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The putative 116 kDa osteoclast specific vacuolar proton pump subunit has ubiquitous tissue distribution

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Abstract

The pharmacological profile of the osteoclast proton pump has been demonstrated to be unique and to be the most active of all acid transport systems thus far studied. The recently reported putative 116 kDa osteoclast specific vacuolar proton pump subunit could possibly explain the unique nature of this proton pump. Here, we demonstrate however, that the osteoclast 116 kDa subunit is not osteoclast specific but has ubiquitous expression in human tissue. © 1998 Elsevier Science B.V.

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Bone resorption is the removal of mineral and organic matrix from bone. It is a process necessary for the remodeling of bone and the long term maintenance of serum calcium levels. The cell lineage responsible for these activities is the osteoclast (Horton and Helfrich, 1992).

The osteoclast degrades bone by the creation of a subosteoclastic resorption lacuna at the site of attachment to the bone matrix. The mineral phase of bone is then degraded by acidification of the resorption lacuna. This acidification is the result of two cellular processes, the enzyme carbonic anhydrase II and a proton pump (Horton and Helfrich, 1992).

The proton pump of the osteoclast is a multi-subunit complex with two distinct functional domains, a cytoplasmic catalytic sector (V_1) and a proton channel (V_0). The V_1 domain is a 500 kDa complex responsible for ATP hydrolysis, whereas, the V_0 domain is a 250 kDa complex responsible for proton translocation (Finbow and Harrison, 1997).

Different forms of the ubiquitous V-type proton pumps, generated by alternative splicing, have been reported, however, to date no new family member genes of the proton pump subunits have yet been identified (Finbow and Harrison, 1997).

The osteoclast proton pump has been reported to possess a unique pharmacological profile and to be the most active of all acid transport systems thus far studied (Chatterjee et al., 1992).

Recently, a putative novel human osteoclast specific 116 kDa vacuolar proton pump subunit (OC-116 kDa) has been cloned and characterized (Genebank acc. no. U45285) (Li et al., 1996). This subunit was reported to be specific to osteoclastoma, unlike the rat and bovine 116 kDa subunit which has ubiquitous distribution. This work suggests that the putative OC-116 kDa subunit of the osteoclast proton pump may, in part, explain the unique pharmacological profile of the osteoclast proton pump.

To confirm the specificity of this subunit, we performed PCR on a large panel of human cDNAs (Table 1), using internal primers based on the published sequence. The cDNA panel comprised 51 Marathon-Ready™ cDNA samples and six in house prepared cDNAs.

The primers used were either: -116-IntF 5′-tggggtggtcctcggagtcttcaa-3′ and 116-IntB 5′-tcatcggagctccagccattcaca-3′ (432 bp fragment) or FLC302 5′ gtgagcagatcggacagaagatcc 3′ and FLC303 5′ cctcggcaatgaggcacttgtgcg 3′ (291 bp fragment).

The PCR conditions for amplification were 95°C for 10 min, then five cycles of 94°C for 30 s, 72°C for 3 min followed by 25 cycles of 94°C for 30 s, 70°C for 3 min followed by a final incubation of 7 min at 70°C. PCR was performed using 1 unit AmpliTaq gold, AmpliTaq gold

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Table 1
Tissue cDNA libraries were the 51 Marathon-Ready™ cDNA libraries (Clontech, Palo Alto, USA) or prepared from total RNA from cell lines or tissues using Trizol reagent

cDNAs prepared in house
Osteoclastoma, osteosarcoma, osteoblast, stromal cell, normal synovial fibroblast, rheumatoid arthritis synovial fibroblast
Marathon-Ready™ cDNAs
Adipocyte, adrenal gland, aorta, bladder, bone marrow, brain, brain cerebellum, brain cerebral cortex, brain hippocampus, brain hypothalamus, colon, colorectal adenocarcinoma, fetal adrenal gland, fetal brain, fetal kidney, fetal liver, fetal lung, fetal skeletal muscle, fetal small intestine, fetal spleen, fetal stomach, fetal thymus, fetus, heart, HeLa, kidney, leukemia chronic myelogenous, leukemia lymphoblastic, leukemia promyelocytic, leukocyte, liver, lung, lung carcinoma, Burkitt's lymphoma, lymph node, mammary gland, melanoma, ovary, pancreas, pituitary gland, placenta, prostate, retina, skeletal muscle, small intestine, spleen, stomach, testis, thymus, thyroid gland, uterus

OligodT primed first-strand cDNA was prepared from 1 μg total RNA using Superscript II reverse transcriptase (all reagents Life Technologies, Inchinnan, UK). The quality of the cDNA was assessed by amplification of an 800-bp fragment of the β-actin gene. PCR amplicons were analysed on a 1% agarose gel and visualized by ethidium bromide staining. Amplicons of expected size for β-actin were obtained for all cDNAs used.

reaction buffer (Perkin-Elmer, Branchburg, NJ, USA), primers (200 nM) and dNTPs (200 μM) and 5 μl cDNA in a total volume of 50 μl using a GeneAmp PCR 9700 (Applied Biosystems, Branchburg, NJ, USA).

All cDNA samples were positive for the 116 kDa subunit with amplicons of expected size.

To confirm the amplicons had the same sequence as the reported OC-116 kDa subunit 5 of the PCR reactions (osteoclastoma, small intestine, stomach, uterus and hypothalamus) were purified using QIAquick™ PCR purification kits (Qiagen, Hilden, Germany) and sequenced using the dye terminator method performed with *Taq* FS polymerase and analysed on an ABI 377 automated sequencer (Applied Biosystems). The DNA sequence data generated for the PCR products from the five cDNA samples matched the 116 kDa gene as 'top hit' when analysed at the nucleotide level in a blast search against Genebank.

To confirm this observation, we designed other primers (FLC300 5′ gacc**ATG**ggctccatgttccggagc 3′ and FLC301 5′ gtgggcc**CTA**gtcatctgtggcagcg 3′) which include the initiation codon and the termination codon and hence, designed to amplify the full-length gene (approx. 2.5 kb). PCR parameters essentially those described above with the following exceptions, 35 cycles were performed rather than 25 and 5% DMSO was included in the reaction.

All cDNA samples showed a PCR band of the expected size. The PCR reactions from three of the cDNA samples (heart, placenta and fetal intestine) were purified as before, subcloned into the pCR® 2.1-TOPO cloning vector (Invitrogen, Carlsbad, CA, USA) and DNA sequenced using both forward and reverse M13 sequencing primers. The resulting sequence data were analysed by blast analysis. The results showed a $>97\%$ match to the 116 kDa gene sequence and demonstrated the presence of the initiation and termination codons.

In a previous publication, OC-116 kDa was demonstrated to be present at high levels in the osteoclastoma tumour but undetectable by Northern analysis in other normal tissues (Li et al., 1996). In contrast, the rat and bovine 116 kDa subunit are expressed ubiquitously (Finbow and Harrison, 1997). The present data reported here clearly demonstrate that the OC-116 kDa subunit is not specific to osteoclast but like that of the rat and bovine 116 kDa subunit has essentially ubiquitous expression.

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