The major ras induced protein in NIH3T3 cells is cathepsin L

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Submitted January 30, 1987

We (1) and others (2,3) have cloned murine cDNA's encoding the most abundant ras induced protein (RIP-1) in NIH3T3 cells, a protease previously called major excreted protein (MEP) (3). It has also been identified as a mouse macrophage cysteine proteinase (MCP) (4). The deduced amino acid sequence of these mouse cDNA clones for RIP-1 (MEP, MCP) show an 81% homology to the N-terminal human cathepsin L sequence recently obtained by direct protein sequencing (5). Mouse MEP and human cathepsin L have the same bond cleavage specificity on oxidized insulin B chain (6). These results suggest that RIP-1 is murine cathepsin L. To verify this hypothesis, human cross-hybridizing RIP-1 cDNA clones were obtained by screening a human kidney λ gt10 library (provided by G. Bell) with a 800 bp RIP-1 cDNA insert (1). Hybridization was in 1% SDS, 1M NaCl and 10% dextran sulfate. Stringency of the final wash was 1% SDS, 2 X $\bar{S}$ SC at 55°C. Inserts from positive clones were subcloned into M13 and sequenced. 38 out of 40 amino acids of N-terminal sequence of human cathepsin L (5) are perfectly predicted by our nucleotide sequence (Fig. 1), thus proving that we have obtained human cathepsin L encoding cDNAs and that RIP-1 is cathepsin L. The discrepancy in the two amino acids may represent possible protein sequencing errors or reflect polymorphism. Our prediction of Glu for position 35 would lead to conservation of this amino acid. Since cathepsin L is a powerful degradative enzyme on extracellular matrix components (5), its identification as the major excreted protein of ras transformed NIH3T3 cells might explain their invasive behavior. Our cathepsin L cDNA clones will be useful for in situ hybridization and Northern blot analysis of human tissues. **References:** (1) Sukhatme, V.P. et al. (1986) Proc. Natl. Acad. Sci. USA, submitted. (2) Denhardt, D.T. et al. (1986) Cancer Res., 46, 4590-4593. (3) Doherty, P.J. et al. (1985) Mol. Cell. Biol., 5, 466-473. (4) Portnoy, D.A. et al. (1986) J. Biol. Chem. 261, 14697-14703. (5) Mason, R.W. et al. (1986) Biochem. J. 240, 373-377. (6) Gal, S. et al. (1986) Biochem. Biophys. Res. Comm. 139, 156-162.

a GAGGCCCCAGATCTGTGGATTGGAGAGAGAAAGGCTACGTGACTCCTGTGAAGAATCAGGGT
b GluAlaProArgSerValAspTrpArgGluLysGlyTyrValThrProValLysAsnGlnGly
c AlaProArgSerValAspTrpArgGluLysGlyTyrValThrProValLysAsnGlnGly

a CAGTGTGGTCTCTGTGGGCTTTTAGTGCTACTGGTGCTCTTGAAGGACAGATGTTCCGGAAA
b GlnCysGlySerCysTrpAlaPheSerAlaThrGlyAlaLeuGluGlyGlnMetPheArgLys
c GlnCysGlySerCysTrpAlaPheSerAlaThrGlyAlaLeuProGlyGlnMetPheTyr

Fig. 1 Nucleotide (a) and deduced amino acid sequence (b) of a human cross-hybridizing RIP-1 cDNA clone and comparison to the N-terminal amino acid sequence (heavy chain) (c) of human cathepsin L (5).