

CONTENTS

	Page
CONTENTS	(6)
LIST OF TABLES	(10)
LIST OF FIGURES	(11)
CHAPTER	
1 INTRODUCTION	1
Review of literature	
1. Embryogenesis.....	2
2. Bone cell	
2.1 Mesenchymal stem cells	3
2.2 Osteoprogenitor cells	3
2.3 Osteoblasts.....	4
2.4 Osteocytes.....	4
2.5 Osteoclasts	5
3. Bone turnover and remodeling	6
4. Systemic regulation of bone metabolism	
4.1 Parathyroid hormone	7
4.2 Vitamin D ₃	8
4.3 Estrogen	8
5. Bone morphogenetic protein as local regulation of bone metabolism	8
6. Expression of BMP in tissue and organ.....	11
7. Intracellular signaling of BMPs.....	15
8. Purification of osteoinductive factors in bone tissue.....	17
Aims of the study.....	21

CONTENTS (Continue)

	Page
2 MATERIALS AND METHODS	
Material	
1. Bone.....	22
2. Bacterial strain.....	22
3. Plasmid vector	23
4. Primer for RT-PCR amplification.....	24
5. Chemicals.	24
6. Restriction enzyme	26
7. Equipment and instruments.....	26
Methods	
Part I Illustration the expression patterns of BMPs family member in primary culture derived from intramembranous and endochondral bone.	
1. Culture of human osteoblastic cells	31
2. Histochemical staining of alkaline phosphatase activity.....	32
3. Analysis the expression patterns of BMPs family member in primary culture derived from intramembranous and endochondral bone	32
4. Agarose gel electrophoresis	34
5. Purify the DNA fragments from the gel	34
6. Ligation of cDNA fragment to pGEM- T Easy	35
7. Transformation of inserted-DNA to competent cells.....	35
8. Purify the plasmid containing inserted-DNA.....	37
9. DNA Sequencing	37
10. Analysis of nucleotide sequencing	38
11. Verify the specificity of the RT-PCR product.....	38
12. Semi-quantitative RT-PCR product	38
13. Statistic Analysis	39

CONTENTS (Continue)

	Page
Part II Identify and compare the member of bone morphogenetic protein expression in human intramembranous and endochondral bone	
1. Tissue samples.....	39
2. Total RNA extraction.....	39
3. RT-PCR using degenerate oligonucleotide primers	40
4. Analysis of the nucleotide sequencing.....	41
5. Analysis the expression of the Bone morphogenetic protein mRNA in fresh human intramembranous and endochondral bone.....	41
6. Verify the specificity of the RT-PCR product	42
7. Statistic Analysis.....	42
Part III Identify the osteoinductive factors extracted from human intramembranous and endochondral bone	
1. Tissue preparation and Extraction.....	43
2. Sequential extraction.....	43
3. The protein determination.....	44
4. SDS-Polyacrylamide Gel Electrophoresis and silver staining.....	44
5. Heparin affinity column chromatography.....	45
3 RESULTS	
Part I Illustrate the expression patterns of BMPs family member in primary culture derived from intramembranous and endochondral bone	
1. Osteoblast culture and osteogenic marker alkaline phosphatase	46
2. Identify osteoblast-like cells by Alkaline phosphate (ALP) staining	47
3. Expression of specific BMPs mRNA members in primary culture of human intramembranous and endochondral bone.....	50
Part II Identity and compare the member of bone morphogenetic protein family expression in human intramembranous and endochondral bone	
1. BMPs member expression in human intramembranous bone	54

CONTENTS (Continue)

	Page
2. Expression of specific BMPs mRNA members in fresh normal human intramembranous and endochondral bone	59
3. Distinct expressed message from fresh human intramembranous and endochondral bone	63
Part III Identify the osteoinductive factors extracted from human intramembranous and endochondral bone	
1. Tissue preparation and sequential extraction	64
2. Protein profile of the intramembranous and endochondral bone.....	66
4 DISCUSSIONS	
Part I Illustrate the expression patterns of BMPs family member in primary culture derived from intramembranous and endochondral bone.....	69
Part II Identify and compare the member of BMPs family expression in human intramembranous and endochondral bone	71
Part III Identify the osteoinductive factors extracted from human intramembranous and endochondral bone.....	74
5 CONCLUSIONS	
Part I Illustrate the expression patterns of BMPs family member in primary culture derived from intramembranous and endochondral bone.....	78
Part II Identify and compare the member of BMPs family expression in human intramembranous and endochondral bone	78
Part III Identify the osteoinductive factors extracted from human intramembranous and endochondral bone.....	79
BIBLIOGRAPHY	81
APPENDIX	102
PUBLICATION	112
VITAE	113

LIST OF TABLES

Table		Page
1	Properties, roles, and location of bone morphogenetic proteins; BMPs	13
2	Reverse transcription polymerase chain reaction (RT-PCR) oligonucleotide primer sequences and expected amplification product sizes.....	28
3	RT- PCR parameters for automated DNA thermal cyclers.....	34
4	PCR parameters for sequencing.....	38
5	RT- PCR parameters for automated DNA thermal cyclers.....	41
6	Preparation of SDS- Polyacrylamide Gel	45
7	Relative abundance of RT-PCR amplified products comparing between intramembranous osteoblastic cell line and endochondral osteoblastic cell line...52	
8	Amplification DNA fragments from degenerated oligonucleotide primers and number of cognate clone which matched by BLAST program.....	56
9	Relative abundance of RT-PCR amplified products comparing between normal intramembranous and endochondral bone.	61
10	Bone samples achieved from patients who underwent necessary surgery	65
11	Molecular weight of osteoinductive factors and bone related component from various species.....	76

LIST OF FIGURES

Figure		Page
1	Amino acid alignment of the mature regions of 20 TGF- β -like proteins beginning at the first invariant cysteine residue.	10
2	BMP is recognized by cell surface receptor serine/threonine kinases, of which the type II receptor phosphorylates and activates the type I receptor kinase, which phosphorylates the C-terminus (C) of a receptor-activated Smad (R-Smad) protein, leading to conformational changes. Phosphorylated Smads hetero-oligomerize with the nonphosphorylated Smad4, translocate to the nucleus, and engage in target gene expression.	16
3	Heparin-Sepharose bound polypeptides extracted from intramembranous (lane 1-3) and endochondral (lane 4-6) tissues. (Scott et al., 1994).	20
4	Physical map of plasmid pGEM-T easy. The figure illustrates plasmid pGEM-T easy containing lacZ gene, multiple cloning sites, origin of replication of E.coli, ampicillin resistant marker gene.	23
5	Alkaline phosphatase histochemical staining of human mandibular bone cells (A-B) and control gingival fibroblast (C).	48
6	Alkaline phosphatase histochemical staining of human iliac crest bone cell (A, B) and control gingival fibroblast (C).	49
7	The expression of specific BMPs members comparing between primary cell culture derived from intramembranous (mandible) and endochondral (iliac and tibia) bone. First lane of each panel showed from intramembranous bone and the second one showed from endochondral bone, and GAPDH as internal control. 51	
8	Relative abundance of RT-PCR amplified products comparing between intramembranous and endochondral osteoblastic cell line.	52
9	Percentage abundance of RT-PCR amplified products of total BMPs in primary osteoblastic cell culture of (A) intramembranous and (B) endochondral bone...53	
10	Amplification products from RT-PCR using degenerated primer showed unequal expression messages of BMPs family.	55

LIST OF FIGURES (Continue)

Figure		Page
11	The graphic showed the alignment of acquired sequence (A1Mand) from intramembranous bone and the known sequence (BMP9).....	57
12	The graphic showed the alignment of acquired sequence (H1Mand) from intramembranous and known sequence (BMP15).....	58
13	The expression of specific BMPs members comparing between fresh normal intramembranous and endochondral bone. Figure 10 presented the band intensity from RT-PCR products from specific BMPs. First lane of each panel showed from intramembranous bone and the second one showed from endochondral bone, and GAPDH as internal control.....	60
14	Percentage abundance of RT-PCR amplified products of total BMPs in fresh normal intramembranous (A) and endochondral (B) bone.....	62
15	Relative abundance of RT-PCR amplified products comparing between fresh normal intramembranous and endochondral bone.	63
16	Intramembranous bones achieved from patients undergoing torectomy (torus palatinus) were cut into small pieces. The bones were composed of both cortical and cancellous part.....	66
17	Standart curve of absorbance (OD750) as a function of initial protein concentration.	67
18	(A) The SDS-PAGE profile of proteins extracted from intramembranous (mandibular and maxilla bone) and endochondral (iliac bone).....	68
19	Diagram proposed the relation of each BMP in process of bone formation through intramembranous and endochondral bone	80