

A novel calcitonin receptor gene in human osteoclasts from normal bone marrow

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Abstract The calcitonin receptor (CTR) gene in human osteoclasts formed in a human bone marrow cell culture system was examined by reverse transcription-polymerase chain reaction (RT-PCR). The RT-PCR results indicated that the 5'-untranslated region (5'UTR) was different between CTR mRNAs in human osteoclasts and in a mammary tumor cell line, MCF-7 cells. We isolated the 5'UTR of the CTR gene from human osteoclasts, whose sequence had only 28.6% identity with that of other CTR genes reported until now. In a radioligand binding assay, COS-1 cells transfected with the osteoclast CTR gene bound to [¹²⁵I]human CT (hCT). These results provided evidence that the CTR gene cloned from human osteoclasts was expressed functionally and its coding protein was identical to MCF-7 cell CTR.

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Key words: Calcitonin receptor; Human; Osteoclast; MCF-7; Calcitonin; Gene

1. Introduction

Calcitonin (CT) [1], first discovered by Copp et al. in 1962, is a 32 amino acid polypeptide hormone that is an important regulator of Ca²⁺ homeostasis through its direct action on osteoclasts inhibiting bone-resorbing activity and on renal tubular cells where it stimulates urinary calcium excretion [2,3]. CT also acts on other tissues, i.e. the central nervous system [4,5], lungs [6], testes [7], placenta [8] and kidney [2]. All physiological functions of CT have yet to be determined, but its primary role is thought to be carried out by its binding to cell surface receptors on osteoclasts. CT has a unique ability of inhibiting bone resorption by reducing the number of osteoclasts and their activities [9] and therefore, it is one of the most common reagents in the clinical treatment of osteoporosis [10], Paget's disease [11] and hypercalcemia induced by malignant tumors [12,13]. However, usage of CT has an important problem which is yet to be solved: resistance to CT response known as the 'escape' phenomenon as a result of continuous administration of the hormone [13,14]. This influence of CT is more significant on human osteoclasts than on cancer cell lines and on other tissue cells in human or other species [15–18]. It is suggested that this problem may result

from a prolonged reduction of the number of CT receptors (CTRs) on osteoclasts [19].

The initial cloning of a cDNA encoding CTR was reported in a porcine renal cell line LLC-PK1 [20]. Since that study, several isoforms of human CTRs (hCTRs) in tumor cell lines have been reported: BIN-67 [21] (ovarian carcinoma), TT [22] (medullary thyroid carcinoma), T47D [23], MCF-7 [24] (mammary carcinoma) and giant cell tumor (GCT) of bone [25]. However, the CTR gene in human osteoclasts has never been reported, mainly due to the difficulties in obtaining and maintaining osteoclasts. Recently, we have established a simple and efficient system for the development of human osteoclasts in normal bone marrow cells. This method has provided us with numerous osteoclasts and enabled us to study the human osteoclast gene in detail [26]. In this report, we describe the cloning of CTR cDNA from human osteoclasts.

2. Materials and methods

2.1. Osteoclast formation

Osteoclasts were isolated from human bone marrow cells obtained from the proximal femur at the time of arthroplasty. A monocyte-rich fraction was isolated from bone marrow cells with Histopaque 1077 (Sigma) as described previously [27] with modifications and cultured in α -minimum essential medium (α -MEM) (Irvine Scientific) supplemented with 20% heat-inactivated horse serum (Gibco BRL) and 10⁻⁸ M 1 α ,25-dihydroxy vitamin D₃ (kindly supplied by Teijin) for 2–3 weeks and osteoclast-like multinucleated cells (MNCs) were formed. These MNCs possessed the osteoclast characteristics, such as multinuclei, staining with tartrate resistant acid phosphatase and reduction of bone resorption activity in response to CT [26]. The MNCs in the culture were treated with 0.002% pronase E (Sigma)/0.02% EDTA in phosphate-buffered saline (PBS(-), pH 7.4) for 5 min to remove stroma cells and adherent osteoclasts were cultured overnight and used for extraction of total RNA.

2.2. Preparation of total RNA from human osteoclasts and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from human osteoclasts was extracted by the acid guanidinium-phenol-chloroform method [28]. Reverse transcription (RT) of total RNA to cDNA and subsequent amplification (PCR) were accomplished using reagents from TaKaRa LA PCR kit (AMV) Ver.1.1 (TaKaRa biomedical). Synthesized primers were shown in Fig. 1. 4.5 μ g of total RNA was reverse-transcribed by AMV reverse transcriptase for 10 min at 30°C, followed by 1 h at 42°C. The reaction was terminated by heating for 5 min at 99°C, followed by chilling to 4°C. RT samples were used for PCR. After the reaction was heated at 94°C for 2 min, 55 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C and extension for 2 min at 72°C were performed in a Perkin-Elmer/Cetus DNA thermal cycler and followed by a final extension at 72°C for 7 min. R2 was used as a reverse primer otherwise mentioned.

2.3. Cloning of the 5'-untranslated region (5'UTR)

Amplification and isolation of the 5'UTRs of CTR genes in osteoclasts and MCF-7 cells was performed in total RNAs of both cells

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Abbreviations: CT, calcitonin; CTR, calcitonin receptor; hCT, recombinant human calcitonin; MNC, multinucleated cell; PCR, polymerase chain reaction; MEM, minimum essential medium

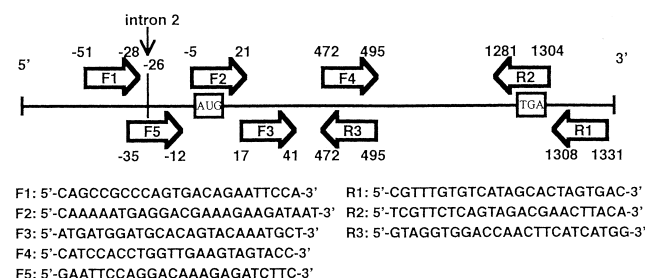


Fig. 1. Primer sequences in RT-PCR referred to the CTR cDNA in T47D cells. Forward primers: F1, F2, F3 and F4. Reverse primers: R1, R2 and R3.

using the rapid amplification of cDNA ends (5'RACE system Ver.2.0, Gibco BRL) method. First strand cDNA was synthesized from 1 µg of total RNA using gene specific primer 1 (GSP1, 5'-GACCCACAA-TAGCCAAATAG-3'). After the purification of this first strand of cDNA, terminal deoxynucleotidyl transferase and dCTP were used to add homopolymeric tails to the 3'-ends of the cDNA. PCR was amplified using GSP2 (5'-CAGGAGTGAAAGCATTGCACA-3') and the abridged anchor primer was provided by this system. Re-amplification of primary PCR product employed nested GSP (5'-GCCATTTTCATAAATTGCGGGCAGC-3') and the abridged universal amplification primer was produced with this method. The amplified fragments were cloned into pCR2.1 (Invitrogen) directly. The nucleotide sequences of these clones were determined in both strands by the dideoxy termination method.

2.4. Cloning of human osteoclast CTR cDNA

The first strand cDNA was synthesized from the isolated total RNA with random 9-mer primers and the product was amplified using the RNA PCR kit (AMV) Ver.2.1 (TaKaRa). The sense primers used to amplify CTR cDNA from osteoclasts and MCF-7 cells were 5'-CCAGTGAGAAGTATGAGAGAGTG-3' and 5'-CAGGAAG-GCGCCGGGAA-3', respectively, and the antisense primer was 5'-ACATTCAAGCAGATGACTCTTGCT-3'. 55 Cycles of denaturation for 20 s at 95°C, annealing for 30 s at 60°C and extension for 1 min at 72°C were performed in a Perkin-Elmer/Cetus DNA thermal cyclor.

2.5. Transfection of COS-1 cells with CTR cDNA including the 5'UTR region

Amplified CTR genes from human osteoclasts and MCF-7 cells were respectively inserted into pCR3.1 (Invitrogen) under its cytomegalovirus promoter. pCMVOC1 and pCMVOC2 represent the expression plasmids inserted with osteoclast CTR cDNA in the sense and antisense direction, respectively, and pCMVMC is the plasmid containing CTR cDNA from MCF-7 cells in the sense direction. Monolayers of COS-1 cells (from the Health Science Research Resources Bank) in 10 cm culture dishes (Corning) were prepared in 5 ml Dulbecco's MEM (DMEM; Gibco BRL) containing 1 g/l glucose and 2 mM L-glutamine. Cells were transfected with 28 µg each of plasmid using FuGENE 6 transfection reagent (Boehringer Mannheim). Cells were incubated at 37°C in a 5% CO₂ atmosphere. 6 h after transfection, 5 ml DMEM containing 10% fetal bovine serum (FBS; Irvine Scientific) was added to the medium in culture. After an additional 18 h, the medium was replaced with 10 ml DMEM/10% FBS. 48 h after transfection, a radioligand binding assay was performed as described below.

2.6. Binding of radiolabelled hCT to COS-1 cells

Radioligand binding assays were performed as described previously [21] with a slight modification. In brief, COS-1 cells transfected with a hCTR gene were washed with PBS(–) once and dispersed by trypsinization. After centrifugation at 1000 rpm for 5 min, cell pellets were suspended in a binding buffer and counted. A portion of 3–5 × 10⁵ cells was divided into a 12 × 75 mm glass tube in a volume of 200 µl of binding buffer (PBS(–), pH 7.4, 11 mM glucose, 1% bovine serum albumin) plus 200 pmol of [¹²⁵I]hCT (Amersham) in the presence of a 10³–10⁵-fold molar excess of unlabelled hCT (generously supplied by Sankyo). The incubation time was 16 h at 4°C. Then, 100 µl of the cell

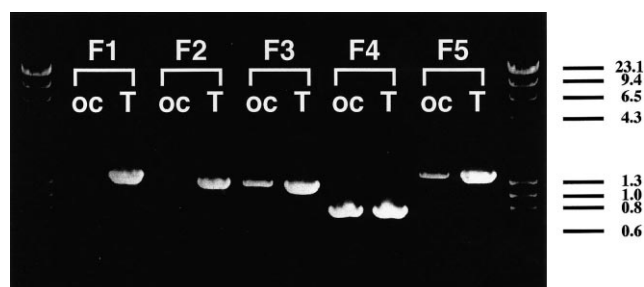


Fig. 2. RT-PCR analysis of CTR mRNA of human osteoclasts and T47D cells (mammary tumor cell line) using various kinds of primers. Total RNA extracted from human osteoclasts (OC) and T47D cells (T) was used.

suspension was layered over 200 µl of 10% sucrose (w/v)-containing binding buffer in a 1.5 ml microfuge tube (Bio-Rad Laboratories) and centrifuged at 10000 rpm for 5 min to precipitate the cells. The sucrose and incubation buffer were then removed by aspiration and the centrifuge tube containing only cells was counted for radioactivity in a gamma counter (Aloka). Saturation ligand binding to COS-1 cells transfected with the hCTR was performed using the same technique in the presence or absence of a 10³-fold molar excess of unlabelled hCT with an increasing concentration of [¹²⁵I]hCT. The K_d value and the number of binding sites were calculated by Scatchard analysis.

AGTTATAAGA	GACAGAAATA	CCAGTGAGAA	GTATGAGAGA	GTGGGTGGGA	GATAATGTTT	60
AGAATCTCTT	TGCTGCCCCG	CAATTATATG	AAATGGCTTG	AAAATATTTA	TGGTCAAAGA	120
CCGAAATATT	TCTTCAAAGA	AGATTAGCTT	TGCTCCATTA	AAAGTAATGA	GTAGAAATAT	180
TAAAAAATA	AAGTTTAAAG	TACCTGAGTA	TCTTGCCAGC	AACTGACCAC	CACTGCTAAA	240
GGTGAGGAGA	GACACAGCTT	TCATCATTTG	GACTGCAGTT	TATTTCAAGG	CAAGAGAGATC	300
TTCAAAAACC	AAAAATGAGG	TTCAACATTA	CAAGCCGGTG	CTTGGCACTG	TTTCTCTCTC	360
TAAATCACCC	AACCCCAATT	CTTCTGCCTT	TTTCAATCA	AACCTATCCA	ACAATAGACC	420
CCAAAGCCATT	TCTTTAGCTC	GTAGGACGAA	AGAAGATGAT	GGATGCACAG	TACAATGCTT	480
ATGACCGAAT	GCAGCAGTTA	CCCGCATACC	AAGGAGAAGG	TCCATATTGC	AATCGCACCT	540
GGGATGGATG	GCTGTGCTGG	GATGACACAC	CGGTGGAGT	ATTGTCTAT	CAGTTCTGCC	600
CAGATTATTT	TCCGATTTT	GATCCATCAG	AAAAGTTTAC	AAAATACCTG	GATGAAAAAG	660
GTGTTTGGTT	TAAACATCCT	GAAAAAATC	GAACCTGGTG	CAACTATACT	ATGTGCAATG	720
CTTCACTCC	TGAGAACTG	AAGAATGCAT	ATGTTCTGTA	CTATTGGCTT	ATTGTGGGTC	780
ATTCTTTGTC	AATTTTCACC	CTAGTGATTT	CCCTGGGGAT	TTTCTGTTT	TTCAGGAGCC	840
TTGGCTGCCA	AAGGTAACCC	CTGCACAAGA	ACATGTTTCT	TACTTACATT	CTGAATTTCTA	900
TGATTATCAT	CATCCACCTG	GTGGAAGTAG	TACCAATGAG	AGAGCTCGTG	CGAAGGGACC	960
CGGTGAGCTG	CAAGATTTTG	CATTTTITCC	ACCAGTACAT	GATGGCTCTG	AACTATTTCT	1020
GGATGCTCTG	TGAAGGGATC	TATCTTCATA	CATCATTTTG	CTTGGCTGTG	TTTACTGAGA	1080
AGCAACGCTT	CGGTGGTAT	TATCTCTTGG	GCTGGGGGTT	CCGCTGGTG	CCAACCACTA	1140
TCCATGCTAT	TACCAGGGCC	GTGTACTTCA	ATGACAACCT	CTGGCTGAGT	GTGGAACACC	1200
ATTGCTTTTA	CATAATCCAT	GGACCTGTCA	TGGCGCACT	TGTGTCAAT	TTCTTCTTTT	1260
TGCTCAACAT	TGTCGGGGTG	CTTGTGACCA	AAATGAGGGA	AACCCATGAG	GCGGAATCCC	1320
ACATGTACCT	GAAGGCTGTG	AAGGCCACCA	TGATCTTTTG	GCCCTGCTGT	GGAAATCCAGT	1380
TTGTCTGCTT	TCCCTGGAGA	CCTTCAACCA	AGATGCTTTG	GAAGATATAT	GATTACGTGA	1440
TGCACTCTCT	GATTCATTTT	CAGGGCTTCT	TTGTGCGAC	CATCTACTGC	TTCTGCAACA	1500
ATGAGTCCA	AACCAACCTG	AAGCCCAAT	GGGCCCAATT	CAAAATTCAG	TGGAACACGC	1560
GTGGGGGAG	GCGCCCTCC	AACGCTCTG	CTGCGCTGC	AGCCGCTGCT	GCGGAGGCTG	1620
GCGACATCCC	AATTTATCAT	TGCCATCAGG	AGCCGAGGAA	TGAACACGCG	AACAACCAAG	1680
GCGAGGAGAG	TGCTGAGATC	ATCCCTTTGA	ATATCATAGA	GCAAGAGTCA	TCTGCTTGA	1740
TGTGAAGCAA	ACACAGTATC	GTGATCACTG	AG			1772

Fig. 3. The nucleotide sequence of the CTR gene in human osteoclasts. The start and stop codons of the coding region are underlined. This coding region was identical to that of CTR cDNA in the MCF-7 cells, mammary tumor cell line. Conversely, 5'UTRs were quite different from each other with an identity of 45.0%, 143/317 base points in the 5'UTR of osteoclast CTR cDNA as described in Fig. 4.

3. Results

3.1. CTR mRNA expressed in human osteoclasts

The detection of CTR mRNA expression in human osteoclasts was related to the forward primer used for RT-PCR amplification (Figs. 1 and 2). When F1 or F2 was used as a forward primer, the amplified fragment was scarcely detected.

However, we detected the amplified fragments when any of the F3–6 primers were employed. In contrast, the amplification was independent of the reverse primer used (data not shown). This fact implied that CTR cDNA in osteoclasts had a common sequence with that in MCF-7 cells in the downstream region of the start codon. However, they differed from each other in the 5'UTR. Then, we cloned both CTR

Osteoclast	AGTT--ATAA	GAGACAG---	--GAATACCA	CTCAGAACTA	TGAGAGACTG	GGTG--GGA	GATAAT----
MCF 7	A-----	-----	-CAGCT----	-----	-GGGAGAGCG	CA-----	GGA AG-----
Porcine	-----	-----	-----	-----	-----	-----	-----
Rat	-----	GGCAC GAGCG	-----	-----	ACAGG	-----	-----
Mouse	AATTCCGCAC	GAGGCTGCAC	CCAGCTCCGG	ATAGGAGGTG	GAGGATAGCC	CCTCCTTGG	AGCAACTTGC
Osteoclast	-----	-----	-----	-----	G TTTAGAATCT	-----	-CTTTGCTG
MCF 7	-----	-----	GGCC-	-----	-----	-----	-----
Porcine	-----	-----	-----	-----	-----	-----	-----
Rat	-----	-----	-----	-----	-----	-----	-----
Mouse	TTTCCTCCCA	GGTGCGCCT	GCCAGCCAG	ACTGCCCCG	CCTGGAATCA	GATTGTCCG	GCTCCTGCTG
Osteoclast	CCCGCAATTT	ATGAAATGG	CTTGA----	-----	AAATA	TTTATGGTCA	AAGACCG----
MCF 7	-----	-----	GGGA A-	-----	-----	GGAAAGCCA	CC--CCACCA
Porcine	-----	-----	GGGA A-	-----	-----	GGACGGCCA	CC--CCCCA
Rat	-----	-----	-----	-----	GCA	AGGAGAGCCA	GTGCCC--A
Mouse	GCTTTCAGTG	GAGAAGGGGA	ATTGTCTCA	ACACCAGTC	AGGAGAGCCA	GCCGCCCA	AGACTCTGGG
Osteoclast	-----	-----	AAATATIT	CT--TCAAA	GAAG----	A TTAGCTTGC	TCCATTAAAA
MCF 7	GGGCTGCGCC	GCGCG-GCTG	GCGGACCTTC	CCGGGTGGA	GAGGTGCGCA	C-GTCCGAC	CTCACCCTGC
Porcine	GGGCTGCGCC	GCGCG-TTTC	TCCGACCTTC	CTGGGTGGA	GAGGTGCGCG	CCGTCCGAC	CTCACCCTGC
Rat	CGGA-----	CG--CAGCTT	GCAGACAAC	CCTGGTTGGA	GAGGTGCTT-	-----	CTGC--TCACCTGA
Mouse	CGACTGCACC	TGGGTGCTT	GCAGACAAC	CTTGGTTGGA	GAGGTGCTTA	CT-TCTGCG	CTCAGCTGC
Osteoclast	G-----	TAATGAG	TAGAAA--TA	TAAAAA----	AA	AAAAGTTTA	A--GTACCTG
MCF 7	GGCTGACATC	TCTGCCCCAG	GAGATGGCG	CTGAAG-CTT	GAGCGCTGA	G-----	-----
Porcine	GGCTGGAATC	TCCAATCCAG	GAGATGGCG	CTGAG-CCT	GAGTTCCTGA	G-----	-----
Rat	GGTTGGC-AQ	CCCC-----	-----	TGCCCA	GTGAAG-TCT	GAGTTCCTGA	GA-----
Mouse	CGTTGGCAT	CCCTGCCT-G	CAGATGCCCA	GTGAAGGTCT	GCTTTCCTGA	GAACACCTGA	GCTGTGCCCA
Osteoclast	AGTATCTTGC	CAGCAACT--	-----	GACCAC	CA-----	-----	CTGCTA
MCF 7	-----	-----	TCC	CTGGAGCCAG	-ACCTGCCAA	CACCTTTG-	-----
Porcine	-----	-----	TCT	CTGGAGCCAG	CACCTGCCAA	TGACCTTCG-	-----
Rat	--CATCCAGC	TAGAGAATTG	CTGCGTCCAG	CTAAGGTAAG	TGCCATT--	-----	CCA
Mouse	GACATCCAGC	AAGAGAATTG	CTGATCCAG	CTAAGGTAAG	TGCCATTAGA	GCGCCTTCCA	GAGGAGAAGA
Osteoclast	GA-----	G ACACAGCTTT	CAT-CATTGG	-----	GACTGCAGTT	TATTCAGGA	CAAGAG---
MCF 7	--TTGAG--	-----	CTG	TGCCAGGCG	CCG----	AGT	GAG-----
Porcine	--CTGAG--	-----	CTG	TGCCAGGCG	CCG----	AGT	GAG-----
Rat	AACCGAGGGA	GCACGGCTTC	TGAACAGCG	ATCTGAAAT	GACTCCACCG	AGATCCAGAA	TGAAAAGGCG
Mouse	AACCGAGGGA	GCACGGCTAC	TACACAGAGG	ATTTGAAAT	GACTCCACCG	AGGTCCAGAG	TGAAAAGGCG
Osteoclast	--ATCTTCAA	AAACCAAAAA	TGAGGTTG	-----	-----	-----	-----
MCF 7	--ATCTTCAA	AAACCAAAAA	TGAGGTTG	-----	-----	-----	-----
Porcine	TCATCTTCAA	AAATCAAAAA	TGAGGTTG	-----	-----	-----	-----
Rat	GAACCTCCGA	AAACCAAGA	TGAGGTTG	-----	-----	-----	-----
Mouse	GAATCTCCGC	AAACCAAGA	TGAGGTTG	-----	-----	-----	-----

Fig. 4. The difference among nucleotide sequences of 5'UTRs of CTR genes in human osteoclasts, MCF-7 cell line, rat brain, mouse brain and porcine kidney cell line (LLC-PK1).

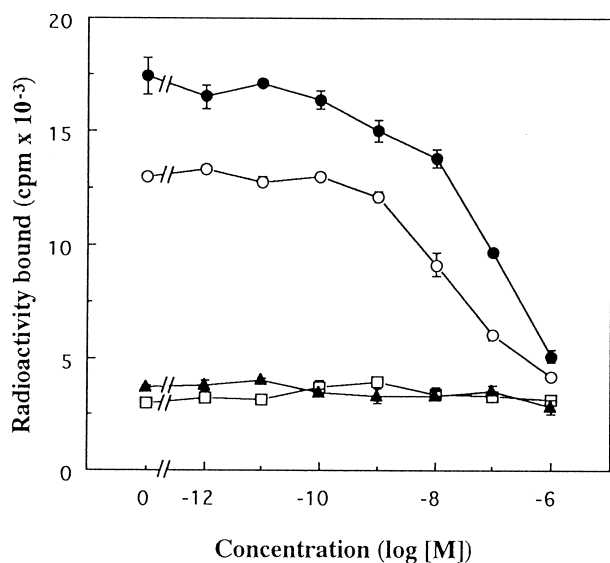


Fig. 5. Radioligand binding assay of hCT in COS-1 cells transfected with CTR cDNA from human osteoclasts and MCF-7 cells. COS-1 cells transfected with four plasmids, pCR3.1 (\square), pCMVOC1 (\circ), pCMVOC2 (\blacktriangle) and pCMVMC (\bullet) bound to [125 I]hCT in the presence or absence of a 10^{-5} -fold molar excess of unlabelled hCT.

cDNAs containing the 5'UTR and coding region from human osteoclasts and MCF-7 cells. The nucleotide sequence of human osteoclast CTR cDNA was shown in Fig. 3.

The deduced coding region of CTR in human osteoclasts was identical to that in MCF-7 cell lines, however, 45% of 5'UTRs (143/317 nucleotide base) in the osteoclast gene were different from that in MCF-7 cells. There were no similarities in 5'UTR of cDNAs in human osteoclasts and others, rat brain, mouse brain and porcine kidney cell line (LLC-PK1) (Fig. 4). The sequence identity was 72.7% between 5'UTRs of MCF-7 and porcine cell lines, 65.5% between rat and mouse tissues, 32.8% among these four genes and 28.6% between these four genes and human osteoclasts, respectively.

3.2. Characteristics of binding of radiolabelled hCT to transfected cells

Binding of [125 I]hCT was assayed in triplicate for each cells transfected with four kinds of plasmids. COS-1 cells, transfected with pCMVOC1, as well as pCMVMC, bound to [125 I]hCT, whose binding decreased by the addition of a 10^{-5} -fold molar excess of unlabeled CT in a dose dependent manner (Fig. 5). However, the [125 I]hCT binding activity of pCMVOC1 was less than that in pCMVMC. COS-1 cells transfected with pCMVOC2 and with pCR3.1 (plasmid alone) did not specifically bind to [125 I]CT.

A saturation binding assay showed specific CTRs in osteoclast CTR-transfected cells (Fig. 6). Specific binding to pCMVOC1-transfected cells (Fig. 6a) was less than that in pCMVMC-transfected cells (Fig. 6b). Scatchard analysis indicated that the K_d values for hCT were similar in pCMVOC1- (~ 9.74 nM) and pCMVMC-transfected COS-1 cells (~ 10.6 nM). Binding sites were 2.7×10^5 sites/cell and 6.4×10^5 sites/cell for pCMVOC1- and pCMVMC-transfected cells, respectively. Expectedly, the protein structure synthesized from the osteoclast CTR gene was identical to that of the MCF-7 CTR gene, but the expression efficiency differed between them.

4. Discussion

Recent studies have detected hCTR genes primarily in human tumor cell lines [21–25] and in renal tissue [29]. hCTR isoforms have been classified into three types, where the majority is human type 1 including tumor cell lines as mentioned above. The nucleotide sequences of CTR cDNAs in BIN-67, T47D, MCF-7 and GCT of bone, which expresses two isoforms [25], indicated that 5'UTRs of CTR cDNAs were similar (Fig. 7). Only the BIN-67 clones had an insertion of 71 bp (between nucleotide positions 46 and 47 of the MCF-7 clones,

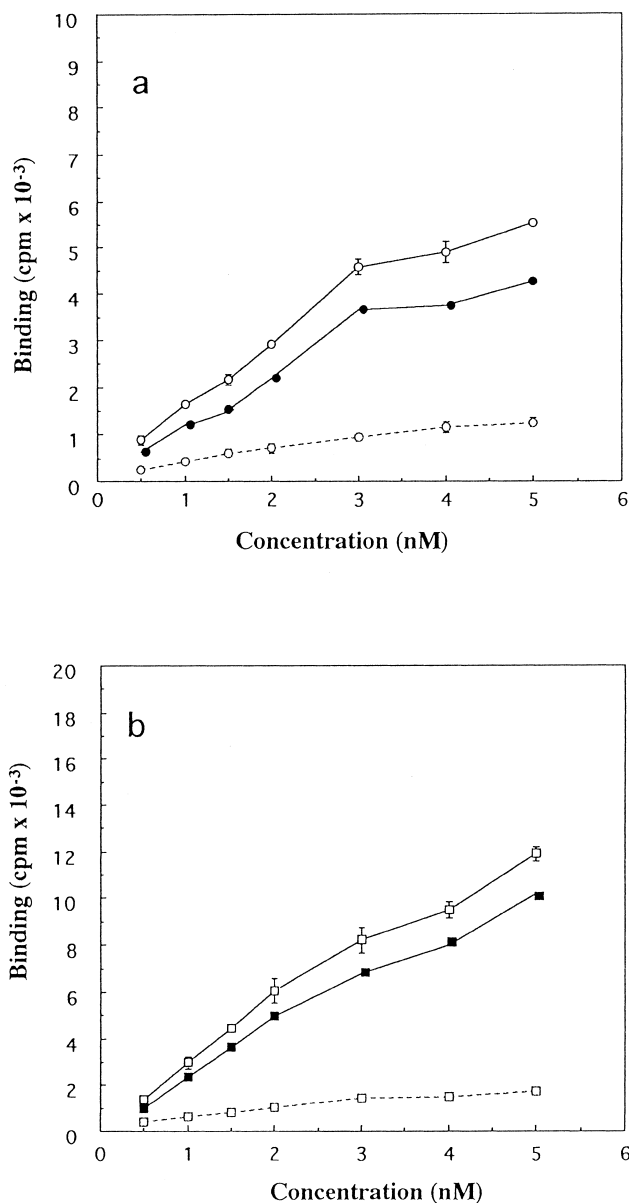


Fig. 6. Saturation binding of [125 I]hCT to COS-1 cells transfected with pCMVOC1 (a) and pCMVMC (b) in the presence or absence of a 10^{-3} -fold molar excess of unlabelled hCT with an increasing concentration of [125 I]hCT. Total, specific and non-specific binding are represented by an open symbol, closed symbol and open symbol with dotted line, respectively. Data represent the means of triplicate measurements and are representative of two separate experiments. Maximum binding of pCMVOC1 was 3759 cpm per assay on average and that of pCMVMC was 8004 cpm.

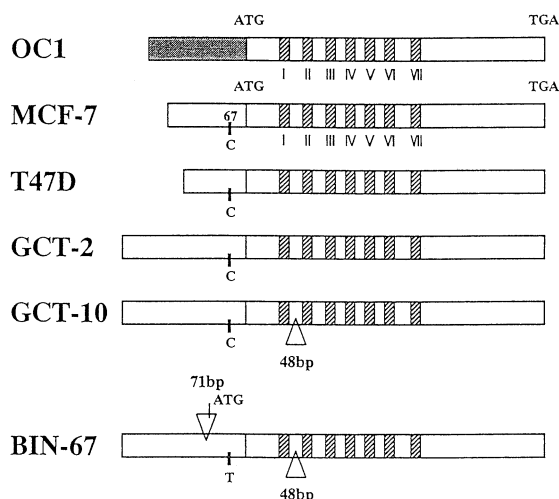


Fig. 7. The bars represent the difference among nucleotide sequences of CTR genes in human osteoclasts (OC) and human tumor cell lines (MCF-7, T-47D, BIN-67) and GCT cells (GCT-2,10). The 5'UTR of CTR cDNA in human osteoclasts (closed squares) was quite different from that in other tumor cells.

at the site of intron 2), which included a potential alternative start codon. 5'UTRs of the two GCT clones are identical and extend further upstream than that of the MCF-7 clones by 104 bp. The 5'UTR of the T47D clones is even shorter by 35 bp. In addition, there is just one other nucleotide in the 5'UTR: position 67 is a T in the BIN-67 clones but a C in the clones of other cell lines. In short, there are no very remarkable changes between 5'UTRs of cDNAs in cancer cell lines and normal renal tissue cells [29]. On the other hand, the 5'UTR of CTR cDNA in human osteoclasts we first cloned was quite different from that in these cDNAs.

The precise role of the distinct 5'UTR of human osteoclast cDNA is unknown. However, the 5'UTR, as well as the 3'UTR, is recognized as an important element in the post-transcriptional regulation of gene expression [30] and several examples showing their importance have been reported [31,32]. Heterogeneity in the 5'UTR of mRNAs leads to an altered translational status of mRNAs. Our study also indicated the difference of translational efficiency between CTR genes from osteoclasts and from MCF-7 cells in COS-1 cells by the difference in [¹²⁵I]hCT binding activity of both CTR genes in a radioligand binding assay (Figs. 5 and 6).

A further hypothesis is that the difference of 5'UTRs might lead to that of promoter regions and that the expression of CTR gene in human osteoclasts would be controlled by distinct promoters other than in human tumor cell lines. For example, the PTH receptor gene, belonging to the G-protein-coupled receptors family including CTR, has at least two promoters [33,34]. Its downstream promoter is ubiquitously expressed, whereas the expression of the upstream promoter is largely restricted to kidney. If there were also the distinct promoters in the human osteoclast CTR gene, they could down-regulate the response to human or salmon CT of human osteoclasts both in vitro and in vivo. This 'escape' phenomenon is found more remarkably in human osteoclasts than in human tumor cell lines. The cellular and molecular mechanisms of this phenomenon are still unknown, but the prolonged suppression of CTR mRNA has been identified in

both short and long term treatment of osteoclast-like MNCs in mouse bone marrow culture with salmon CT [19,35]. Although the specimens in these experiments were not carried out in human, it is still likely that the regulation of CTR mRNA expression is probably an important process and these regulators would be distinct promoters in human osteoclasts.

The regulation mechanism of CTR in human osteoclasts remains to be solved. Additional research is needed to analyze the upper-stream regions, that is, promoter and enhancer regions in the CTR gene of human osteoclasts.

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