MOLECULAR ENGINEERING OF STEROIDOGENESIS INDUCING PROTEIN-LIKE PROTEIN

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Abstract: Steroidogenesis inducing protein (SIP) is a novel growth factor that was initially identified in human ovarian follicular fluid. SIP has been sequenced on its N-terminal side; and parts of the peptide sequence were uncovered. Three peptide sequences derived from SIP are almost completely homologous to p205, a protein found in synovial fluid from patients with rheumatoid arthritis. To test the functional importance of these peptides, we employed a gain-of-function strategy to detect the SIP-like bioactivities in a chimeric protein (SIP-like protein), in which the three peptides were arranged in a similar order as was postulated in SIP. As part of this project, we present here the process of construction of the SIP-like protein. A selected clone encodes immunoglobulin heavy constant γ 1 (IGHG1) that contains complete sequences of peptides 1 and 3. IGHG1 gene was inserted into pcDNA3.1 expression vector; its C-terminus was modified by addition of myc and 6His epitopes. The variable region of IGHG1 was aligned with the sequence of peptide 2; the comparable portion was then replaced by the peptide 2 sequence. The gene encoding SIP-like protein was constructed with overlap extension PCR technique and restriction enzyme digestion; the resulting clone was verified by DNA sequencing. The plasmids were transiently transfected into COS-1 cells. Western blot analysis with anti-myc antibody and anti-6His antibody detected overexpression of IGHG1 or SIP-like protein in total cell lysates. Stable cell lines expressing IGHG1 or SIP-like protein were subsequently generated. Recombinant proteins were purified with Ni²⁺ affinity column. In conclusion, we have successfully obtained recombinant IGHG1 and SIP-like protein.