukicevic & Grgurevic, 2009), kidney fibrosis, and more. For the last years BMP-2 and BMP-7 are used as therapeutics in orthopedics, harnessing their regenerative potential as growth factors.

From the onset of medicine scholars have been aware of the bone regenerative potential. In 1965. Urist was first to show that demineralized bone matrix (DBM) can induce bone growth if implanted into extraskeletal site. Active component from DBM was named *bone morphogenetic protein* by Urist & Strates (1971). Purification, cloning and sequencing of BMP was done almost 20 years later by Wozney et al. (1988). They showed that BMP is not a single protein but a family of growth factors.

From introduction of the BMP term through cloning and sequencing of individual BMPs in late 1980s, scientific output in the field has constantly grown and has exceeded 1500 papers in 2010. (Figure 1)

BMPs are part of transforming growth factor β (TGF- β) superfamily of proteins. In humans TGF- β superfamily constitutes of 37 proteins. (Figure 2) Beside BMPs, TGF- β superfamily includes TGF- β proteins, inhibins (INH), growth/differentiation factors (GDF) and few others: artemin (ARTN), glial cell line-derived neurotrophic factor (GDNF), left-right determination factor 1 (LFTY1), LFTY2, muellerian-inhibiting factor (MIS), nodal homolog (NODAL), neurturin (NRTN) and persephin (PSPN).

BMPs are functionally and structurally very conserved throughout animal kingdom. Their biological importance is reflected through functional and structural redundancy of different BMPs in single species. BMPs are translated as pre-propeptides. Signal peptide targets them for secretion out of cell. Prodomain is two thirds to four fifths of total peptide length and

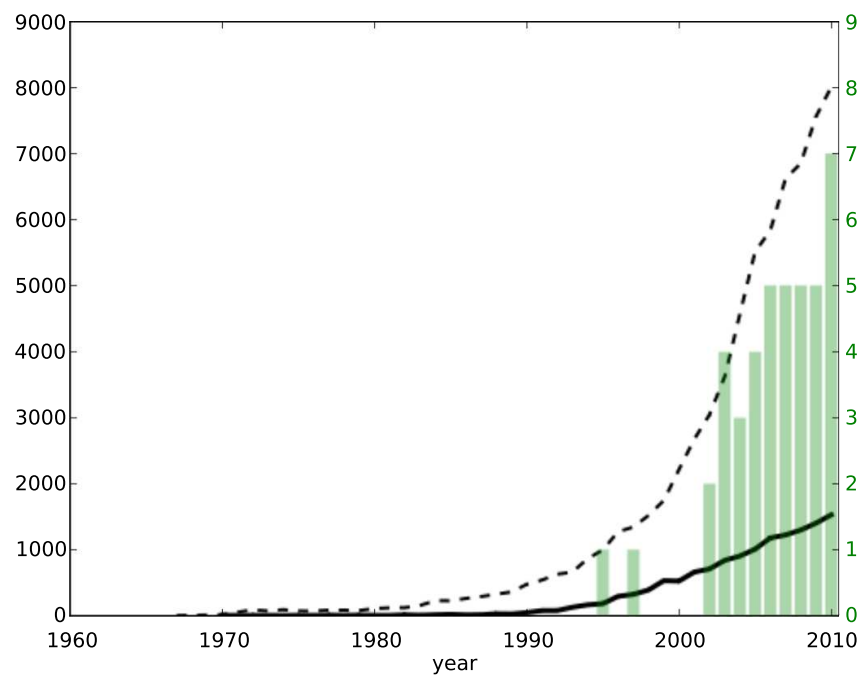


Fig. 1. Scientific output on *bone morphogenetic protein* (solid black line), (*mass spectrometry*) *protein OR proteomics* (dashed black line) and (*mass spectrometry*) *AND bone morphogenetic protein* (green bars). Data gathered from Pubmed on June 2011.

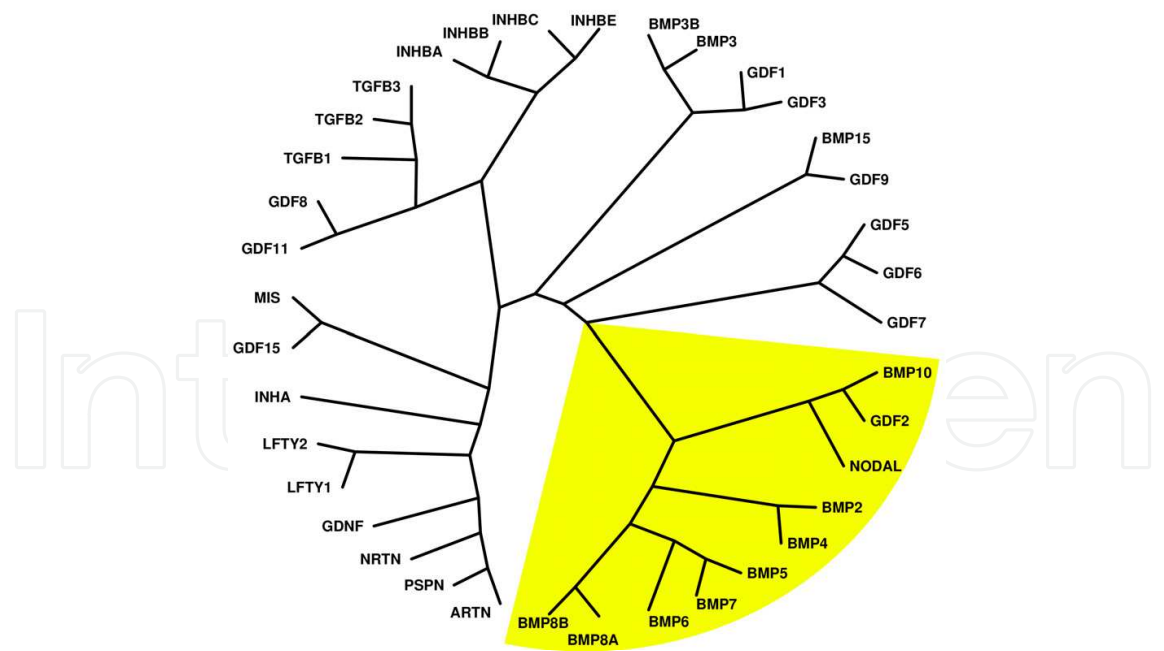


Fig. 2. Transforming growth factor β protein superfamily. Amino acid sequences were aligned by ClustalW and plotted by Cytoscape with PhyloTree plugin.

is cleaved off to produce mature peptide chain. Mature domain is highly conserved and has number of shared features. (Figure 3) There are seven conserved cysteines starting from position 446 (by unified position numbering displayed above aligned sequences), and then on

positions 475, 479, 511, 512, 545 and 547. Counting from first conserved cysteine there are 30 completely conserved positions which is almost one third length of mature domain.

From purification and sequencing studies done in late 1980s, it was clear that the active form of a BMP is a homodimer made up of two mature domains connected by disulphide bridge. The first BMP crystal structure was resolved by Griffith et al. (1996). Until present there are more then 30 structures related to BMPs deposited into Protein Data Bank.

BMP mature domain is folded in the hand like structure. (Figure 4) Two fingers, made up of two beta sheets, are protruding from the cystine knot, which is made up of three disulphide bridges. On the opposite side of the cystine knot is alpha helix forming the heel of the hand. Active homodimer is made up of two hand structures pointing in the opposing directions palms facing toward each other.

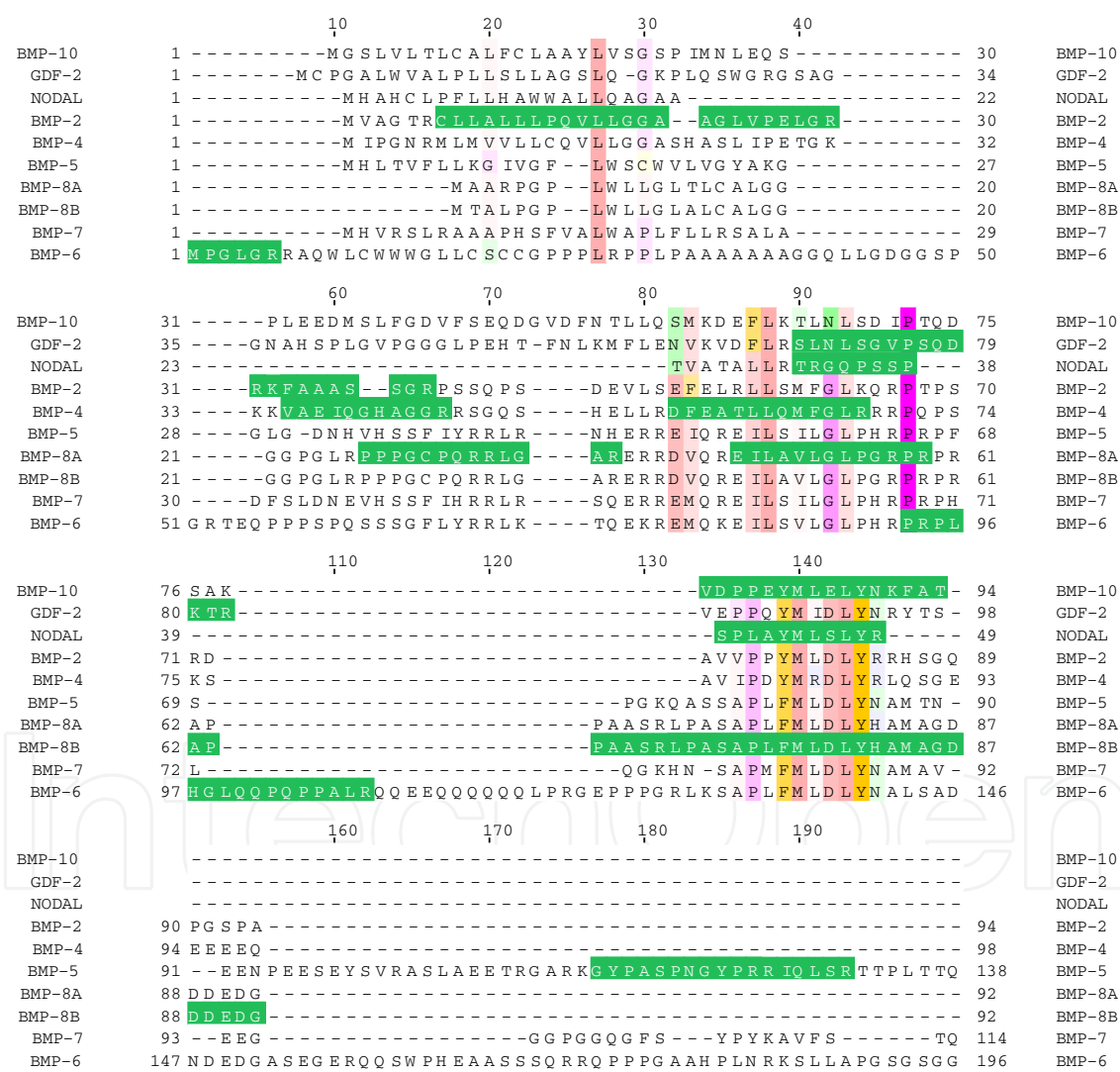


Fig. 3. BMPs sequence alignment. Ten most conserved BMPs aligned by ClustalW algorithm with most conserved residues color coded by their physicochemical properties (Zappo color scheme). Green sequence features are MS identified peptides deposited in PRIDE proteomics database.

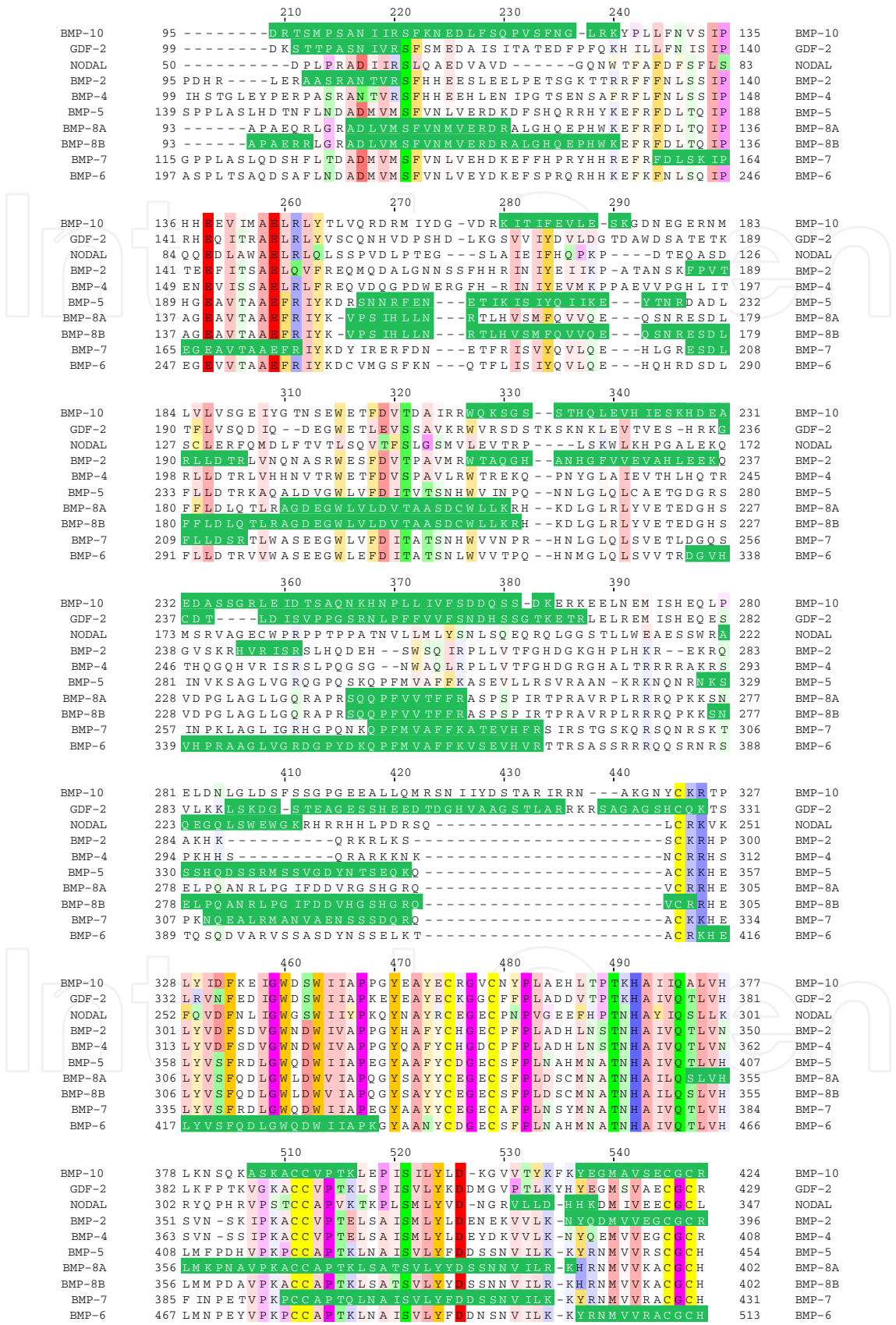


Fig. 3. ...continued.

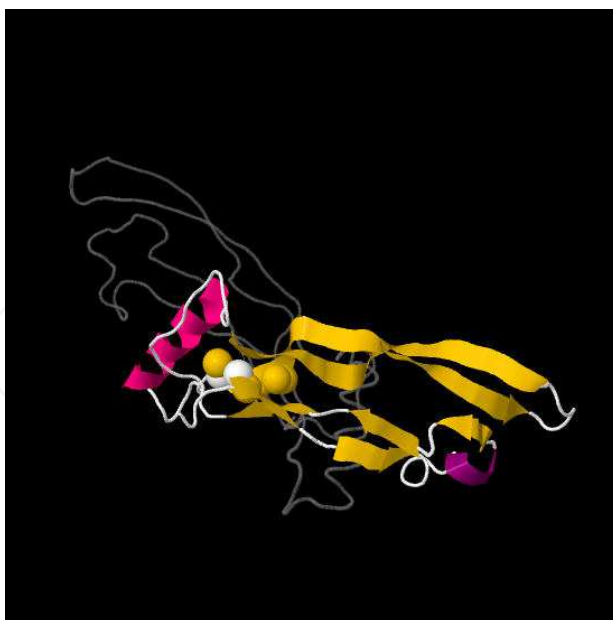


Fig. 4. Crystal structure of the mature homodimer BMP6. (Saremba et al., 2008) One monomer is cartoon rendered with sulfur atoms of cystine knot spacefill rendered. The structure with 2R52 accession number was downloaded from Protein Data Bank, and rendered by RasMol.

1.2 Mass spectrometry of proteins

Mass spectrometry (MS) of proteins exploded with the discovery of soft ionization techniques in late 1980s. Fenn et al. (1989) produced one of the first mass spectra of different proteins using electrospray ionization (ESI). They also introduced an algorithm for deconvolution of multiple charged envelope in the mass spectra of proteins. Hillenkamp & Karas (1990) did a similar thing with the matrix assisted laser dissociation ionization (MALDI).

Identification of proteins by peptide mass fingerprint (PMF) was introduced by Henzel et al. (1993). Proteins, separated by two dimensional gel electrophoresis (2DE), are in gel protease digested (usually by trypsin). Peptide masses are then measured by mass spectrometer. The measured peptide mass fingerprint is used for searching the protein sequence database by automated computer algorithm. The first large scale analysis using PMF was done by Shevchenko et al. (1996). They identified 128 different yeast genes from 150 2D gel spots. This scientific approach became *proteomics* after term *proteome* coined by Kahn (1995).

Protein identification is now done preferentially using tandem mass spectrometry. To avoid errors and to speed up process, algorithms for the protein database searching using tandem mass spectra of peptides had to be invented. (Eng et al., 1994; Perkins et al., 1999)

Except for the identification of proteins, mass spectrometry based quantification became reality with development of isotope-coded affinity tag (ICAT) strategy. (Gygi et al., 1999) After introduction, there have been devised different stable isotope labeling quantification strategies. Ong et al. (2002) developed stable isotope labeling by amino acids in cell culture (SILAC).

At last, proteomics community realized the importance of high mass resolving power, high mass accuracy, scanning speed and dynamic range as crucial parameters on which number and quality of the protein identifications depends. Mann & Kelleher (2008)

2. Bone morphogenetic proteins by mass spectrometry

Before 2000 there were only two papers published reporting on separation of the glycoforms of rhBMP-2 by capillary electrophoresis coupled to MS. (Yeung et al., 1997; Yim et al., 1995) From 2000 onward, there is a continuum of scientific research on the BMPs using a mass spectrometry. There are two main approaches to the research in the field: (a) isolation and identification of a BMP or related molecule from different biological sources; and (b) proteomic study of a system perturbed using a BMP or related molecule. We have reviewed papers based on their biological focus.

2.1 Bone and cartilage

BMPs have been discovered and studied first as bone and cartilage growth factors. After development and growth, bones are constantly remodeled to meet their body function. Resorption of the bone is done by osteoclasts and formation by osteoblasts. Therapeutic regeneration and skeletal tissue engineering depends on three elements: extracellular matrix, cells and growth factors. (Reddi, 1998)

Role of BMP-1-3 in bone fracture repair has recently been studied in our group. (Grgurevic et al., 2011) We have shown *in vivo* enhancement of bone healing by systemic and local administration of BMP-1-3. We used rat fractured femur and critical size defect of rabbit ulnae models and demonstrated a synergistic effect of BMP-1-3 and BMP-7 on bone regeneration. BMP-1-3 stimulates osteoblast differentiation *in vitro*. By combining isolation of growth factors with proteomic global approach we have identified potential biomarkers of acute bone fracture from plasma of 25 patients. (Grgurevic et al., 2007) To overcome inherent limitation of proteomics to cope with plasma protein dynamic range of more than 10 orders of magnitude, we have devised a growth factors enrichment strategy. Members of TGF- β protein superfamily have characteristic heparin binding domain as part of their structure, and can be affinity enriched using heparin chromatography. We enriched growth factors from 80 mL of pulled plasma, and then separated them on minigel electrophoresis. To overcome loading capacity of analytical gels, proteins were loaded into consecutive wells, and afterwards combined in 12 fractions by electrophoretic mobility. After in-gel digestion, peptides were separated and measured by on-line nano LC coupled to Orbitrap working in data dependent scanning mode. Top five MS signals were isolated and fragmented by CID in linear IT. Global proteomic approach allowed us to identify 213 proteins, of which we have singled out 12 bone and cartilage related proteins. Four of them have never before been found in the circulation: Transforming growth factor β -induced protein ig-h3 (β ig-h3), cartilage acidic protein 1 (CRTAC1), procollagen C proteinase enhancer protein (PCPE-1) and TGF- β receptor type 3 (TGF- β -R-3). Nakasaki et al. (2008) have identified insulin-like growth factor I (IGF-I) as potent chemotactic factor for osteoblasts and showed IGF-I involvement in fracture healing. They used five step sequential chromatography to purify IGF-I from 18 L of MC3T3-E1 (mouse osteoblast-like cell line) serum free conditioned media (CM). After precipitation of CM with 80% saturated ammonium sulphate, they used gelatine-, Blue-, heparin-affinity chromatography, hydroxyapatite chromatography and gel filtration to purify IGF-I more than 200 fold. IGF-I was separated by silver stained SDS-PAGE, in gel digested, and measured by micro LC coupled to 3DIT working in data dependant mode.

Kim et al. (2009a) have studied transdifferentiation of murine premyoblast C2C12 cell line induced by BMP-2. They invented the Two-Stage Double-Technique Hybrid (TSDTH) proteomic strategy, for the analysis of early phosphoproteome and late proteome changes.

Authors have enriched more than 150 µg of phosphoproteins from 2 mg of the total protein cell lysate using commercial kit. Using a phosphoprotein enrichment, as opposed to a phosphopeptide enrichment, one should get a better sequence coverage of the individual phosphorylated proteins. Phosphoproteins were separated by SDS-PAGE, in gel digested, and analysed by micro LC coupled to linear IT. They measured MS3 fragmentation spectra of phosphopeptides showing dominant neutral loss of phospho moiety (minus 98 Th/49 Th/32.7 Th for singly, doubly or triply charged species respectively) using data dependant mode. For the proteome quantification, authors metabolically labelled all cell proteins using SILAC approach. After combining samples, they separated proteins using SDS-PAGE, in gel digested them, and analysed them by micro LC coupled to LTQ-FT working in data dependent mode. Top five signals from high resolution survey scan were measured individually using single ion monitoring (SIM) scan in FT-ICR analyser, and then CID fragmented in linear IT. From 1321 phosphoproteins identified and 433 proteins quantified authors selected 374 BMP-2 specific phosphoproteins and 54 differentially expressed proteins. By pathway enrichment analysis authors found IGF and calcium signaling pathways as well as TGF- β /BMP signaling proteins to be potentially involved in the early and long-term actions of BMP-2.

At present, BMP-2 and BMP-7 are the only BMPs commercially used for regenerative purposes. We have identified lysine 60 of BMP-6 as amino acid crucial for BMP-6 lower susceptibility to noggin inhibition in comparison to BMP-7. (Song et al., 2010) This is important because of the present therapeutic use of BMP-7 in large doses. In BMP-6, lysine 60 forms intramolecular hydrogen bonds with asparagine 65 connecting finger 1 and 2 in that way that it increases overall rigidity of the molecule.

Osteoporosis is one of the most prevalent bone disease. (Raisz, 2005) We have studied effects of systemically administered BMP-6 to osteoporotic rats. (Simic et al., 2006) We could not demonstrate the presence of BMPs in bone extract of ovariectomized rats. We next demonstrated that systemically administered recombinant human mature BMP-6 accumulates in the skeleton. BMP-6, systemically applied, restores the bone inductive capacity, microarchitecture, and quality of the skeleton in osteoporotic rats. Next, using gene expression profiling, we found that BMP-6 exerts its osteoinductive effect at least in part through the IGF-I and epidermal growth factor (EGF) pathways. (Grasser et al., 2007) Hong et al. (2010) have studied impairment of osteoblasts differentiation in context of the glucocorticoid-induced osteoporosis. They treated MC3T3-E1 cells with high dose of dexamethasone, which inhibited cell differentiation and proliferation and induced apoptosis. Authors used SILAC labelling with heavy/light lysine, SDS-PAGE protein separation and nano LC coupled to LTQ-Orbitrap proteomic approach. They found upregulation of tubulins (TUBA-1A, TUBB-2B, and TUBB-5), Ras GTPase-activating-like protein 1 (IQGAP1), S100 proteins (S100-A11, S100-A6, S100-A4, and S100-A10), myosins (MYH-9 and MYH-11), and apoptosis and stress proteins (BAX), and downregulation of ATP synthases (ATP5O, ATP5H, ATP5A1, and ATP5F1), G3BP-1, and Ras-related proteins (RAB-1A, RAB-2A, and RAB-7).

Hong et al. (2010a) have studied early osteoblast differentiation. They induced differentiation of mouse osteoprogenitor MC3T3-E1 cells by rhBMP-2 and have studied cells in an early differentiation stage critical for transformation of premature osteoblasts to mature osteoblasts. At that stage osteoblasts express ALP but do not form mineralized nodules yet. By label-free quantitative proteomic approach they identified numerous differentially expressed proteins important for actin skeleton regulation and/or focal adhesion. They used SDS-PAGE proteins

separation, and nano LC coupled to LTQ-Orbitrap peptide analysis. By western blot they have confirmed upregulation of IQGAP1, isoform 1 of gelsolin (GSN), moesin, radixin, and cofilin 1 (CFL-1), and upregulation of focal adhesion proteins FLN-A, LAMA1, LAMA5, COL1A1, COL3A1, COL4A6, and COL5A2, as well as the downregulation of COL4A1, COL4A2, and COL4A4.

Park et al. (2009) have studied heparin-binding growth factor 2 (HBGF-2) effect on osteoblasts. Authors used 2DE/MALDI-TOF proteomic approach. They identified asparaginyl-tRNA synthetase (NARS), eukaryotic peptide chain release factor subunit 1 (ETF1), GDP-forming succinyl-CoA synthetase (SUCLG2), heat shock protein 84 (HSP 84), sorting nexin 9 (SNX9) and neutral α -glucosidase AB (GANAB) to be upregulated, and tropomyosin 2 to be downregulated. NARS, member of aminoacyl-tRNA synthetases (AARS), was upregulated more than 900 fold. NARS upregulation increased the cell proliferation. NARS downregulation suppressed cell proliferation and induced cell apoptosis. Also downregulation of NARS increased serum deprivation induced apoptosis. Reduction of NARS also reduced p-Akt activity and increased caspase-3. Authors shown that HBGF-2 induced NARS promotes osteoblast survival through PI3K/Akt pathway.

Kodaira et al. (2006) have purified BMP-like factor from fetal bovine serum (FBS), which they found to both inhibits myogenesis and stimulates differentiation of osteoblasts. They started with 5 L of FBS, which they separated by heparin, strong anion exchange (SAX), heparin again, strong cation exchange (SCX) and reverse phase chromatography. Using LC-MS/MS authors identified this factor as BMP4, and found it to be in over 100 kDa complex in circulation. Behnam et al. (2006) identified dermatopontin (DPT) as the most prominent BMP-2 co-purified demineralized bone matrix protein, using alkaline urea extraction of BM, separation of insoluble fraction by hydroxyapatite chromatography, SDS-PAGE of BMP active fraction and MALDI-TOF analysis. Kubota et al. (2002) have studied signal transduction from osteoclasts to osteoblasts. They have isolated and identified osteoblastogenesis inhibitory factor (OBIF) from RANKL treated RAW264.7 cell line conditioned medium. This OBIF inhibited osteoblastogenesis of MC3T3-E1 cell line, induced by BMP-4. After three step chromatography (heparin affinity, anion exchange and reverse phase chromatography) from 1.8 L of conditioned medium, they have managed to identify OBIF as platelet-derived growth factor BB (PDGF BB) homo-dimer using nano LC coupled to LCQ ion trap, working in data dependant mode..

2.1.1 Chondrogenesis

Ji et al. (2010) have studied mesenchymal stem cells chondrogenesis. They induced chondrogenic differentiation of C3H10T1/2 cells, murine embryonic mesenchymal cell line, by treatment with BMP-2. They validated chondrogenesis by following glycosaminoglycan and collagen type II. By isobaric tags for relative and absolute quantitation (iTRAQ) labeling and 2D nano LC-MS/MS (QSTAR XL) analysis they identified 100 differentially expressed proteins out of 1753 identified and quantified proteins. 83 proteins were downregulated, and 17 upregulated. This is in concordance with “stem state” concept, which assumes wider set of proteins expressed in cells which are less differentiated. Collagen types II and XI are upregulated, as are PAPSS 2 and LOX, enzymes involved in posttraslational modification of chondrocytes extracellular matrix components. Most enriched category of downregulated proteins is energy metabolism. Chondrocytes produce ATPs by anaerobic glycolysis, because cartilage is avascular and hypoxic. IGF-I was upregulated and was shown to exhibit additive

effect on chondrogenesis with BMP-2. Fibulin-5, an ECM protein, was upregulated. BTF3L4, general transcription factor, was upregulated. Kim et al. (2010) have studied chondrogenesis of adipose tissue-derived stem cells (ASCs). They identified 756 proteins using nano LC coupled to LCQ MS/MS approach. From 33 chondrogenic factors or proteins identified authors especially mentioned type 2 collagen, biglycan, IGF-binding protein and TGF- β 1.

2.2 Stem cells

One of the approaches in the regenerative medicine is the use of stem cells as cell therapies. (Keller, 2005; Murry & Keller, 2008; Wobus & Boheler, 2005) Their self renewal capability, the unlimited potential for differentiation, and signals that control differentiation fate are under broad scientific scrutiny. We have reviewed the role of BMP-6 in mesenchymal stem cell differentiation. (Vukicevic & Grgurevic, 2009) BMP-6 roles have been reviewed in bone remodeling, bone to pancreas coupling and kidney development.

Lee et al. (2010) have studied BMP-2 induced differentiation of bone marrow stem cells (BMSC). They identified and quantified 449 proteins using SILAC LC-MS/MS approach. Proteins were separated by SDS-PAGE and in gel digested. Peptides were separated by LC and analysed by LTQ-FT, working in the data dependant mode. Top five signals from the survey scan were measured in the FT analyser using SIM, and CID fragmented in the linear IT. 12 proteins were upregulated, 7 proteins were downregulated, and 19 proteins were only detected in BMP-2 induced cells. Authors singled out β -catenin (identified only in BMP-2 induced differentiated cells with 4 peptides). Wnt/ β -catenin pathway is known to be important in osteoblast differentiation. They ruled out Smad and ERK BMP pathways on β -catenin upregulation. They identified PI3K pathway as crucial for BMP-2 induced BMSC differentiation. Willert et al. (2003) have for the first time isolated Wnt3a in an active form. They have expressed Wnt3a in mouse L cells, purified it from 2 L of conditioned medium by three step chromatography (Blue sepharose, gel filtration and heparin cation exchange) in the presence of CHAPS, due to hydrophobic nature of the Wnt protein. They have pinpointed hydrophobicity to lipid modification, and have identified cysteine 77 as place of palmitation, by MudPIT.

Hoof et al. (2009) studied phosphorylation dynamics during BMP4 induced differentiation of hESCs. They used SILAC labeling and phosphopeptide enrichment by SCX-TiO₂ chromatography. High resolution Orbitrap, working in the data dependant top two mode, was used for the measurement of peptides in four time points (0, 30, 60, 240 min). Authors identified 5222 proteins, of which 1399 were phosphorylated on 3067 sites (2431 serines, 582 threonines, and 54 tyrosines). Half of the quantified phosphopeptides are regulated within the first hour of BMP-4 initiated differentiation. 586 identified proteins are regulated by the core transcriptional network. By a GO analysis prominent protein groups are associated with the epigenetic modification, transcription and translation. Authors found increased phosphorylation of SMADs, PI3K/AKT pathway activation and transient activation of JNKs. Regulators of pluripotency are also altered, but SOX2 did not changed its phosphorylation status. By analysis of the predicted kinases responsible for measured phosphorylation, authors found that CDK1/2 has central role.

Yocum et al. (2008) have identified and verified several potential markers of noggin-induced neural and BMP-4-induced epidermal ectoderm differentiation of hESCs. They developed hESCs cell culture system (on gelatin-coated dishes) free of mouse embryonic fibroblasts (MEF) feeder layer or conditioned media. In this way targeted proteome is not contaminated

by proteins from MEFs and noggin/BMP4 effect on hESCs is not influenced by possible MEFs response. They used 2D LC/MALDI-TOF/TOF (ABI 4800 TOF/TOF) for global analysis and MRM on 4000 Q Trap for verification and targeted analysis. Beside verification of tubulin β -III and cytokeratin-8, previously known markers for neuronal and epithelial differentiation respectively, they proposed nuclear autoantigenic sperm protein (NASP) as marker for pluripotency and dihydropyrimidinase-related protein (DRP) 2 and 4 as markers for the early neuronal differentiation.

Nunomura et al. (2005) have identified 200 membrane proteins from mouse embryonic stem cells using biotin based cell surface labeling, biotin-avidin affinity peptide purification and 2D LC-MS/MS analysis on Q-Tof 2. Among 82 proteins involved in different cell signaling pathways, they identified leukemia inhibitory factor receptor (LIF-R), interleukin-6 receptor subunit β (IL-6R β), ciliary neurotrophic factor receptor (CNTFR- α), BMPR-1a and integrin α 6 β 1.

Prowse et al. (2005) identified 102 proteins secreted by human neonatal fibroblasts, which are used for maintenance of undifferentiated growth of human embryonic stem cells, using 2D LC-MS and 2DE/MALDI-TOF. Among others they have identified proteins involved in cell adhesion, cell proliferation and inhibition of cell proliferation, Wnt signaling and inhibition of BMPs.

Kurisaki et al. (2005) have studied effects of the removal of leukemia inhibitory factor (LIF) from mouse embryonic stem cells. LIF maintains pluripotency of mouse ESCs, by activating JAK/STAT3 pathway. Only in cooperation with BMPs, can LIF inhibit differentiation of mouse ESCs. BMPs activate expression of Id genes and suppress neuronal differentiation. Authors identified more than 100 proteins by DIGE and MALDI-TOF analysis on AXIMA-CFR Plus and/or 4700 Proteomics Analyzer.

2.3 Cancer

BMPs, as part of the TGF- β protein superfamily, are involved in the cancer pathophysiology. (Massague, 2008) Klose et al. (2011) have shown BMP-7 induced cell cycle arrest at G₁/S checkpoint of human glioma-derived Gli36 Δ EGFR-LITG cells. Kim et al. (2009) have studied PRL-3-mediated metastasis using B16 and B16F10 mouse melanoma cell lines. Using 2D DIGE and MS they identified the heat shock protein 70, fascin-1, septin-6, ATP synthase beta subunit, and bone morphogenetic protein receptor type IB, as proteins that differ in cancer cells with low and high metastatic potential. We showed the inhibitory role of BMP-7 to bone metastases of breast cancer (Buijs et al., 2007) and prostate cancer (Buijs et al., 2007a). The cancer metastasis can be triggered by TGF- β stimulating epithelial-to-mesenchymal transition. Recombinant BMP-7 is inhibiting cancer bone metastases growth, so we proposed it as novel therapy for bone metastases. Rivera et al. (2007) have identified 43 differentially expressed proteins in melanoma cell line with breast cancer metastasis-suppressor 1 (BRMS1) gene silenced or over-expressed compared to wild type. They used 2D-DIGE for relative quantification and MALDI-TOF/TOF (ABI 4800 TOF/TOF Analyzer) for protein identification from preparative 2D gel. Among others they identified and confirmed BMPR-II. van Gils et al. (2005) reviewed serum and urine markers in European prostate cancer P-Mark project. BMP-6 was considered as bone metastasis prognostic serum marker, but later dismissed due to technical challenges in BMP-6 serum detection. Cheung et al. (2004) have identified 24 kDa SELDI-TOF signal specific to early prostate carcinogenesis as dimeric form of mature GDF-15. By laser capture micro-dissection

(LCM) they have isolated normal, early cancer (Gleason grade 2 to 4) and high-grade prostatic intraepithelial neoplasia (hPIN) epithelial cells from 22 patients. For SELDI-TOF analysis, authors used a copper IMAC chemistry, and proteins measured by Ciphergen Protein Biology System II spectrometer (Ciphergen Biosystems, Inc.). For the protein identification, authors first separated proteins by SDS-PAGE. 24 kDa band was analysed by oMALDI Q-TOF (ABI Q-Star).

2.4 Kidney

We have previously reviewed the role of BMPs on the development and homeostasis of kidneys. (Simic & Vukicevic, 2005) BMPs have been connected to kidney biology already in 1990s. The BMPs role in kidney development has been discovered by early localization studies using immunohistochemistry (Vukicevic et al., 1994a), autoradiography (Vukicevic et al., 1990) and *in situ* hybridization (Helder et al., 1995; Vukicevic et al., 1994). We have shown that BMP-7 is crucial for metanephric mesenchyme differentiation during kidney development. (Vukicevic et al., 1996)

BMP-7 is effective in treatment of acute renal failure, as we have shown in a ischemia/reperfusion rat model. (Vukicevic et al., 1998) Wang & Hirschberg (2011) have identified Y-box protein-1 (YB-1) as BMP-7 transcriptional activator in context of chronic kidney disease (CKD) using LC-MS/MS. They used promotor region of BMP7 gene to fish out factors from MDCK nuclear fraction. Proteins were separated by SDS-PAGE, and analysed by nano LC coupled to LTQ-Orbitrap XL. We have identified BMP-6, GDF-15 and BMP-1-3 from plasma of healthy volunteers and patients with CKD. (Grgurevic et al., 2011a) We showed an increased renal fibrosis in rats with CKD after systemic administration of BMP-1-3. Administration of anti BMP-1-3 antibody reduced the fibrosis.

2.5 Iron

Iron content is tightly controlled by individual cell and also systemically by whole organism. (Hentze et al., 2004; 2010) We found that BMP-6 is an endogenous regulator of iron metabolism *in vivo*. (Andriopoulos et al., 2009) *Bmp6* knockout mouse has reduced hepcidin expression and tissue iron overload, which resembles hemochromatosis. Hepcidin has a central role in maintaining iron body levels. Recombinant BMP6, when administered to mouse with hemochromatosis reduced the serum iron in a dose-dependent manner. Next, we found that BMP-6 treatment reduces hemochromatosis in *Hfe* knockout mouse. (Corradini et al., 2010) We also showed BMP-6 effect on TMPRSS6 expression, which is a negative regulator of hepcidin. (Meynard et al., 2011)

Kartikasari et al. (2008) used SELDI-TOF MS to detect and quantify hepcidin-25, 25 residues long hepcidin isoform which regulates the iron homeostasis. Authors used cation exchange chip for protein binding, and Ciphergen Protein Biology System IIc TOF mass spectrometer for the measurements. They found synergistic upregulation of hepcidin-25 by BMP-2/9 and IL-6, suggesting a crosstalk between iron and inflammatory pathways.

2.6 Other

BMP-15 is oocyte secreted growth factor important for female fertility. (Elvin et al., 2000) Saito et al. (2008) have characterized rhBMP-15 expressed in human embryonic kidney 293 cells by MS. Authors used MALDI-TOF (Reflex 3, Bruker) for the protein molecular weight measurement and micro LC coupled to LTQ-Orbitrap for the CID fragmentation and the

neutral loss measurements. They found N-terminal amino acid to be pyroglutamic acid and C-terminal end to be truncated. 16 kDa BMP-15 form is phosphorylated on serine 6 and 17 kDa BMP-15 form is O-glycosylated on threonine 10. Tibaldi et al. (2010) have identified serine 6 from mature BMP-15 and BMP-9 to be phosphorylated by Golgi apparatus casein kinase (G-CK). Phosphorylation sites were identified using phosphopeptide enrichment and nano LC coupled to LCQ XL, or MALDI-TOF/TOF (ABI 4800 Plus) analysis.

Li et al. (2007) have studied ubiquitination of Smad1 mediated by carboxyl terminus of Hsc70-interacting protein (CHIP). They have confirmed N-terminus mono-ubiquitination of Smad1 by MALDI-TOF analysis of *in vitro* ubiquitinated Smad1.

Pulmonary arterial hypertension (PAH) was reviewed by Rubin (1997) and Rabinovitch (2008). Abdul-Salam et al. (2010) found 25 differentially expressed out of more than 300 identified proteins by SDS-PAGE and LC-MS/MS from lung tissue of 8 patients and 8 control subjects. They found for the first time increased expression of chloride intracellular channel 4 (CLIC4), receptor for advanced glycation end products, and periostin. Meyrick et al. (2008) found 16 differentially expressed proteins in familial pulmonary arterial hypertension (FPAH) patients compared to obligate carrier from the same family with known BMPR-2 mutation. They used 2D-DIGE/MALDI-TOF(/TOF) of EBV-transformed B lymphocytes. They connected adapter protein growth factor receptor bound protein (GRB2) to signal transduction of BMPR-2 receptor.

Pappano et al. (2003) have identified *in vivo* substrates of BMP-1 and mammalian Tolloid-like metalloproteinases (mTLL) using proteomics. They compared *in vivo* procollagen C-proteinase (pCP) activity of wild type *vs Bmp1 Tll* doubly homozygous null mice. Using nano ESI-QToF sequencing, they have identified four 2DE spots (absent in *Bmp1^{-/-} Tll^{-/-}* mouse embryo fibroblasts, MEF, conditioned media) as C-propeptides of the pro α 1 chain of type I procollagen, of the pro α 1 chain of type III procollagen, of the pro α 2 chain of type III procollagen and for the first time proline- and arginine-rich protein (PARP) subdomain of the N-terminal globular sequences of the pro α 1 chain of type XI collagen.

The low resolution structure of procollagen C-proteinase enhancer 1 (PCPE-1), an extracellular matrix glycoprotein that can stimulate the pCP action of tolloid metalloproteinases, was determined by Bernocco et al. (2003). Using MALDI-TOF they have determined MW of PCPE-1 to be 48628 Da. Wajih et al. (2004) have studied intracellular processing and transport of the matrix γ -carboxyglutamic acid protein (MGP) in human vascular smooth muscle cells (VSMCs) infected with adenovirus carrying the MGP construct. MGP is an inhibitor of arterial wall and cartilage calcification through the binding of BMP-2. They have identified bovine fetuin instead of MGP using SDS-PAGE followed by nano LC coupled to LCQ Deca XL ion trap MS. Using glutathion-S-transferase (GST) pull-down followed by 2DE/MALDI-TOF analysis, Hassel et al. (2004) identified 33 proteins interacting with BMPRII. Wermter et al. (2007) studied the substrate selectivity of BMP-1 using the full-length *vs.* isolated proteolytic domain of BMP-1. They managed to over-express BMP-1 catalytic domain in *E. coli* and to refold it properly. By MALDI-TOF analysis of reduced *vs.* unreduced tryptic peptides they confirmed the proper formation of three disulphide bridges (C65-C85, C43-C199 and C63-C66). Number of ligands that are binding to activin type II receptor (ActRII) and ActRIIB from human and mouse sera have been identified using affinity purification, SDS-PAGE and nano LC coupled to linear IT (LTQ). (Souza et al., 2008) They confirmed that in addition to myostatin, BMP-11 and activins-A, -B and -AB could regulate the muscle growth by inhibiting myoblast-to-myotube differentiation. Meleady et al. (2008) have compared Chinese hamster

ovary (CHO) cells expressing rhBMP-2 to cell co-expressing soluble exogenous paired basic amino acid cleaving enzyme (PACEsol), which improves post-translational processing of the mature rhBMP-2. They used 2D-DIGE/MALDI-TOF and found 60 differentially expressed proteins.

3. Conclusion

Growth factors MS based characterization from biofluids is almost exclusively done by CID on plethora of different tandem instruments. Very low levels of growth factors in circulation is limiting their MS based identification due to huge dynamic range of plasma/serum proteins. Different biochemical purification strategies are used to enrich individual growth factors enough to be able to analyse it by MS. Most of them use heparin affinity chromatography as one purification step. We have successfully identified number of growth factors from biofluids using heparin based enrichment, followed by the SDS-PAGE protein separation and peptide analysis on nano LC coupled to LTQ-Orbitrap.

4. Future prospects

Disparity between liters of starting material used in the isolation and identification studies of individual growth factors from biological fluids, and an unmet need to use small amounts of biological samples for high throughput clinically relevant studies, is ending by development of growth factors enrichment strategies and of course by development of new and better mass spectrometers, and/or new proteomic strategies.

In the future we can expect more and more proteomic studies with BMPs and other growth factors to be identified, quantified and/or characterized from different clinically relevant samples. This will help to better understand particular diseases, discover new biomarkers and to develop new therapeutic strategies.

5. Abbreviations

2DE	Two dimensional gel electrophoresis
β ig-h3	Transforming growth factor-β-induced protein ig-h3 (β ig-h3) (Kerato-epithelin) (RGD-containing collagen-associated protein) (RGD-CAP)
AARS	Aminoacyl-tRNA synthetases
ARTN	Artemin (Enovin) (Neublastin)
ASC	Adipose tissue-derived stem cells
ATP5F1	ATP synthase B chain, mitochondrial precursor
ATP5H	ATP synthase D chain, mitochondrial
ATP5O	ATP synthase O subunit, mitochondrial precursor
ATPA1	ATP synthase subunit alpha, mitochondrial precursor
BAX	Apoptosis regulator BAX
bFGF	Heparin-binding growth factor 2 (HBGF-2) (Basic fibroblast growth factor) (bFGF)
BMP	Bone morphogenetic protein

BMP-1	Bone morphogenetic protein 1 (BMP-1) (EC 3.4.24.19) (Mammalian tolloid protein) (mTld) (Procollagen C-proteinase) (PCP)
BMP-10	Bone morphogenetic protein 10 (BMP-10)
BMP-15	Bone morphogenetic protein 15 (BMP-15) (Growth/differentiation factor 9B) (GDF-9B)
BMP-2	Bone morphogenetic protein 2 (BMP-2) (Bone morphogenetic protein 2A) (BMP-2A)
BMP-3	Bone morphogenetic protein 3 (BMP-3) (Bone morphogenetic protein 3A) (BMP-3A) (Osteogenin)
BMP-3B	Bone morphogenetic protein 3B (BMP-3B) (Bone-inducing protein) (BIP) (Growth/differentiation factor 10) (GDF-10)
BMP-4	Bone morphogenetic protein 4 (BMP-4) (Bone morphogenetic protein 2B) (BMP-2B)
BMP-5	Bone morphogenetic protein 5 (BMP-5)
BMP-6	Bone morphogenetic protein 6 (BMP-6) (VG-1-related protein) (VG-1-R) (VGR-1)
BMP-7	Bone morphogenetic protein 7 (BMP-7) (Osteogenic protein 1) (OP-1) (Eptotermine α)
BMP-8A	Bone morphogenetic protein 8A (BMP-8A)
BMP-8B	Bone morphogenetic protein 8B (BMP-8) (BMP-8B) (Osteogenic protein 2) (OP-2)
BMPR-II	Bone morphogenetic protein receptor type-2 (BMP type-2 receptor) (BMPR-2) (EC 2.7.11.30) (Bone morphogenetic protein receptor type II) (BMP type II receptor) (BMPR-II)
BMSC	Bone marrow stem cell
BRMS1	Breast cancer metastasis-suppressor 1
BTF3L4	Transcription factor BTF3 homolog 4 (Basic transcription factor 3-like 4)
C3H10T1/2	Murine embryonic mesenchymal cell line
CDK1	Cyclin-dependent kinase 1 (CDK1) (EC 2.7.11.22) (EC 2.7.11.23) (Cell division control protein 2 homolog) (Cell division protein kinase 1) (p34 protein kinase)
CFL-1	Cofilin-1 (18 kDa phosphoprotein) (p18) (Cofilin, non-muscle isoform)
CHIP	E3 ubiquitin-protein ligase CHIP (EC 6.3.2.-) (Antigen NY-CO-7) (CLL-associated antigen KW-8) (Carboxy terminus of Hsp70-interacting protein) (STIP1 homology and U box-containing protein 1)
CID	Collision induced dissociation
CKD	Chronic kidney disease
CLIC4	Chloride intracellular channel protein 4 (Intracellular chloride ion channel protein p64H1)
CNTFR- α	Ciliary neurotrophic factor receptor subunit α (CNTF receptor subunit α) (CNTFR- α)
COL1A1	Collagen α -1(I) chain (α -1 type I collagen)

CRMP-2	Dihydropyrimidinase-related protein 2 (DRP-2) (Collapsin response mediator protein 2) (CRMP-2) (N2A3) (Unc-33-like phosphoprotein 2) (ULIP-2)
CRTAC1	Cartilage acidic protein 1 (68 kDa chondrocyte-expressed protein) (CEP-68) (ASPIC)
Da	Dalton
DBM	Demineralized bone matrix
DIGE	Difference gel electrophoresis
DPT	Dermatopontin (Tyrosine-rich acidic matrix protein) (TRAMP)
DRP-2	Dihydropyrimidinase-related protein 2 (DRP-2) (Collapsin response mediator protein 2) (CRMP-2) (N2A3) (Unc-33-like phosphoprotein 2) (ULIP-2)
EBV	Epstein-Barr virus
EGF	Epidermal growth factor
ESI	Electrospray ionisation
ETF1	Eukaryotic peptide chain release factor subunit 1 (Eukaryotic release factor 1) (eRF1) (Protein Cl1) (TB3-1)
FLN-A	Filamin-A (FLN-A) (Actin-binding protein 280) (ABP-280) (α -filamin) (Endothelial actin-binding protein) (Filamin-1) (Non-muscle filamin)
FT	Fourier transform mass spectrometry
G3BP-1	Ras GTPase-activating protein-binding protein 1
GANAB	Neutral α -glucosidase AB (EC 3.2.1.84) (α -glucosidase 2) (Glucosidase II subunit α) (α glucosidase 2 α neutral subunit)
GDF	Growth/differentiation factor
GDF-1	Embryonic growth/differentiation factor 1 (GDF-1)
GDF-11	Growth/differentiation factor 11 (GDF-11) (Bone morphogenetic protein 11) (BMP-11)
GDF-15	Growth/differentiation factor 15 (GDF-15) (Macrophage inhibitory cytokine 1) (MIC-1) (NSAID-activated gene 1 protein) (NAG-1) (NSAID-regulated gene 1 protein) (NRG-1) (Placental TGF- β) (Placental bone morphogenetic protein) (Prostate derived factor)
GDF-2	Growth/differentiation factor 2 (GDF-2) (Bone morphogenetic protein 9) (BMP-9)
GDF-3	Growth/differentiation factor 3 (GDF-3)
GDF-5	Growth/differentiation factor 5 (GDF-5) (Cartilage-derived morphogenetic protein 1) (CDMP-1) (Radotermis)
GDF-6	Growth/differentiation factor 6 (GDF-6) (Growth/differentiation factor 16)
GDF-7	Growth/differentiation factor 7 (GDF-7)
GDF-8	Growth/differentiation factor 8 (GDF-8) (Myostatin)
GDF-9	Growth/differentiation factor 9 (GDF-9)
GDNF	Glial cell line-derived neurotrophic factor (hGDNF) (Astrocyte-derived trophic factor) (ATF)

GRB2	Growth factor receptor-bound protein 2 (Adapter protein GRB2) (Protein Ash) (SH2/SH3 adapter GRB2)
GSN	Gelsolin
GST	Glutathion-S-transferase
HBGF-2	Heparin-binding growth factor 2 (HBGF-2) (Basic fibroblast growth factor) (bFGF)
hPIN	High-grade prostatic intraepithelial neoplasia
HSP 84	Heat shock protein HSP 90- β (HSP 90) (Heat shock 84 kDa) (HSP 84) (HSP84)
ICAT	Isotope-coded affinity tag
ICR	Ion cyclotron resonance
IGF-I	Insulin-like growth factor I (IGF-I) (Mechano growth factor) (MGF) (Somatomedin-C)
IL-6R β	Interleukin-6 receptor subunit β (IL-6 receptor subunit β) (IL-6R subunit β) (IL-6R- β) (IL-6R β) (Interleukin-6 signal transducer) (Membrane glycoprotein 130) (gp130) (Oncostatin-M receptor subunit α) (CD antigen CD130)
IMAC	Immobilized metal ion affinity chromatography
INH	Inhibin
INH- α	Inhibin α chain
INH- β -A	Inhibin β A chain (Activin β -A chain) (Erythroid differentiation protein) (EDF)
INH- β -B	Inhibin β B chain (Activin β -B chain)
INH- β -C	Inhibin β C chain (Activin β -C chain)
INH- β -E	Inhibin beta E chain (Activin β -E chain)
IQGAP1	Ras GTPase-activating-like protein 1
IT	Ion trap
iTRAQ	Isobaric tags for relative and absolute quantitation
LAMA1	Laminin subunit α -1 (Laminin A chain) (Laminin-1 subunit α) (Laminin-3 subunit α) (S-laminin subunit α) (S-LAM α)
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LCM	Laser capture microdissection
LCQ	3D ion trap from Thermo
LFTY1	Left-right determination factor 1 (Left-right determination factor B) (Protein lefty-1) (Protein lefty-B)
LFTY2	Left-right determination factor 2 (Endometrial bleeding-associated factor) (Left-right determination factor A) (Protein lefty-2) (Protein lefty-A) (Transforming growth factor β -4) (TGF- β -4)
LIF	Leukemia inhibitory factor
LIF-R	Leukemia inhibitory factor receptor (LIF receptor) (LIF-R) (D-factor/LIF receptor) (CD antigen CD118)
LOX	Protein-lysine 6-oxidase (EC 1.4.3.13) (Lysyl oxidase)

LTQ	Linear trap quadrupole
MALDI	Matrix assisted laser desorption/ionisation
MC3T3-E1	Mouse osteoblast precursor-like cell line
MEF	Mouse embryo fibroblasts
MGP	Matrix γ -carboxyglutamic acid protein
MIS	Muellerian-inhibiting factor
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MS3	Triple stage mass spectrometry
MudPIT	Multidimensional protein identification technology
MYH	Myosin
NARS	Asparaginyl-tRNA synthetase, cytoplasmic (EC 6.1.1.22) (Asparagine-tRNA ligase) (AsnRS)
NASP	Nuclear autoantigenic sperm protein
NODAL	Nodal homolog
NRTN	Neurturin
OBIF	Osteoblastogenesis inhibitory factor
oMALDI	Orthogonal MALDI
PAPSS 2	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (PAPS synthase 2) (PAPSS 2) (Sulfurylase kinase 2) (SK 2) (SK2) [Includes: Sulfate adenylyltransferase (EC 2.7.7.4) (ATP-sulfurylase) (Sulfate adenylyl transferase) (SAT); Adenylyl-sulfate kinase (EC 2.7.1.25) (3'-phosphoadenosine-5'-phosphosulfate synthase) (APS kinase) (Adenosine-5'-phosphosulfate 3'-phosphotransferase) (Adenylylsulfate 3'-phosphotransferase)]
PCPE-1	Procollagen C-endopeptidase enhancer 1 (Procollagen COOH-terminal proteinase enhancer 1) (PCPE-1) (Procollagen C-proteinase enhancer 1) (Type 1 procollagen C-proteinase enhancer protein) (Type I procollagen COOH-terminal proteinase enhancer)
PDGF	Platelet-derived growth factor
PMF	Peptide mass fingerprint
PRL-3	Protein tyrosine phosphatase type IVA 3 (EC 3.1.3.48) (PRL-R) (Protein-tyrosine phosphatase 4a3) (Protein-tyrosine phosphatase of regenerating liver 3) (PRL-3)
PSPN	Persephin (PSP)
Q-Tof	Quadrupole time-of-flight mass spectrometer
QSTAR	Q-Tof from Applied Biosystems
Q Trap	Hybrid triple quadrupole/linear ion trap mass spectrometer
RAB-1A	Ras-related protein Rab-1A (YPT1-related protein)

RANKL	Tumor necrosis factor ligand superfamily member 11 (Osteoclast differentiation factor) (ODF) (Osteoprotegerin ligand) (OPGL) (Receptor activator of nuclear factor kappa-B ligand) (RANKL) (TNF-related activation-induced cytokine) (TRANCE) (CD antigen CD254) [Cleaved into: Tumor necrosis factor ligand superfamily member 11, membrane form; Tumor necrosis factor ligand superfamily member 11, soluble form]
RAW264.7	Mouse osteoclast-like myeloma cell line
SAX	Strong anion exchange
SCX	Strong cation exchange
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SELDI	Surface-enhanced laser desorption/ionization
SILAC	Stable isotope labeling by amino acids in cell culture
SIM	Single ion monitoring
SNX9	Sorting nexin-9 (SH3 and PX domain-containing protein 1) (Protein SDP1) (SH3 and PX domain-containing protein 3A)
SUCLG2	Succinyl-CoA ligase [GDP-forming] subunit β , mitochondrial (EC 6.2.1.4) (GTP-specific succinyl-CoA synthetase subunit β) (Succinyl-CoA synthetase β -G chain) (SCS- β G)
TGF- β	Transforming growth factor β
TGF- β -1	Transforming growth factor β -1 (TGF- β -1) [Cleaved into: Latency-associated peptide (LAP)]
TGF- β -2	Transforming growth factor beta-2 (TGF- β -2) (BSC-1 cell growth inhibitor) (Cetermin) (Glioblastoma-derived T-cell suppressor factor) (G-TSF) (Polyergin)
TGF- β -R-3	Transforming growth factor β receptor type 3 (TGF- β receptor type 3) (TGF-R-3) (β glycan) (Transforming growth factor β receptor III) (TGF- β receptor type III)
Th	Thomson
TMPRSS6	Transmembrane protease serine 6 (EC 3.4.21.-) (Matriptase-2)
TOF/TOF	Tandem time-of-flight mass spectrometry
VSMC	Vascular smooth muscle cells
YB-1	Nuclease-sensitive element-binding protein 1 (CCAAT-binding transcription factor I subunit A) (CBF-A) (DNA-binding protein B) (DBPB) (Enhancer factor I subunit A) (EFI-A) (Y-box transcription factor) (Y-box-binding protein 1) (YB-1)

6. References

- Abdul-Salam, V. B., Wharton, J., Cupitt, J., Berryman, M., Edwards, R. J. & Wilkins, M. R. (2010). Proteomic analysis of lung tissues from patients with pulmonary arterial hypertension., *Circulation* 122(20): 2058–2067.
 URL: <http://dx.doi.org/10.1161/CIRCULATIONAHA.110.972745>

- Andriopoulos, B., Corradini, E., Xia, Y., Faasse, S. A., Chen, S., Grgurevic, L., Knutson, M. D., Pietrangelo, A., Vukicevic, S., Lin, H. Y. & Babitt, J. L. (2009). BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism., *Nat Genet* 41(4): 482–487.
URL: <http://dx.doi.org/10.1038/ng.335>
- Behnam, K., Murray, S. S. & Brochmann, E. J. (2006). BMP stimulation of alkaline phosphatase activity in pluripotent mouse C2C12 cells is inhibited by dermatopontin, one of the most abundant low molecular weight proteins in demineralized bone matrix., *Connect Tissue Res* 47(5): 271–277.
URL: <http://dx.doi.org/10.1080/03008200600995908>
- Bernocco, S., Steiglitz, B. M., Svergun, D. I., Petoukhov, M. V., Ruggiero, F., Ricard-Blum, S., Ebel, C., Geourjon, C., Deleage, G., Font, B., Eichenberger, D., Greenspan, D. S. & Hulmes, D. J. S. (2003). Low resolution structure determination shows procollagen C-proteinase enhancer to be an elongated multidomain glycoprotein., *J Biol Chem* 278(9): 7199–7205.
URL: <http://dx.doi.org/10.1074/jbc.M210857200>
- Buijs, J. T., Henriquez, N. V., van Overveld, P. G. M., van der Horst, G., Que, I., Schwaninger, R., Rentsch, C., Dijke, P. T., Cleton-Jansen, A.-M., Driouch, K., Lidereau, R., Bachelier, R., Vukicevic, S., Clezardin, P., Papapoulos, S. E., Cecchini, M. G., Lowik, C. W. G. M. & van der Pluijm, G. (2007). Bone morphogenetic protein 7 in the development and treatment of bone metastases from breast cancer., *Cancer Res* 67(18): 8742–8751.
URL: <http://dx.doi.org/10.1158/0008-5472.CAN-06-2490>
- Buijs, J. T., Rentsch, C. A., van der Horst, G., van Overveld, P. G. M., Wetterwald, A., Schwaninger, R., Henriquez, N. V., Dijke, P. T., Borovecki, F., Markwalder, R., Thalmann, G. N., Papapoulos, S. E., Pelger, R. C. M., Vukicevic, S., Cecchini, M. G., Lowik, C. W. G. M. & van der Pluijm, G. (2007). BMP7, a putative regulator of epithelial homeostasis in the human prostate, is a potent inhibitor of prostate cancer bone metastasis in vivo., *Am J Pathol* 171(3): 1047–1057.
URL: <http://dx.doi.org/10.2353/ajpath.2007.070168>
- Chen, D., Zhao, M. & Mundy, G. R. (2004). Bone morphogenetic proteins., *Growth Factors* 22(4): 233–241.
URL: <http://dx.doi.org/10.1080/08977190412331279890>
- Cheung, P. K., Woolcock, B., Adomat, H., Sutcliffe, M., Bainbridge, T. C., Jones, E. C., Webber, D., Kinahan, T., Sadar, M., Gleave, M. E. & Vielkind, J. (2004). Protein profiling of microdissected prostate tissue links growth differentiation factor 15 to prostate carcinogenesis., *Cancer Res* 64(17): 5929–5933.
URL: <http://dx.doi.org/10.1158/0008-5472.CAN-04-1216>
- Corradini, E., Schmidt, P. J., Meynard, D., Garuti, C., Montosi, G., Chen, S., Vukicevic, S., Pietrangelo, A., Lin, H. Y. & Babitt, J. L. (2010). BMP6 treatment compensates for the molecular defect and ameliorates hemochromatosis in Hfe knockout mice., *Gastroenterology* 139(5): 1721–1729.
URL: <http://dx.doi.org/10.1053/j.gastro.2010.07.044>
- Elvin, J. A., Yan, C. & Matzuk, M. M. (2000). Oocyte-expressed TGF-beta superfamily members in female fertility., *Mol Cell Endocrinol* 159(1-2): 1–5.

- Eng, J. K., McCormack, A. L. & Yates III, J. R. (1994). An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database, *Journal of the American Society for Mass Spectrometry* 5(11): 976 – 989.
URL: <http://www.sciencedirect.com/science/article/pii/1044030594800162>
- Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F. & Whitehouse, C. M. (1989). Electrospray ionization for mass spectrometry of large biomolecules., *Science* 246(4926): 64–71.
- Grasser, W. A., Orlic, I., Borovecki, F., Riccardi, K. A., Simic, P., Vukicevic, S. & Paralkar, V. M. (2007). BMP-6 exerts its osteoinductive effect through activation of IGF-I and EGF pathways., *Int Orthop* 31(6): 759–765.
URL: <http://dx.doi.org/10.1007/s00264-007-0407-9>
- Grgurevic, L., Macek, B., Durdevic, D. & Vukicevic, S. (2007). Detection of bone and cartilage-related proteins in plasma of patients with a bone fracture using liquid chromatography-mass spectrometry., *Int Orthop* 31(6): 743–751.
URL: <http://dx.doi.org/10.1007/s00264-007-0404-z>
- Grgurevic, L., Macek, B., Healy, D. R., Brault, A. L., Erjavec, I., Cipicic, A., Grgurevic, I., Rogic, D., Galesic, K., Brkljacic, J., Stern-Padovan, R., Paralkar, V. M. & Vukicevic, S. (2011a). Circulating bone morphogenetic protein 1-3 isoform increases renal fibrosis., *J Am Soc Nephrol* 22(4): 681–692.
URL: <http://dx.doi.org/10.1681/ASN.2010070722>
- Grgurevic, L., Macek, B., Mercep, M., Jelcic, M., Smoljanovic, T., Erjavec, I., Dumic-Cule, I., Prgomet, S., Durdevic, D., Vnuk, D., Lipar, M., Stejskal, M., Kufner, V., Brkljacic, J., Maticic, D. & Vukicevic, S. (2011). Bone morphogenetic protein (BMP)1-3 enhances bone repair., *Biochem Biophys Res Commun* 408(1): 25–31.
URL: <http://dx.doi.org/10.1016/j.bbrc.2011.03.109>
- Griffith, D. L., Keck, P. C., Sampath, T. K., Rueger, D. C. & Carlson, W. D. (1996). Three-dimensional structure of recombinant human osteogenic protein 1: structural paradigm for the transforming growth factor beta superfamily., *Proc Natl Acad Sci U S A* 93(2): 878–883.
- Gygi, S., Rist, B., Gerber, S., Turecek, F., Gelb, M. & Aebersold, R. (1999). Quantitative analysis of complex protein mixtures using isotope-coded affinity tags, *Nat Biotechnol* 17(10): 994–999.
- Hassel, S., Eichner, A., Yakymovych, M., Hellman, U., Knaus, P. & Souchelnytskyi, S. (2004). Proteins associated with type II bone morphogenetic protein receptor (BMPRII) and identified by two-dimensional gel electrophoresis and mass spectrometry., *Proteomics* 4(5): 1346–1358.
URL: <http://dx.doi.org/10.1002/pmic.200300770>
- Helder, M. N., Ozkaynak, E., Sampath, K. T., Luyten, F. P., Latin, V., Oppermann, H. & Vukicevic, S. (1995). Expression pattern of osteogenic protein-1 (bone morphogenetic protein-7) in human and mouse development., *J Histochem Cytochem* 43(10): 1035–1044.
- Hentze, M. W., Muckenthaler, M. U. & Andrews, N. C. (2004). Balancing acts: molecular control of mammalian iron metabolism., *Cell* 117(3): 285–297.
- Hentze, M. W., Muckenthaler, M. U., Galy, B. & Camaschella, C. (2010). Two to tango: regulation of mammalian iron metabolism., *Cell* 142(1): 24–38.
URL: <http://dx.doi.org/10.1016/j.cell.2010.06.028>

- Henzel, W. J., Billeci, T. M., Stults, J. T., Wong, S. C., Grimley, C. & Watanabe, C. (1993). Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases., *Proc Natl Acad Sci U S A* 90(11): 5011–5015.
- Hillenkamp, F. & Karas, M. (1990). Mass spectrometry of peptides and proteins by matrix-assisted ultraviolet laser desorption/ionization., *Methods Enzymol* 193: 280–295.
- Hogan, B. L. (1996). Bone morphogenetic proteins: multifunctional regulators of vertebrate development., *Genes Dev* 10(13): 1580–1594.
- Hong, D., Chen, H.-X., Yu, H.-Q., Liang, Y., Wang, C., Lian, Q.-Q., Deng, H.-T. & Ge, R.-S. (2010). Morphological and proteomic analysis of early stage of osteoblast differentiation in osteoblastic progenitor cells., *Exp Cell Res* 316(14): 2291–2300.
URL: <http://dx.doi.org/10.1016/j.yexcr.2010.05.011>
- Hong, D., Chen, H.-X., Yu, H.-Q., Wang, C., Deng, H.-T., Lian, Q.-Q. & Ge, R.-S. (2010). Quantitative proteomic analysis of dexamethasone-induced effects on osteoblast differentiation, proliferation, and apoptosis in MC3T3-E1 cells using SILAC., *Osteoporos Int* .
URL: <http://dx.doi.org/10.1007/s00198-010-1434-8>
- Hoof, D. V., Munoz, J., Braam, S. R., Pinkse, M. W. H., Linding, R., Heck, A. J. R., Mummery, C. L. & Krijgsveld, J. (2009). Phosphorylation dynamics during early differentiation of human embryonic stem cells., *Cell Stem Cell* 5(2): 214–226.
URL: <http://dx.doi.org/10.1016/j.stem.2009.05.021>
- Ji, Y.-hua, Ji, J.-ling, Sun, F.-yong, Zeng, Y.-ying, He, X.-hui, Zhao, J.-xian, Yu, Y., Yu, S.-he & Wu, W. (2010). Quantitative proteomics analysis of chondrogenic differentiation of C3H10T1/2 mesenchymal stem cells by iTRAQ labeling coupled with on-line two-dimensional LC/MS/MS., *Mol Cell Proteomics* 9(3): 550–564.
URL: <http://dx.doi.org/10.1074/mcp.M900243-MCP200>
- Kahn, P. (1995). From genome to proteome: looking at a cell's proteins., *Science* 270(5235): 369–370.
- Kartikasari, A. E. R., Roelofs, R., Schaeps, R. M. J., Kemna, E. H. J. M., Peters, W. H. M., Swinkels, D. W. & Tjalsma, H. (2008). Secretion of bioactive hepcidin-25 by liver cells correlates with its gene transcription and points towards synergism between iron and inflammation signaling pathways., *Biochim Biophys Acta* 1784(12): 2029–2037.
URL: <http://dx.doi.org/10.1016/j.bbapap.2008.08.004>
- Keller, G. (2005). Embryonic stem cell differentiation: emergence of a new era in biology and medicine., *Genes Dev* 19(10): 1129–1155.
URL: <http://dx.doi.org/10.1101/gad.1303605>
- Kim, B.-G., Lee, J.-H., Ahn, J.-M., Park, S. K., Cho, J.-H., Hwang, D., Yoo, J.-S., Yates, J. R., Ryoo, H.-M. & Cho, J.-Y. (2009). 'Two-stage double-technique hybrid (TSDTH)' identification strategy for the analysis of BMP2-induced transdifferentiation of premyoblast C2C12 cells to osteoblast., *J Proteome Res* 8(10): 4441–4454.
URL: <http://dx.doi.org/10.1021/pr900231a>
- Kim, B.-S., Kang, K.-S. & Kang, S.-K. (2010). Soluble factors from ASCs effectively direct control of chondrogenic fate., *Cell Prolif* 43(3): 249–261.
URL: <http://dx.doi.org/10.1111/j.1365-2184.2010.00680.x>

- Kim, S. H., Kim, Y., Kim, M., Kim, D. S., Lee, S. C., Chi, S.-W., Lee, D. H., Park, S. G., Park, B. C., Bae, K.-H. & Kang, S. (2009). Comparative proteomic analysis of mouse melanoma cell line B16, a metastatic descendant B16F10, and B16 overexpressing the metastasis-associated tyrosine phosphatase PRK-3., *Oncol Res* 17(11-12): 601–612.
- Klose, A., Waerzeggers, Y., Monfared, P., Vukicevic, S., Kaijzel, E. L., Winkeler, A., Wickenhauser, C., Lowik, C. W. G. M. & Jacobs, A. H. (2011). Imaging bone morphogenetic protein 7 induced cell cycle arrest in experimental gliomas., *Neoplasia* 13(3): 276–285.
- Kodaira, K., Imada, M., Goto, M., Tomoyasu, A., Fukuda, T., Kamijo, R., Suda, T., Higashio, K. & Katagiri, T. (2006). Purification and identification of a BMP-like factor from bovine serum., *Biochem Biophys Res Commun* 345(3): 1224–1231.
URL: <http://dx.doi.org/10.1016/j.bbrc.2006.05.045>
- Kubota, K., Sakikawa, C., Katsumata, M., Nakamura, T. & Wakabayashi, K. (2002). Platelet-derived growth factor BB secreted from osteoclasts acts as an osteoblastogenesis inhibitory factor., *J Bone Miner Res* 17(2): 257–265.
URL: <http://dx.doi.org/10.1359/jbmr.2002.17.2.257>
- Kurisaki, A., Hamazaki, T. S., Okabayashi, K., Iida, T., Nishine, T., Chonan, R., Kido, H., Tsunasawa, S., Nishimura, O., Asashima, M. & Sugino, H. (2005). Chromatin-related proteins in pluripotent mouse embryonic stem cells are downregulated after removal of leukemia inhibitory factor., *Biochem Biophys Res Commun* 335(3): 667–675.
URL: <http://dx.doi.org/10.1016/j.bbrc.2005.07.128>
- Lee, J.-H., Kim, B.-G., Ahn, J.-M., Park, H.-J., Park, S.-K., Yoo, J.-S., Yates, J. R. & Cho, J.-Y. (2010). Role of PI3K on the regulation of BMP2-induced beta-Catenin activation in human bone marrow stem cells., *Bone* 46(6): 1522–1532.
URL: <http://dx.doi.org/10.1016/j.bone.2010.02.013>
- Li, R.-F., Shang, Y., Liu, D., Ren, Z.-S., Chang, Z. & Sui, S.-F. (2007). Differential ubiquitination of Smad1 mediated by CHIP: implications in the regulation of the bone morphogenetic protein signaling pathway., *J Mol Biol* 374(3): 777–790.
URL: <http://dx.doi.org/10.1016/j.jmb.2007.09.082>
- Mann, M. & Kelleher, N. L. (2008). Precision proteomics: The case for high resolution and high mass accuracy, *Proc Natl Acad Sci U S A* 105(47): 18132–18138.
- Massague, J. (2008). TGFbeta in Cancer., *Cell* 134(2): 215–230.
URL: <http://dx.doi.org/10.1016/j.cell.2008.07.001>
- Meleady, P., Henry, M., Gammell, P., Doolan, P., Sinacore, M., Melville, M., Francullo, L., Leonard, M., Charlebois, T. & Clynes, M. (2008). Proteomic profiling of CHO cells with enhanced rhBMP-2 productivity following co-expression of PACEsol., *Proteomics* 8(13): 2611–2624.
URL: <http://dx.doi.org/10.1002/pmic.200700854>
- Meynard, D., Vaja, V., Sun, C. C., Corradini, E., Chen, S., Lopez-Otin, C., Grgurevic, L., Hong, C. C., Stirnberg, M., Gutschow, M., Vukicevic, S., Babitt, J. L. & Lin, H. Y. (2011). Regulation of TMPRSS6 by BMP6 and iron in human cells and mice., *Blood* .
URL: <http://dx.doi.org/10.1182/blood-2011-04-348698>
- Meyrick, B. O., Friedman, D. B., Billheimer, D. D., Cogan, J. D., Prince, M. A., Phillips, J. A. & Loyd, J. E. (2008). Proteomics of transformed lymphocytes from a family with

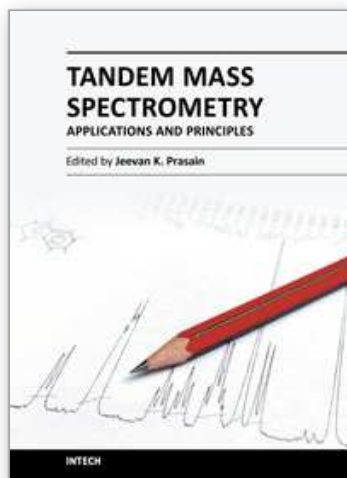
- familial pulmonary arterial hypertension., *Am J Respir Crit Care Med* 177(1): 99–107.
URL: <http://dx.doi.org/10.1164/rccm.200703-499OC>
- Murry, C. E. & Keller, G. (2008). Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development., *Cell* 132(4): 661–680.
URL: <http://dx.doi.org/10.1016/j.cell.2008.02.008>
- Nakasaka, M., Yoshioka, K., Miyamoto, Y., Sasaki, T., Yoshikawa, H. & Itoh, K. (2008). IGF-I secreted by osteoblasts acts as a potent chemotactic factor for osteoblasts., *Bone* 43(5): 869–879.
URL: <http://dx.doi.org/10.1016/j.bone.2008.07.241>
- Nunomura, K., Nagano, K., Itagaki, C., Taoka, M., Okamura, N., Yamauchi, Y., Sugano, S., Takahashi, N., Izumi, T. & Isobe, T. (2005). Cell surface labeling and mass spectrometry reveal diversity of cell surface markers and signaling molecules expressed in undifferentiated mouse embryonic stem cells., *Mol Cell Proteomics* 4(12): 1968–1976.
URL: <http://dx.doi.org/10.1074/mcp.M500216-MCP200>
- Ong, S., Blagoev, B., Kratchmarova, I., Kristensen, D., Steen, H., Pandey, A. & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics, *Mol Cell Proteomics* 1(5): 376–386.
- Pappano, W. N., Steiglit, B. M., Scott, I. C., Keene, D. R. & Greenspan, D. S. (2003). Use of Mmp1/Tll1 doubly homozygous null mice and proteomics to identify and validate in vivo substrates of bone morphogenetic protein 1/tolloid-like metalloproteinases., *Mol Cell Biol* 23(13): 4428–4438.
- Park, S. J., Kim, S. H., Choi, H. S., Rhee, Y. & Lim, S.-K. (2009). Fibroblast growth factor 2-induced cytoplasmic asparaginyl-tRNA synthetase promotes survival of osteoblasts by regulating anti-apoptotic PI3K/Akt signaling., *Bone* 45(5): 994–1003.
URL: <http://dx.doi.org/10.1016/j.bone.2009.07.018>
- Perkins, D. N., Pappin, D. J., Creasy, D. M. & Cottrell, J. S. (1999). Probability-based protein identification by searching sequence databases using mass spectrometry data., *Electrophoresis* 20(18): 3551–3567.
URL: <http://dx.doi.org/10.1002/2-2>
- Prowse, A. B. J., McQuade, L. R., Bryant, K. J., Dyk, D. D. V., Tuch, B. E. & Gray, P. P. (2005). A proteome analysis of conditioned media from human neonatal fibroblasts used in the maintenance of human embryonic stem cells., *Proteomics* 5(4): 978–989.
URL: <http://dx.doi.org/10.1002/pmic.200401087>
- Rabinovitch, M. (2008). Molecular pathogenesis of pulmonary arterial hypertension., *J Clin Invest* 118(7): 2372–2379.
URL: <http://dx.doi.org/10.1172/JCI33452>
- Raisz, L. G. (2005). Pathogenesis of osteoporosis: concepts, conflicts, and prospects., *J Clin Invest* 115(12): 3318–3325.
URL: <http://dx.doi.org/10.1172/JCI27071>
- Reddi, A. H. (1998). Role of morphogenetic proteins in skeletal tissue engineering and regeneration., *Nat Biotechnol* 16(3): 247–252.
URL: <http://dx.doi.org/10.1038/nbt0398-247>
- Rivera, J., Megias, D. & Bravo, J. (2007). Proteomics-based strategy to delineate the molecular mechanisms of the metastasis suppressor gene BRMS1., *J Proteome Res*

- 6(10): 4006–4018.
URL: <http://dx.doi.org/10.1021/pr0703167>
- Rubin, L. J. (1997). Primary pulmonary hypertension., *N Engl J Med* 336(2): 111–117.
URL: <http://dx.doi.org/10.1056/NEJM199701093360207>
- Saito, S., Yano, K., Sharma, S., McMahon, H. E. & Shimasaki, S. (2008). Characterization of the post-translational modification of recombinant human BMP-15 mature protein., *Protein Sci* 17(2): 362–370.
URL: <http://dx.doi.org/10.1110/ps.073232608>
- Saremba, S., Nickel, J., Seher, A., Kotzsch, A., Sebald, W. & Mueller, T. D. (2008). Type I receptor binding of bone morphogenetic protein 6 is dependent on N-glycosylation of the ligand., *FEBS J* 275(1): 172–183.
URL: <http://dx.doi.org/10.1111/j.1742-4658.2007.06187.x>
- Shevchenko, A., Jensen, O., Podtelejnikov, A., Sagliocco, F., Wilm, M., Vorm, O., Mortensen, P., Shevchenko, A., Boucherie, H. & Mann, M. (1996). Linking genome and proteome by mass spectrometry: Large-scale identification of yeast proteins from two dimensional gels, *Proc Natl Acad Sci U S A* 93(25): 14440–14445.
- Simic, P., Culej, J. B., Orlic, I., Grgurevic, L., Draca, N., Spaventi, R. & Vukicevic, S. (2006). Systemically administered bone morphogenetic protein-6 restores bone in aged ovariectomized rats by increasing bone formation and suppressing bone resorption., *J Biol Chem* 281(35): 25509–25521.
URL: <http://dx.doi.org/10.1074/jbc.M513276200>
- Simic, P. & Vukicevic, S. (2005). Bone morphogenetic proteins in development and homeostasis of kidney., *Cytokine Growth Factor Rev* 16(3): 299–308.
URL: <http://dx.doi.org/10.1016/j.cytogfr.2005.02.010>
- Song, K., Krause, C., Shi, S., Patterson, M., Suto, R., Grgurevic, L., Vukicevic, S., van Dinther, M., Falb, D., Dijke, P. T. & Alaoui-Ismaili, M. H. (2010). Identification of a key residue mediating bone morphogenetic protein (BMP)-6 resistance to noggin inhibition allows for engineered BMPs with superior agonist activity., *J Biol Chem* 285(16): 12169–12180.
URL: <http://dx.doi.org/10.1074/jbc.M109.087197>
- Souza, T. A., Chen, X., Guo, Y., Sava, P., Zhang, J., Hill, J. J., Yaworsky, P. J. & Qiu, Y. (2008). Proteomic identification and functional validation of activins and bone morphogenetic protein 11 as candidate novel muscle mass regulators., *Mol Endocrinol* 22(12): 2689–2702.
URL: <http://dx.doi.org/10.1210/me.2008-0290>
- Tibaldi, E., Arrigoni, G., Martinez, H. M., Inagaki, K., Shimasaki, S. & Pinna, L. A. (2010). Golgi apparatus casein kinase phosphorylates bioactive Ser-6 of bone morphogenetic protein 15 and growth and differentiation factor 9., *FEBS Lett* 584(4): 801–805.
URL: <http://dx.doi.org/10.1016/j.febslet.2009.12.052>
- Urist, M. R. (1965). Bone: formation by autoinduction., *Science* 150(698): 893–899.
- Urist, M. R. & Strates, B. S. (1971). Bone morphogenetic protein., *J Dent Res* 50(6): 1392–1406.
- van Gils, M. P. M. Q., Stenman, U. H., Schalken, J. A., Schroder, F. H., Luijck, T. M., Lilja, H., Bjartell, A., Hamdy, F. C., Pettersson, K. S. I., Bischoff, R., Takalo, H., Nilsson, O., Mulders, P. F. A. & Bangma, C. H. (2005). Innovations in serum and urine markers in prostate cancer current European research in the P-Mark project., *Eur Urol*

- 48(6): 1031–1041.
URL: <http://dx.doi.org/10.1016/j.eururo.2005.06.009>
- Vukicevic, S., Basic, V., Rogic, D., Basic, N., Shih, M. S., Shepard, A., Jin, D., Dattatreymurty, B., Jones, W., Dorai, H., Ryan, S., Griffiths, D., Maliakal, J., Jelic, M., Pastorcic, M., Stavljenic, A. & Sampath, T. K. (1998). Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat., *J Clin Invest* 102(1): 202–214.
URL: <http://dx.doi.org/10.1172/JCI2237>
- Vukicevic, S. & Grgurevic, L. (2009). BMP-6 and mesenchymal stem cell differentiation., *Cytokine Growth Factor Rev* 20(5-6): 441–448.
URL: <http://dx.doi.org/10.1016/j.cytogfr.2009.10.020>
- Vukicevic, S., Helder, M. N. & Luyten, F. P. (1994). Developing human lung and kidney are major sites for synthesis of bone morphogenetic protein-3 (osteogenin)., *J Histochem Cytochem* 42(7): 869–875.
- Vukicevic, S., Kopp, J. B., Luyten, F. P. & Sampath, T. K. (1996). Induction of nephrogenic mesenchyme by osteogenic protein 1 (bone morphogenetic protein 7)., *Proc Natl Acad Sci U S A* 93(17): 9021–9026.
- Vukicevic, S., Latin, V., Chen, P., Batorsky, R., Reddi, A. H. & Sampath, T. K. (1994). Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes., *Biochem Biophys Res Commun* 198(2): 693–700.
URL: <http://dx.doi.org/10.1006/bbrc.1994.1100>
- Vukicevic, S., Paralkar, V. M., Cunningham, N. S., Gutkind, J. S. & Reddi, A. H. (1990). Autoradiographic localization of osteogenin binding sites in cartilage and bone during rat embryonic development., *Dev Biol* 140(1): 209–214.
- Vukicevic, S. & Sampath, K. T. (eds) (2008). *Bone morphogenetic proteins: From Local to Systemic Therapeutics*, Springer.
URL: <http://www.springer.com/978-3-7643-8551-4>
- Wajih, N., Borrás, T., Xue, W., Hutson, S. M. & Wallin, R. (2004). Processing and transport of matrix gamma-carboxyglutamic acid protein and bone morphogenetic protein-2 in cultured human vascular smooth muscle cells: evidence for an uptake mechanism for serum fetuin., *J Biol Chem* 279(41): 43052–43060.
URL: <http://dx.doi.org/10.1074/jbc.M407180200>
- Wang, S. & Hirschberg, R. (2011). Y-box protein-1 is a transcriptional regulator of BMP7., *J Cell Biochem* 112(4): 1130–1137.
URL: <http://dx.doi.org/10.1002/jcb.23027>
- Wermter, C., Howel, M., Hintze, V., Bombosch, B., Aufenvenne, K., Yiallourous, I. & Stocker, W. (2007). The protease domain of procollagen C-proteinase (BMP1) lacks substrate selectivity, which is conferred by non-proteolytic domains., *Biol Chem* 388(5): 513–521.
URL: <http://dx.doi.org/10.1515/BC.2007.054>
- Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R. & Nusse, R. (2003). Wnt proteins are lipid-modified and can act as stem cell growth factors., *Nature* 423(6938): 448–452.
URL: <http://dx.doi.org/10.1038/nature01611>

- Wobus, A. M. & Boheler, K. R. (2005). Embryonic stem cells: prospects for developmental biology and cell therapy., *Physiol Rev* 85(2): 635–678.
URL: <http://dx.doi.org/10.1152/physrev.00054.2003>
- Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. & Wang, E. A. (1988). Novel regulators of bone formation: molecular clones and activities., *Science* 242(4885): 1528–1534.
- Yeung, B., Porter, T. J. & Vath, J. E. (1997). Direct isoform analysis of high-mannose-containing glycoproteins by on-line capillary electrophoresis electrospray mass spectrometry., *Anal Chem* 69(13): 2510–2516.
- Yim, K., Abrams, J. & Hsu, A. (1995). Capillary zone electrophoretic resolution of recombinant human bone morphogenetic protein 2 glycoforms. An investigation into the separation mechanisms for an exquisite separation., *J Chromatogr A* 716(1-2): 401–412.
- Yocum, A. K., Gratsch, T. E., Leff, N., Strahler, J. R., Hunter, C. L., Walker, A. K., Michailidis, G., Omenn, G. S., O'Shea, K. S. & Andrews, P. C. (2008). Coupled global and targeted proteomics of human embryonic stem cells during induced differentiation., *Mol Cell Proteomics* 7(4): 750–767.
URL: <http://dx.doi.org/10.1074/mcp.M700399-MCP200>

IntechOpen



Tandem Mass Spectrometry - Applications and Principles

Edited by Dr Jeevan Prasain

ISBN 978-953-51-0141-3

Hard cover, 794 pages

Publisher InTech

Published online 29, February, 2012

Published in print edition February, 2012

Tandem Mass Spectrometry - Applications and Principles presents comprehensive coverage of theory, instrumentation and major applications of tandem mass spectrometry. The areas covered range from the analysis of drug metabolites, proteins and complex lipids to clinical diagnosis. This book serves multiple groups of audiences; professional (academic and industry), graduate students and general readers interested in the use of modern mass spectrometry in solving critical questions of chemical and biological sciences.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Genadij Razdorov and Slobodan Vukicevic (2012). The Use of Mass Spectrometry in Characterization of Bone Morphogenetic Proteins from Biological Samples, Tandem Mass Spectrometry - Applications and Principles, Dr Jeevan Prasain (Ed.), ISBN: 978-953-51-0141-3, InTech, Available from:

<http://www.intechopen.com/books/tandem-mass-spectrometry-applications-and-principles/the-use-of-mass-spectrometry-in-characterization-of-bone-morphogenetic-proteins-from-biological-samp>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen