Acta Biochimica Polonica

Vol. 41 No. 2/1994

QUARTERLY

Distribution of nonhistone proteins and glycoproteins in chromatin from larynx cancer

Andrzej Stepulak, Marta Stryjecka-Zimmer and Maria Sanecka-Obacz

Department of Physiological Chemistry, Medical Academy, Lubartowska 85, 20–123 Lublin, Poland

Key words: nonhistone proteins, glycoproteins, larynx cancer

Nonhistone chromatin proteins exhibit many predicted properties of gene regulatory macromolecules. These heterogeneous and tissue specific proteins take part in chromatin packing and regulation of transcription.

The nonhistone proteins undergo numerous posttranslational modifications that are important for the functional state of these proteins and affect gene activity [1–4].

It is believed that alterations of cellular phenotype during differentiation and neoplastic transformation are accompanied by changes in the composition of nonhistone proteins, some of these changes may have regulatory meaning [5–10].

The aim of the present work was to examine qualitative and quantitative changes in the content of nonhistone proteins and their posttranslational glycosylation in larynx cancer in humans.

All experiments were performed on squamous cell cancer (*Carcinoma planoepitheliale*) obtained after total laryngectomy of 30 patients of 38–70 year of age at the Department of Otolaryngology Medical School of Lublin.

As control material macroscopically normal mucosa from the lingual surface of the epiglottis was used. The control mucosa was at least 1.5 cm away from tumor margin. Freshly removed tumor tissues and normal mucosa were used immediately or stored at –30°C. All the solutions used for isolation of nuclei or preparation of chromatin contained 0.5 mM phenylmethylsulfonyl fluoride.

Nuclei were obtained by the method of Blobel & Potter [11].

The nuclear chromatin was isolated according to Spelsberg & Hnilica [12] except that an additional treatment with 0.5% Triton X-100 was applied to remove membrane ghosts and omittence of the 0.3 M NaCl wash.

Total chromatin proteins were separated by electrophoresis on SDS-polyacrylamide linear gradient gel (5%–15%) according to Laemmli [13]. The gels were stained for proteins with Coomassie Brillant Blue and for glycoproteins with Schiff reagent according to Zacharius *et al.* [14]. In addition we have used Concanavalin A combined with horseradish peroxidase [15] for selective recognition of specific sugar residues. For determination of this lectin binding glycoproteins isolated chromatin were first separated on SDS-PAGE and than electrophoretically transferred onto nitrocellulose sheets [16]. Protein concentration was assayed by the method of Bradford [17]. DNA concentration in chromatin was determined spectrophotometrically at 260 nm.

Polyacrylamide gel electrophoresis patterns of chromatin proteins from tumors and control mucosa after Coomassie Blue staining are shown in Fig. 1A, and polyacrylamide gel densitometric scans in Fig. 1B.

Analysis of electrophoretic gels showed considerable similarity in chromatin protein patterns between of the 30 control mucosa tested cases, whereas those patterns were different (especially quantitative) from those of the larynx cancer chromatin proteins.

Twenty one (from 30 cases) examined tumors showed similar chromatin protein electrophoretic patterns. In 9 cases we observed loss

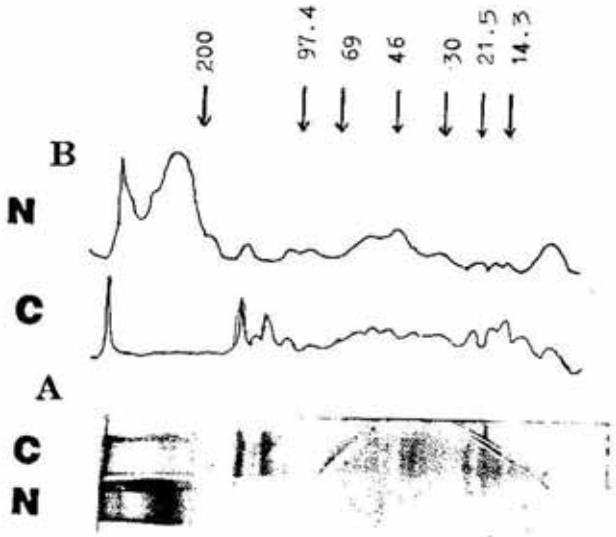

Fig. 1. Electrophoretic analysis of chromatins from control mucosa (N) and cancer (C).
A, SDS-polyacrylamide gel electrophoresis; B, densitometric scans of the stained gel. Slab gel was stained with Coomassie Brillant Blue. All samples represent 40 µg of chromatin (as DNA) applied to the gel. Molecular mass standards: myosin (200 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.3 kDa).

or reduction of several or a few additional protein fractions. Probably, the tumors contain mixed cell populations either of various phenotypes, or differing in their proportions of dividing and nondividing cells.

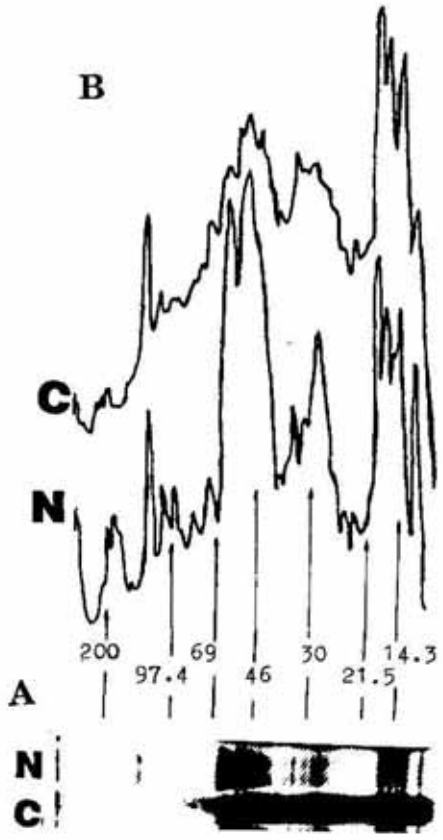

Fig. 2A. SDS-polyacrylamide gel electrophoresis of chromatin from control mucosa (N) and cancer (C) (xerocopy of original slab). Fig. 2B. Densitometric scans of the stained gel.
Slab gel was stained with Schiff reagent. All samples represent 100 µg of chromatin (as DNA) applied to gel. Molecular mass standards as in Fig. 1.

Our data indicate that the main significant difference between chromatin proteins from larynx cancer and control mucosa concerns their reactivity with the periodic acid — Schiff reagent (PAS). When polyacrylamide gels were stained with PAS the larynx cancers revealed quite different patterns as compared with the control mucosa cells (Fig. 2).

Additional glycoprotein components of molecular mass about 320 kDa, 270–200 kDa (heterogeneous), 180 kDa, absent from tumor cells, were evidenced only in the chromatin

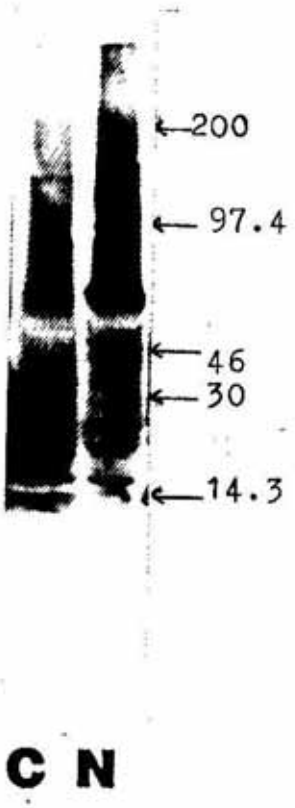

Fig. 3. Protein blott analysis of chromatin glycoproteins from control mucosa (N) and cancer (C).
The chromatin samples (40 µg as DNA) were resolved by SDS-PAGE in 5%–15% polyacrylamide gradient gel, electroblotted onto nitrocellulose and probed with Concanavalin A combined with horseradish peroxidase. Molecular mass standards as in Fig. 1.

proteins from control mucosa.

In the larynx cancer chromatins there appeared some new or more intense glycoprotein bands with molecular mass about 125 kDa, 115 kDa, 100 kDa and 90 kDa.

We have also compared the Concanavalin A binding capacity of chromatin from normal and transformed cells (Fig. 3). Striking qualitative and quantitative changes occured in these specific lectin binding components of chromatin during neoplastic transformation.

The presence of glycoproteins on the cell surface and within nuclei has been reported [18–24]. Differences exist in the glycosylation of surface [23] and chromatin protein between the transformed and nontransformed cells [6, 18, 24]. The glycoproteins were therefore distinguished as the component may be capable of initiating and successively modifying genomic activity involved in the mechanism of nucleus–cytoplasm interactions.

Our results provide additional support for the role of specific types of glycosylation in neoplastic tissues. Further studies are directed to determine the number and size of major lectin binding chromatin associated glycoproteins, and classify some of these proteins according to their sugar substituents.

REFERENCES

1. Allfrey, V.G. & Boffa, L.C. (1979) in *The Cell Nucleus* (Busch, H., ed.) vol. 7, pp. 552–562, New York, Academic Press Inc.
2. Stein, G.S., Plumb, M., Stein, J.L., Philips, I.R. & Shephard, E.A. (1983) in *Chromosomal Nonhistone Proteins* (Hnilica, L.S., ed.) vol. 1, pp. 127–186, CRC Press, Boca Raton.
3. Chiu, J.F. (1985) in *Progress in Non-Histone Protein Research* (Bekhor, I., Mirell, C.J., Liew, C.C., eds.) vol. 1, pp. 1–28, CRC Press, Boca Raton.
4. Halikowski, M.J. & Liew, C.C. (1987) *Biochem. Cell Biol.* **66**, 349–366.
5. Glass, W.F. & Briggs, R.C. (1981) *Science* **211**, 70–72.
6. Stryjecka-Zimmer, M., Schmidt, W.N., Briggs, R.C. & Hnilica, L.S. (1982) *Int. J. Biochem.* **14**, 591–600.
7. Wen, B.G., Burkhard, A.L., Cote, G.J., Huang, D.P. & Chiu, J.F. (1987) *Mol. Cell Biochem.* **76**, 75–84.
8. Babin, J.K. & Anderson, J.N. (1988) *Cancer Res.* **48**, 5495–5502.
9. Kiliańska, Z., Krajewska, W.M., Xie, R., Kłyszejko-Stefanowicz, L. & Chiu, J.F. (1991) *J. Cell Biochem.* **45**, 303–310.
10. Krajewska, W.M., Lipińska, A., Gaczyński, M. & Kłyszejko-Stefanowicz, L. (1992) *Int. J. Biochem.* **24**, 759–767.
11. Blobel, G. & Potter, V.R. (1966) *Science* **154**, 1662–1665.
12. Spelsberg, T.C. & Hnilica, L.S. (1971) *Biochim. Biophys. Acta* **228**, 202–211.
13. Laemmli, U.K. (1970) *Nature* **227**, 680–685.
14. Zacharius, R.M., Zell, T.E., Morrison, J.H. & Woodlock, J.J. (1969) *Anal. Biochem.* **30**, 148–152.
15. Faye, L. & Chrispeels, M.J. (1985) *Anal. Biochem.* **149**, 218–224.
16. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354.
17. Bradford, M.A. (1976) *Anal. Biochem.* **72**, 248–256.
18. Goldberg, A.H., Yeoman, L.C. & Busch, H. (1978) *Cancer Res.* **38**, 1052–1056.
19. Reeves, R. & Hung, S.C. (1983) *J. Biol. Chem.* **258**, 679–687.
20. Reeves, R. (1984) *Biochim. Biophys. Acta* **782**, 679–687.
21. D'Erme, M. & Caiafa, P. (1987) *Cell Mol. Biol.* **33**, 755–765.
22. Kinoshita, S., Yoshii, K. & Tonegawa, Y. (1988) *Exp. Cell Res.* **175**, 148–157.
23. Itzkowitz, S.H. (1992) *J. Cell Bioch., Suppl.* **16G**, 97–101.
24. Kearse, K.P. & Hart, G.W. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1701–1705.