

Full length cDNA sequence of porcine secreted phosphoprotein-I (SPP-I, osteopontin)

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To isolate mRNA encoding proteins that are expressed in a bone-specific manner, fetal porcine calvaria cDNA was hybridized with an excess of poly A⁺ mRNA fetal porcine skin (1) and the single stranded cDNA, enriched in bone-specific sequences, used to screen a fetal porcine calvaria cDNA library constructed according to the method of Okayama and Berg (2). One of the clones obtained from this screen hybridized to an mRNA species of approximately 1.5 kb that was expressed almost exclusively in bone, with low levels of expression evident in kidney, skin and placenta. The cDNA insert of this clone was subcloned in both orientations into the BamHI site of pT7T3 18U (Pharmacia) and both strands sequenced after generating a series of nested deletions using timed exonuclease III digestions (3). Sequence analysis and comparison with rat (4), mouse (5) and human (6) SPP-I sequences revealed that the clone represented a full-length cDNA of porcine SPP-I that included 97 bases of 5' untranslated region (Fig. 1) and that SPP-I is highly conserved at an N-linked glycosylation site, the poly aspartate region, the RGD cell binding sequence and at the N and C-terminal regions of the molecule.

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AGCGACGGAGGCCAGAGGGGACGACTGACAGCCGATCAGCAITGCTCCGGGACTGGACTCTTCGGGGGGTGCAGACCAAGGAAAT 90
CATTACCATGAGAATTGCACTGATAGCTCTCTGGCTTGGGGCTTGGCTTGGCTTCCAGTTAAACGAGACTAATTTCTGGCAGCTCGGA 180
M R I A V I A F C L W G F A S A | L P V K Q T T N S G S S E
GGAAAAAGCTGCTTTCACAAATACACAGATGCTGTAGCCACATTGTAAAGCCTGACCCATCTCAGAAAGCAGACTTCTTCCAGCGCCACA 270
E K L L S N K Y T D A V A T L L K P D P S Q K Q T F L A P Q
GAATACATTTCTCCGGAGGAAACGGACGACTTCAACAAGAGACCTGCCAAGCAAGTCCAAAGAAAGCCCTGAGCAAAACAGACGATGT 360
N T I S S E E T D D F K Q E T L P S K S Ⓢ E S P E Q T D D V
GGACGACGAGCAAGCAAGCAACCGCTGGACAGCAGGACACCGGACTCCGAGGAAGCTGATCAGCTGACGACCGCTGACCGATCCGACGA 450
D D D D D E D H V D S R D T D S E E A D H A D D A D R S D E
GTCTCATCTCCGATGATTCOGATGAGCTGGTCAACGATTTCCCAACCGACACCCCAAGCAACCGGCTGCTCCGGCTGTCCCAACGGG 540
S H H S D E S D E L V T D F P T D T P A T D V T P A V P T G
AGACCCCAATGATGGCCGGGGATAGTGTGGTCTATGGACTGAGGTCAAAATCTAAGAAGTTCCCGCAGATCCGAAAGCCGACGAGTGA 630
D P N D G R G D S V V Y G L R S K S K K F R R S E A Q Q L D
TGCCACAGAGGAAGCACTCACCTCACATGTGGAAGTGGAGAGCGGATGGTAOCCCAAGCCATCTCTGTTGGCCAGCGCTGACAGT 720
A T E E D L T S H V E S E E T D G T P K A I L V A Q R L H V
GGCTTCTGACTTGGACAGCCAAAGAGGACAGTCAAGGACGATGACCGGATGACCGGATGTGGAAACCCGACGACGAGGAGCAGT 810
A S D L D S Q E K D S Q E T S Q P D D R S V E T R S Q E Q S
CAAAGAATAACAGATCAAGACTATGAGGCAATGAGCAATTCATGATGATGAGAGTCAAGGAAATCCAGAAATCCAGCAAGAAAT 900
K E Y T I K T Y D G S N E H S N V I E S Q E N P K V S Q E F
CCACAGCCATGAAAGCAAGCTGGTCCGACACTTAAAGCGGAAAGAGCAAAACACTGACACACTTATAGACAGGATATAAATTTTCAAT 990
H S H E D K L V P D S K S E E D K H L K L R V S H E L E S A
GTCTCTGAGATCAACTGAGAAATACATGTCTTACTTTGCTTTAGTAAAAAGAAAAAACACACTGTGACGAGCTGGGAGGAAAT1080
S S E I N *
TATGAAAGGCTTATTTCTCAGCTTAGTGGTGAATGTATATGTGTGGCTCTGGAAACAATAATATTTTGTATCATTAGTTAGTTG1170
TGCTTCTGATGTAACCCCTTCTCACTAAAGCTTCCAGGTTTATGTCAGCGCTTTCCTGATAGAAATACAAGCCCTCAGCTGATTTG1260
TAATGTTTGTGATCCTTTATGATGAAATTTATGTAGAAAGCAAAACACTGACACACTTATAGACAGGATATAAATTTTCAATGT1350
CACTCTGATCTTTTGTATTTAAATAGTGTATATTTTGTGATTATTTTGTGGTGTGAATAAATATCTCCGATGTA...poly A 1430
    
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Fig. 1. Sequence of the full length cDNA for porcine calvaria SPP-I. The nucleotide sequence of porcine SPP-I is shown with the predicted amino acid sequence displayed underneath. The predicted start of translation is indicated as 98 and the site of cleavage of the signal peptide is shown (|) as is the N-linked glycosylation site (Ⓢ), the poly aspartate region (underlined) and the RGD cell binding site (bold type).

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