〔Cancer Res., **49**, 3494 (1989)〕

Hormonal Regulation of Synthesis and Secretion of pS2 Protein Relevant to Growth of Human Breast Cancer Cells (MCF-7).

NORIYOSHI KIDA, TOMOAKI, YOSHIMURA, KAZUTOSHI MORI, KYOZO HAYASHI*

We have recently identified human epidermal growth factor-like immunoreactive factor synthesized and secreted by human breast cancer cells (MCF-7) as secretory protein encoded by the pS2 gene, the transcription of which is directly induced by estrogen. We demonstrated in this paper that synthesis and secretion of pS2 protein as well as pS2 mRNA were induced about 5-fold specifically by physiological concentrations of estrogen, which stimulated growth of the cells abous 5-fold. Stimulative effects of estrogen on both cell growth and synthesis/secretion of pS2 protein were inhibited completely by actinomycin D, cycloheximide, and antiestrogen. These results show that induction of pS2 by estrogen is not involved in the growth-stimulating effect of estrogen.

〔J. Clin. Chem. Nutr., **7**, 269 (1989)〕

Improved Procedure for Purification of Human Platelet-Derived Growth Factor.

KYOZO HAYASHI,* MASASHI ITOH, MASAYUKI KUROBE

A relative simple method for purification of human platelet-derived growth factor (PDGF) was developed. PDGF was purified from clinically outdated, platelet-rich plasma by means of freezing and thawing extraction and successive chromatography on CM-Sephadex, Sephacryl S-200, and Phenyl Sepharose. Further purification of the PDGF obtained showed two silver-stained bands following polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of sodium dodecyl sulfate. Amino acid sequence analysis of these two components separated by SDS-PAGE demonstrated that the sequences coincided with those of PDGF A and B chains previously reported and predicted from the nucleotide sequences of the cloned cDNA's of PDGF A and B chain genes. The purified PDGF stimulated a nanogram level of thymidine incorporation into DNA of previously quiesecnt Balb 3T3 cells.

〔FEBS Letters, **254**, 106 (1689)〕

Neutralizing Monoclonal Antibody Specific for α-Bungarotoxin : Preparation and Characterization of the Antibody, and Localization of Antigenic Region of α-Bungarotoxin.

RYOICHI KASE, HISAYO KITAGAWA, KYOZO HAYASHI,* KENJIRO TANOUE, FUYUHIKO INAGAKI

Neurotoxins isolated from the venom of *Elapidae* and *Hydrophiidae* families are immunogenic polypeptides with three or four antigenic antibodies. We prepared an α-bungarotoxin-specific monoclonal antibody that neutralized the biological activity of the toxin *in vivo*. The antigenic determinant combining specifically with this antibody was determined on the basis of cross-reaction experiments using three other long neurotoxins and peptide fragments of α-bungarotoxin. The antigenic determinant was located on the peptide fragment containing S34-S35-R36-G37-K38, which forms a part of the expected site that binds to the acetylcholine receptor proteins.