A TRANSFORMING GROWTH FACTOR-BETA BINDING PROTEIN EXPRESSED ON THE SURFACE OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS IS RECOGNIZED BY THE MONOCLONAL ANTIBODY SH-2

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Introduction:

Several studies have shown that bone marrow derived mesenchymal stem cells (MSCs) can differentiate into multiple tissues including cartilage, bone, tendon, muscle, and fat. The SH-2 antibody recognizes a protein which is expressed on the surface of MSCs prior to differentiation. Expression of the SH-2 antigen is lost during osteogenic differentiation and no direct staining has been demonstrated in bone, articular chondrocytes, tendon, or skeletal muscle (1). The specific identity of this cell surface marker for MSCs and the functional role that it plays during the differentiation process are the subject of this study.

Methods:

MSCs were isolated from fresh human bone marrow aspirates and expanded in culture as previously described (2). Confluent cell layers were washed with cold Tyrode's buffer to remove fetal bovine serum, and then incubated at 4°C, for 2 hours with 1 µg/ml of SH-2 antibody in Tyrode's buffer. The cell layers were washed to remove unbound antibody and then scraped into 10 ml buffer and pelleted by centrifugation. The cell pellet was solubilized at a density of 30×10^6 cells/ml by adding cell lysis buffer consisting of 20 mM Tris, pH 8.0, 0.14 M NaCl, 0.5 % CHAPS with added protease inhibitors. The solubilized extract was clarified by centrifugation and the antibody-antigen complex was immunoprecipitated with Protein G-Sepharose. The protein complex was eluted from the Protein G-Sepharose by boiling for 5 minutes in SDS-PAGE sample buffer containing mercaptoethanol. After electrophoresis on 12 % SDS PAGE gels, the Coomassie-G stained bands were excised and stored at -20°C until ready for use. The protein bands from multiple gels were chopped into 1mm cubes and digested with trypsin using a standard in-gel digestion protocol (5). Control samples from an unstained portion of the gel were also digested. The digest was separated on a Vydac C18 (2.1mm X 250mm) column using a HP 1090 LC system. Purified peptides were sequenced using a HP G1005A Sequencing System and their mass was obtained using a HP G2025A Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometer. An estimate of the amount of the SH-2 antigen extracted was obtained by staining the SDS-PAGE gel with the fluorescent dye Sypro-Orange and quantitation of the staining intensity on a Storm Phospho-Imaging System.

Results:

Quantitative fluorometric analysis of the amount of SH-2 present on human MSCs indicated that the abundance of the protein was approximately 1 µg per 10^9 cells. SDS-PAGE analysis of the immunoprecipitated protein complex showed two distinct bands (Fig. 1), at 80 and 92 kDa. The yield of the 80 kDa species varied from preparation to preparation, and the 92 kDa band was the most prominent. Comparison of the tryptic map of the 92 kDa band (Fig. 2) with that of the control gel digest identified several candidate peptides for N-terminal sequence analysis.

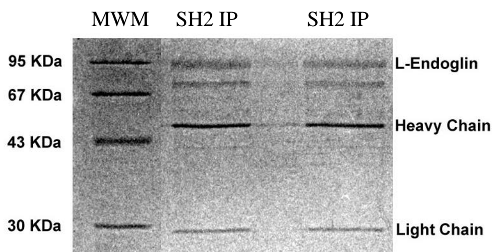

Fig. 1 SDS-PAGE of the SH2 immunoprecipitation (shown in duplicate) from human MSCs

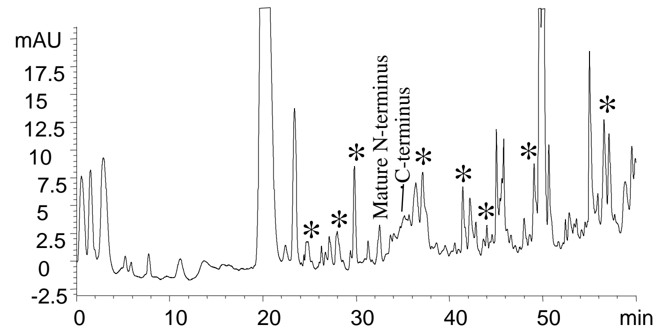

Fig. 2 Reverse Phase HPLC profile of a tryptic digest of the 92 kDa band from an immunoprecipitation of the SH2 antigen

Sequence data were obtained for 12 peptides and a search of the GenBank protein database using a BLITZ search identified this protein as endoglin. The peptide sequences obtained in this study showed complete homology to the published human endoglin sequence (GenBank Accession: JO5481). Tryptic peptides derived from both N- and C-termini were identified. The detection of the C-terminal peptide identifies the 92 kDa species as the long form of endoglin (L-endoglin). The mass values measured for all peptides by MALDI-TOF were within 0.06 % of the calculated masses.

Discussion:

Immunoprecipitation of the SH-2 antigen from a total of 5 X 10^9 human MSCs yielded sufficient material for in-gel digestion, purification and sequence analysis of a number of peptides. The sequence information identified the antigen as human endoglin. Endoglin is an RGD-containing glycoprotein that was first identified on the surface of endothelial cells (4). Like betaglycan it is a TGF-β type III receptor. Endoglin is a type I integral membrane protein which forms a disulfide linked homodimeric complex comprised of subunits of 95 kDa. Two isoforms of endoglin have been described: a 95 kDa long form (L-endoglin) and a shorter 82 kDa species (S-endoglin). The latter has a truncated cytoplasmic domain and is generally present in lower abundance. The 80 kDa species observed in these studies may represent S-endoglin but this has not been confirmed.

The binding specificity of endoglin has been studied (4): it binds TGF-β1 and -β3 with high affinity (K_D = 50 pM) but not TGF-β2. This is in contrast to betaglycan, which has been shown to bind all three isoforms of TGF-β with strong affinity ($K_D \sim 10^{-10}$ M) (5). The regulation of the chondrogenic differentiation of human MSCs is modulated by TGF-β1 and TGF-β3 (6) and since endoglin is present on undifferentiated MSCs it is very likely that it plays a central role in this process.

References:

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