

# Human Zn- $\alpha_2$ -glycoprotein cDNA cloning and expression analysis in benign and malignant breast tissues

José P. Freije, Antonio Fueyo, José Uría and Carlos López-Otín

*Departamento de Biología Funcional, Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain*

Received 23 July 1991

Two cDNA clones coding for Zn- $\alpha_2$ -glycoprotein (Zn- $\alpha_2$ -gp) have been isolated from a human breast library and their nucleotide sequences determined. The deduced amino acid sequence contains the coding information for a hydrophobic signal peptide and the 278 residues of the mature protein. Comparison of this sequence with that from the protein purified from plasma reveals four differences: two amino acid changes (Gln-67 and Glu-222) and insertion of two residues (Ile-75 and Phe-76). Northern-blot analysis showed that the Zn- $\alpha_2$ -gp gene is expressed in liver and normal breast, but not in placenta, ovary and thyroid. A comparative analysis in mammary tissues from women with different diseases revealed enhanced expression of Zn- $\alpha_2$ -gp gene in benign breast lesions and a variable expression level in breast cancers.

Zn- $\alpha_2$ -glycoprotein; Breast cystic disease; Breast cancer

## 1. INTRODUCTION

Zn- $\alpha_2$ -glycoprotein (Zn- $\alpha_2$ -gp) is a human protein originally isolated from plasma and whose name derives from its electrophoretic mobility in the  $\alpha_2$ -region and from its ability to bind zinc ions [1]. The protein shows a molecular mass of about 40 kDa, contains 12% carbohydrates and displays a considerable charge heterogeneity on agarose gel electrophoresis and on isoelectrofocusing [2,3]. The complete amino acid sequence of human plasma Zn- $\alpha_2$ -gp has been recently elucidated [4]. It consists of a single polypeptide chain of 276 amino acid residues, with a high degree of similarity to antigens of the major histocompatibility complex.

Although the biological function of Zn- $\alpha_2$ -gp is unknown, the finding of high concentrations of this protein in cyst fluid from women with gross cystic breast disease has suggested that Zn- $\alpha_2$ -gp may play a role in the induction of the pathological process characteristic of the disease [5]. In addition, immunohistochemistry analysis performed on tissue specimens obtained from patients with breast carcinoma has led to identifying a subtype of tumors which synthesize and secrete this protein [6,7]. A better understanding of these possible implications of Zn- $\alpha_2$ -gp in the development of benign and malignant breast diseases could be facilitated by the availability of the gene encoding this protein. In this work, we report the isolation and sequencing of cDNA clones for human Zn- $\alpha_2$ -gp. In addition,

we have used these cDNAs as a probe in order to study Zn- $\alpha_2$ -gp expression in different normal and pathological human tissues.

## 2 MATERIALS AND METHODS

### 2.1. Materials

Restriction endonucleases and other reagents used for molecular cloning were obtained from Boehringer-Mannheim. [ $\alpha$ - $^{32}$ P]dCTP (3000 Ci/mmol), [ $\alpha$ - $^{32}$ P]ATP (3000 Ci/mmol) and [ $\alpha$ - $^{35}$ S]dATP (600 Ci/mmol) were purchased from Amersham International. A human breast cDNA library in  $\lambda$ gt11 was obtained from Clontech Laboratories Inc. Oligonucleotides were synthesized by the phosphoramidite method in an Applied Biosystems DNA synthesizer, model 381A and purified by polyacrylamide gel electrophoresis according to standard procedures [8]. Double-stranded DNA probes were radiolabeled to high specific activity with [ $\alpha$ - $^{32}$ P]dCTP by using a commercial random-priming kit from Boehringer-Mannheim.

### 2.2. Isolation and analysis of cDNA clones

Two different oligonucleotides (20-mer) with sequences AA(AG)CA(TC)GTNGT(AG)GA(TC)GTNCC and TC(TC)TC(TC)TTCCA(AG)TC(TC)TCCAT based on the amino acid sequence of Zn- $\alpha_2$ -gp (residues 19–25 and 61–66, respectively) were synthesized and used to screen a cDNA library made from human breast tissue. A total of  $5 \times 10^5$   $\lambda$  plaques were analyzed according to the method of Benton and Davis [9]. Recombinant phages from isolated positive plaques were grown and DNA was prepared from them according to Maniatis et al. [8]. The restriction map of inserts was obtained following standard procedures [8].

### 2.3. Sequence analysis

Restriction fragments were inserted in the polylinker region of phage vectors M13mp18 or M13mp19 [10]. Single-stranded templates were isolated and sequenced using either the universal M13 primer or synthetic oligonucleotide primers and the Sequenase kit (United States Biochemical Corp.). The nucleotide sequence was determined at least twice in both strands. The DNA sequences were analysed by computer programs DM5 [11] and ALIGN [12] on an IBM PC-AT computer.

Correspondence address: C. López-Otín, Departamento de Biología Funcional, Facultad de Medicina, Universidad de Oviedo, 33006 Oviedo, Spain. Fax: (34) (8) 523 2255.



using as a probe the *Eco*RI restriction fragment which contains the 3'-end of the cDNA. As shown in Fig. 3, a single hybridizing band corresponding to a mRNA species of about 1300 nucleotides was identified in breast, liver and in both benign and malignant breast tumors. The strongest signal was obtained with RNA from patients with fibroadenoma, being the result in Fig. 3, a representative example of 4 different specimens. According to this, enhanced expression of Zn- $\alpha_2$ -gp could be considered as a marker for benign mammary lesions. The variable intensity of the band detected at breast carcinoma tissues may be related to specific behavioural characteristics of the tumors and agrees with immunohistological studies indicating the existence of a subset of tumors which maintain the ability to produce this protein [6].

The molecular basis for the increased levels of Zn- $\alpha_2$ -gp in benign tumors or in cyst fluid from breast gross cystic disease are presently unknown, but the mechanism is probably mediated by hormonal factors, since several data suggest that these lesions result from an hormonal imbalance [5]. In relation to this, it is remarkable that Zn- $\alpha_2$ -gp and other major cyst fluid proteins like apolipoprotein D [14] are induced by androgens in breast cancer cells [15,16] which suggests that androgens could be directly involved in the enhanced expression of Zn- $\alpha_2$ -gp.

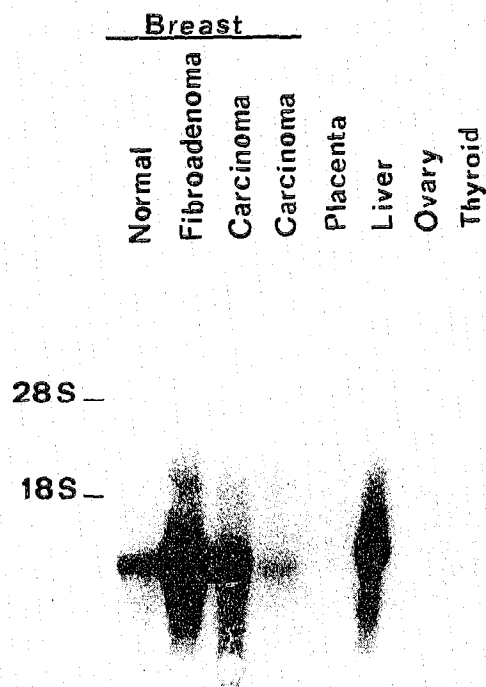


Fig. 3. Expression of Zn- $\alpha_2$ -gp mRNA in human tissues. About 10  $\mu$ g of total RNA from the tissues indicated in the upper part of the figure were analysed by Northern-blot using a Zn- $\alpha_2$ -gp probe. The positions of 28 S- and 18 S-rRNA bands are indicated.

During the preparation of this manuscript, a report describing the characterization of a Zn- $\alpha_2$ -gp clone from a prostate cDNA library has been published [17]. Examination of this nucleotide sequence revealed some minor discrepancies with that presented in this work (changes at positions 26 and 35 and variable extensions at the 3'-flanking region). The absence of data on the 5'-untranslated sequence precludes a more detailed comparison of this region with the corresponding one to the breast cDNA clone characterized in this work. The isolation and characterization of genomic DNA clones containing this part of the gene will be useful to elucidate its structure as well as the mechanisms controlling the expression of the gene in normal and pathological breast tissues.

**Acknowledgements:** We are grateful to Dr. S. Gascón for his support, Drs. P.S. Lazo and M. Balbin for helpful discussions and Dr. F. Vizoso for providing tissue specimens. This work was supported by a grant from CICYT-Spain. J.P.F. is recipient of a fellowship from the Ministerio de Educación y Ciencia, Spain.

## REFERENCES

- [1] Bürgi, W. and Schmid, K. (1961) *J. Biol. Chem.* 236, 1066-1074.
- [2] Schmid, K. and Takahashi, S. (1964) *Nature* 203, 407-408.
- [3] Kamboh, M.I. and Ferrell, R.E. (1986) *Biochem. Genet.* 24, 849-857.
- [4] Araki, T., Gejyo, F., Takagaki, K., Haupt, H., Schwick, H.G., Bürgi, W., Marti, T., Schaller, J., Rickli, E., Brossmer, R., Atkinson, P.H., Putnam, F.W. and Schmid, K. (1988) *Proc. Natl. Acad. Sci. USA* 85, 679-683.
- [5] Haagensen, D.E. and Mazoujian, G. (1986) in: *Diseases of the Breast* (Haagensen, C.D. ed) pp. 474-500, W.B. Saunders, Philadelphia, PA.
- [6] Bundred, N.J., Miller, W.R. and Walker, R.A. (1987) *Histopathology* 11, 603-610.
- [7] Mazoujian, G. and Haagensen, D.E. (1990) *Ann. NY Acad. Sci.* 586, 188-197.
- [8] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- [9] Benton, W.D. and Davis, R.W. (1977) *Science* 196, 180-182.
- [10] Messing, J. and Vieira, J. (1982) *Gene* 19, 269-276.
- [11] Mount, D.M. and Conrad, B. (1986) *Nucleic Acids Res.* 14, 443-454.
- [12] Pearson, W.R. and Lipman, D.J. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2444-2448.
- [13] Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156-159.
- [14] Balbin, M., Freije, J.M.P., Fueyo, A., Sánchez, L.M. and López-Otin, C. (1990) *Biochem. J.* 271, 803-807.
- [15] Chabos, D., Haagensen, D., Parish, T. and Rochefort, H. (1987) *Cancer Res.* 47, 2787-2792.
- [16] Simard, J., Dauvois, S., Haagensen, D.E., Levesque, C., Merand, Y. and Labrie, F. (1990) *Endocrinology* 126, 3223-3231.
- [17] Ueyama, H., Niwa, M., Tada, T., Sasaki, M. and Ohkubo, I. (1991) *Biochem. Biophys. Res. Commun.* 177, 696-703.