Table 2. Genes regulated during osteoblastic differentiation of OYL primary cells by DNA microarray analysis

OYL Cells GeneName	OYL Cells Accession Number	OYL Cells fold change
metallothionein 1L	F26137	13.4
RAN binding protein 2	D42063	10.7
metallothionein 1L	X76717	10.4
ZFP		10.1
metallothionein 1G	J03910	5.8
matrix Gla protein	AW997681	5.3
matrix Gla protein	X53331	4.6
ESTs, Highly similar to	R99207	4.5
Sequence 39 from Patent WO9947655.	AX017495	4.5
crystallin, alpha B	S45630	4.0
metallothionein 1L	X97261	4.0
activated leucocyte cell adhesion molecule	AA777157	3.5
fibroblast growth factor 7 (keratinocyte growth factor)	AI075338	3.4
cardiac ankyrin repeat protein	X83703	3.2
glutaredoxin (thioltransferase)	X76648	3.1
Homo sapiens cDNA: FLJ21685 fis, clone COL09372	AK025338	3.1
integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	NM_002205	2.9
Sequence 12 from Patent WO9954460.	AX013690	2.7
adrenomedullin	S73906	2.5
transgelin	M95787	2.5
cardiac ankyrin repeat protein	X83703	2.4
collagen, type VIII, alpha 1	NM_001850	2.1
glucocorticoid-induced leucine zipper	BE295817	2.1
laminin, alpha 2 (merosin, congenital muscular dystrophy)	M59832	2.1
collagen, type XI, alpha 1	J04177	2.1
transgelin	D17409	2.0

Fold change, up regulated gene after 48 h OS induction.

Table 3. Genes regulated during osteoblastic differentiation of non-OPLL primary cells by DNA microarray analysis

non-OPLL Cells GeneName	non-OPLL Cells Accession Number	non-OPLL Cells fold change
small inducible cytokine subfamily A (Cys-Cys), member 15	AF088219	8.0
Sequence 39 from Patent WO9947655.	AX017495	6.4
metallothionein 1G	J03910	5.7
Human metallothionein-II pseudogene (mt-IIps).	J00272	5.4
metallothionein 1L	F26137	5.1
collagen, type VIII, alpha 1	NM_001850	5.0
RAN binding protein 2	D42063	5.0
ZFP		4.7
matrix Gla protein	AW997681	4.6
ESTs, Highly similar to	R99207	4.5
metallothionein 1L	X76717	4.4
cardiac ankyrin repeat protein	X83703	4.2
matrix Gla protein	X53331	4.2
crystallin, alpha B	S45630	4.1
integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	NM_002205	3.9
collagen, type XI, alpha 1	J04177	3.8
dual specificity phosphatase 1	X68277	3.7
cardiac ankyrin repeat protein	X83703	3.6
nidogen (enactin)	NM_002508	3.5
insulin receptor substrate 2	AB000732	3.4
adrenomedullin	NM_001124	3.2
integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	X06256	3.2
DKFZP564G013 protein	AF155135	3.1
alcohol dehydrogenase 2 (class I), beta polypeptide	AF153821	2.9
MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D)	L16794	2.9
adrenomedullin	S73906	2.9
Sequence 12 from Patent WO9954460.	AX013690	2.7
Sequence 58 from Patent WO9947669.	AX017303	2.7
alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	NM_001150	2.4
CEGP1 protein	CAB92285	2.3
activated leucocyte cell adhesion molecule	AA777157	2.3
glucocorticoid-induced leucine zipper	BE295817	2.3
fibroblast growth factor 7 (keratinocyte growth factor)	AI075338	2.2
glutaredoxin (thioltransferase)	X76648	2.2
quinone oxidoreductase homolog	AF010309	2.1
milk fat globule-EGF factor 8 protein	NM_005928	2.1
follistatin-like 1	U06863	2.0

Fold change, up regulated gene after 48 h OS induction.

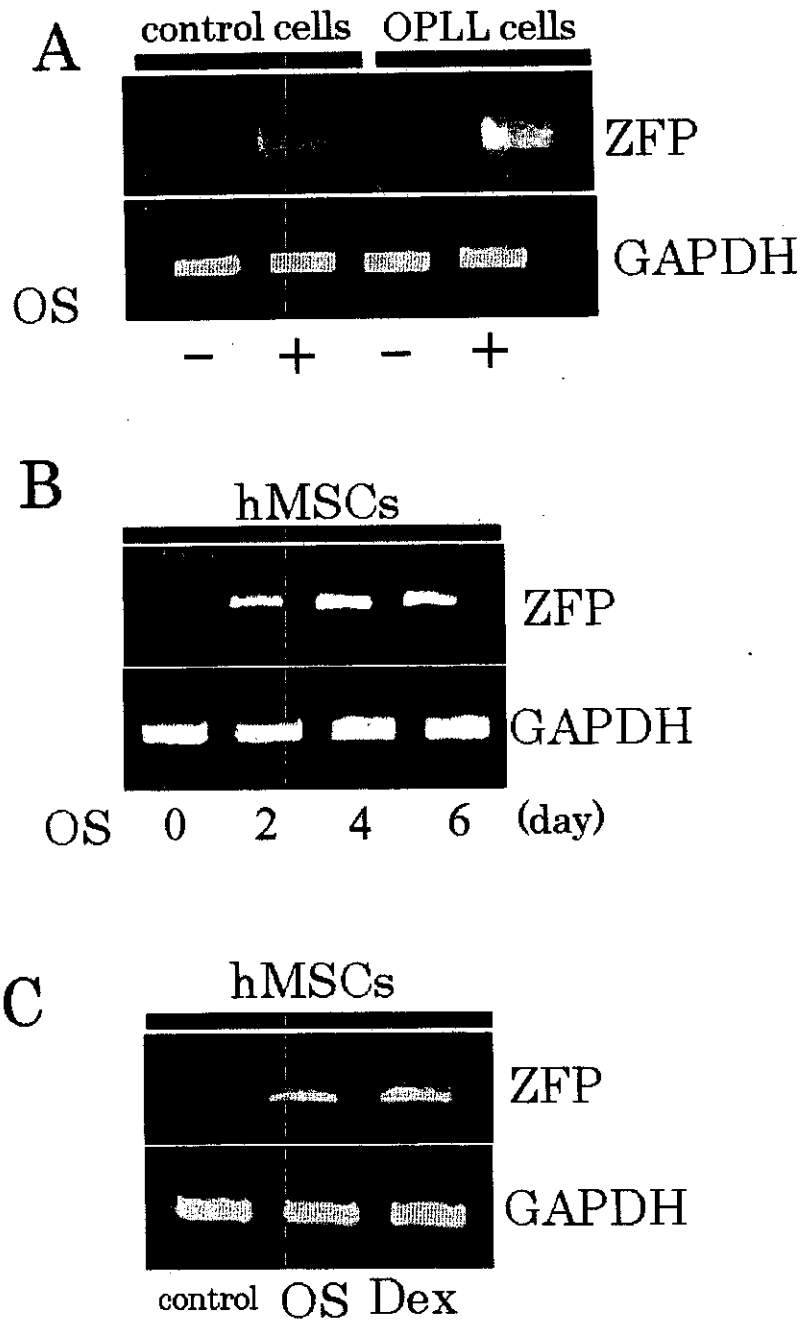

図２　分化誘導剤（OS）、デキサメタゾンによるZFPの発現亢進

A) OPLLもしくはcontrolからの靭帯細胞を分化誘導剤で処理した。４８時間後にtotal RNAを抽出し、ZFPの発現量をRT-PCRで確認した。OPLL靭帯細胞は、control靭帯細胞より高い発現を示した。B) ヒト間葉系幹細胞（hMSC）を分化誘導剤で２、４、６日間培養しtotal RNA抽出後、RT-PCRでZFPの発現量を調べた。C) 分化誘導剤中に含まれるデキサメタゾン（Dex）によってもZFPの発現が亢進された。

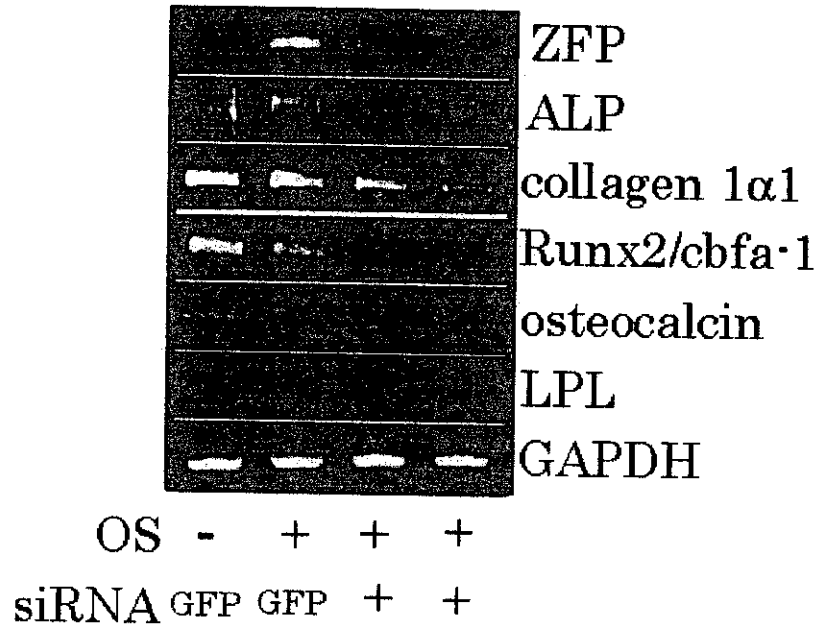

図３ ZFP siRNA の骨芽細胞への抑制効果

ZFP の siRNA を hMSC にリポフェクトアミンを用いて導入した。導入２４時間後に分化誘導剤で４８時間処理し、total RNA を抽出した。RT-PCR を行い、ZFP, ALP, collagen 1α1, Runx2/cbfa-1, osteocalcin, LPL, GAPDH の発現量を調べた。

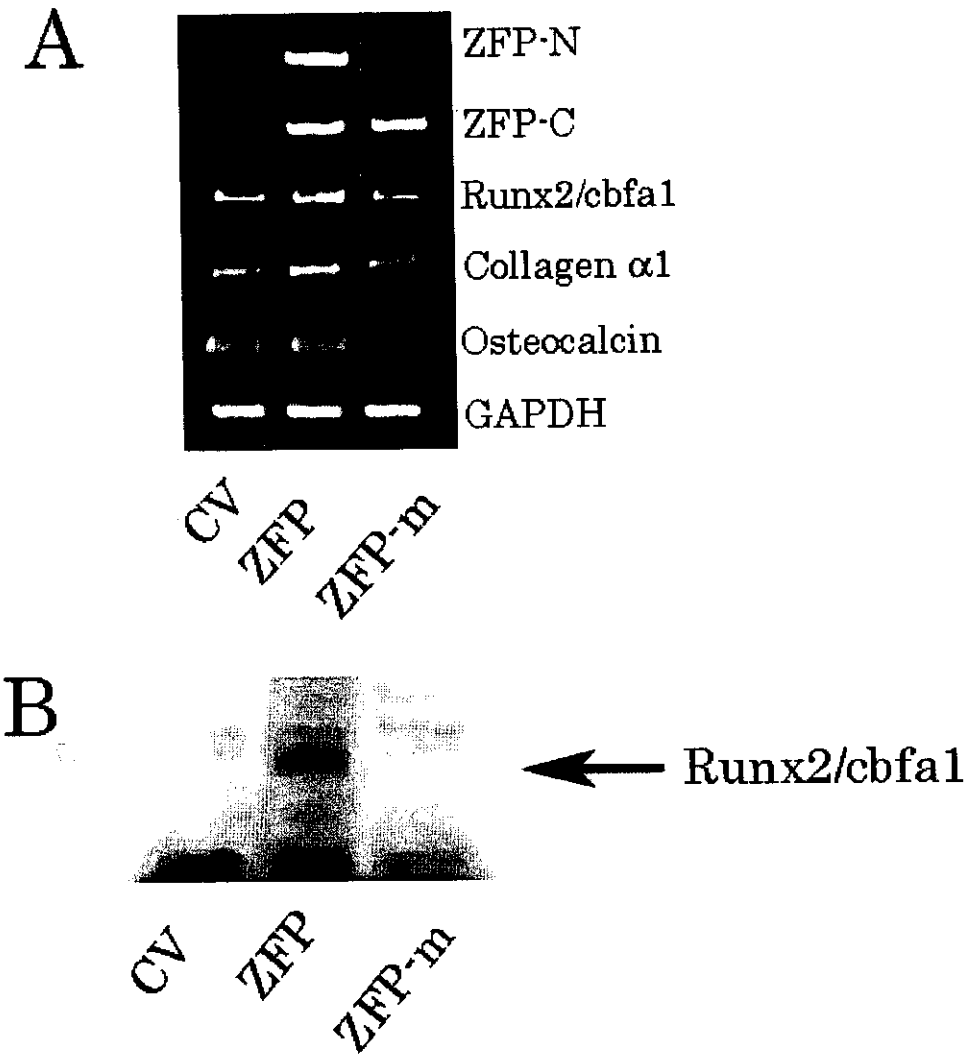

図４ ZFP が C2C12 細胞の骨芽細胞系遺伝子の発現に及ぼす影響

A) ZFP、N 末端欠損 ZFP をマウス未分化細胞株 C2C12 細胞に強制発現させた。 これらの細胞から total RNA を抽出し、Runx2/cbfa1, collagen α1, osteocalcin の発現量を調べた。 B) ZFP、N 末端欠損 ZFP をマウス未分化細胞株 C2C12 細胞に強制発現させ、total cell lysate を用いて Runx2 のタンパク量をイムノブロット解析にて比較した。

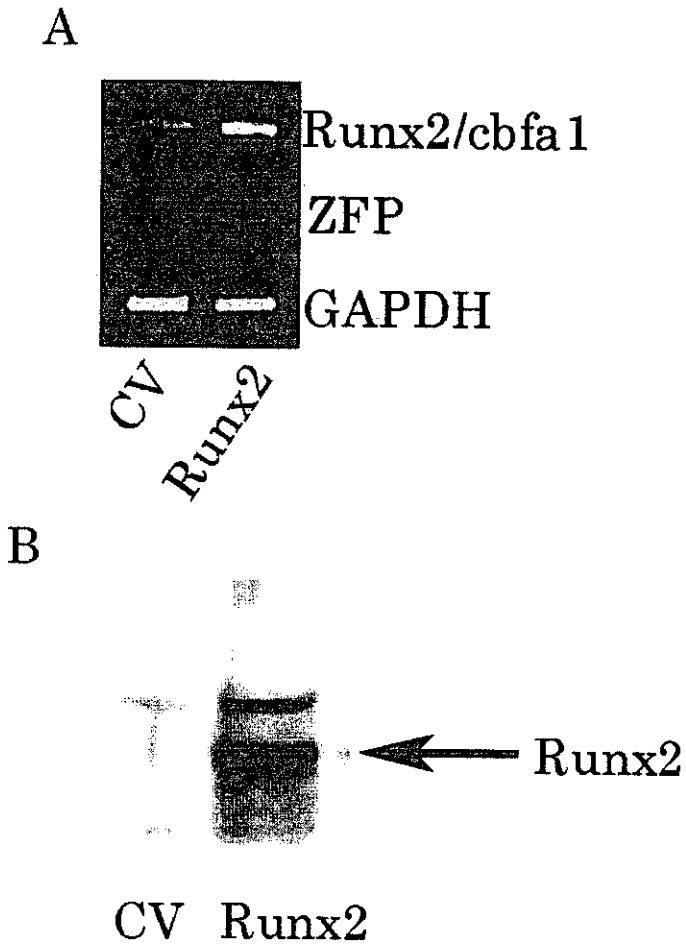

図５ Runx2/cbfa1 が ZFP の発現に及ぼす影響

A) Runx2/cbfa1 をマウス未分化細胞株 C2C12 細胞に強制発現させた。 これらの細胞から total RNA を抽出し、ZFP の発現量を調べた。 B) Runx2/cbfa1 をマウス未分化細胞株 C2C12 細胞に強制発現させ、total cell lysate を用いて Runx2 のタンパク量をイムノブロット解析した。

別添６

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Onda H, Kasuya H, Yoneyama T, Hori T, Nakajima T, Inoue I.	Endoglin is not a major susceptibility gene for intracranial aneurysm among Japanese.	*Stroke*		in press	2003
Shimo-onoda K, Tanaka T, Furushima K, Nakajima T, Toh S, Harata S, Yone K, Komiya S, Adachi H, Nakamura E, Fujimiya H, Inoue I.	Akaike's information criterion for an alternative measure of linkage disequilibrium.	*J Hum Genet*	47	649-655	2002
Maeda S, Nobukuni T, Shimo-onoda K, Hayashi K, Yone K, Komiya S, Inoue I.	Sortilin is up-regulated during osteoblastic differentiation of mesenchymal stem cells and promotes extracellular matrix mineralization.	*J Cell Pysiol*	193	73-79	2002
Kobayashi Y, Nakajima T, Inoue I.	Molecular modeling of the dimeric structure of human lipoprotein lipase and functional studies of the carboxyl-terminal domain.	*Eur J Biochem*	269	4701-4710	2002
Ohmori H, Makita Y, Funamizu M, Hirooka K, Hosoi T, Orimo H, Suzuki T, Ikari K, Nakajima T, Inoue I, Hata A.	Linkage and association analyses of the osteoprotegerin gene locus with human osteoporosis.	*J Hum Genet*	47	400-406	2002
Rohrwasser A, Zhang S, Dillon HF, Inoue I, Callaway CW, Hillas E, Lalouel JM.	Contribution of Sp1 to initiation of transcription of angiotensinogen.	*J Hum Genet*	47	249-56	2002
Nakajima T, Jorde LB, Ishigami T, Umemura S, Emi M, Lalouel J-M, Inoue I.	Nucleotide diversity and haplotype structure of the human angiotensinogen gene in two populations.	*Am. J. Hum. Genet.*	70	108-123	2002

Furushima K, Shimo-onoda K, Maeda S, Nobukuni T, Ikari K, Koga H, Komiya S, Nakajima T, Harata S, Inoue I.	Large scale screening for candidate genes of ossification of the posterior longitudinal ligament of the spine.	*J. Bone Miner. Res.*	17	128-137	2002
Tsuda E, Ishibashi Y, Okamura Y, Toh S.	ligament-hamstring reflex arc after anterior cruciate ligament reconstruction.	*Restoration of anterior cruciate Knee Surg Sports Traumatol Arthrosc*	2	63-7	2003
Otsuka H, Ishibashi Y, Tsuda E, Sasaki K, Toh S.	Comparison of three techniques of anterior cruciate ligament reconstruction with bone-patellar tendon-bone graft: differences in anterior tibial translation and tunnel enlargement with each technique.	Am J Sports Med	31(2)	282-8	2003
Ohishi H, Furukawa KI, Iwasaki K, Ueyama K, Okada A, Motomura S, Harata S, Toh S.	Role of prostaglandin I2 in the gene expression induced by mechanical stress in spinal ligament cells derived from patients with ossification of the posterior longitudinal ligament.	*J Pharmacol Exp Ther*		in press	2003
Toh S, Miura H, Arai K, Yasumura M, Wada M, Tsubo K.	Scaphoid fractures in children: problems and treatment.	*J Pediatr Orthop*	23 (2)	216-21	2003
Toh S, Tsubo K, Nishikawa S, Inoue S, Nakamura R, Narita S.	Osteosynthesis for nonunion of the lateral humeral condyle.	*Clin Orthop*	405	230-41	2002
Nishikawa S, Toh S.	Anatomical study of the carpal attachment of the triangular fibrocartilage complex.	*J Bone Joint Surg Br*	84 (7)	1062-5	2002
Vallejo GI, Toh S, Arai H, Arai K, Harata S.	Results of the latissimus dorsi and teres major tendon transfer on to the rotator	*Scand J Plast Reconstr Surg Hand Surg*	36(4)	207-11	2002

	cuff for brachial plexus palsy at birth.				
Ikeda R, Furukawa T, Mitsuo R, Noguchi T, Kitazono M, Okumura H, Sumizawa T, Haraguchi M, Che XF, Uchimiya H, Nakajima Y, Ren XQ, Oiso S, Inoue I, Yamada K, Akiyama S.	Thymidine phosphorylase inhibits apoptosis induced by cisplatin.	*Biochem Biophys Res Commun*	301	358-63	2003

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以降は雑誌/図書に掲載された論文となりますので、
P.25-P.27の「研究成果の刊行に関する一覧表」をご参照ください。