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Nonhistone protein distribution in larynx cancer

Skład białek niehistonowych w komórkach raka krtani

INTRODUCTION

Nonhistone chromatin proteins (NHCP) exhibit many predicted properties of gene regulatory macromolecules. These heterogenous and tissue-specific proteins take part in chromatin packing and regulation of transcription as well as play an important role in signalling pathways leading to apoptosis or cell survival [6]. It is believed that alterations of cellular phenotype during differentiation and neoplastic transformation are accompanied by changes in the amount and composition of nonhistone proteins, some of these changes may have regulatory meaning [8, 10, 25]. One of the best characterised NHCP — HMG (High Mobility Group) proteins bind to the narrow groove of AT-rich regions in double-stranded DNA and act as architectural proteins that bring many of the transcription factors into precise three-dimensional shapes, and therefore play a key role in transcriptional regulation of gene expression [15]. Recent studies have shown that some members of HMG proteins family, especially HMGI(Y) and HMGI(C) are frequently expressed in transformed cells at levels that correlate with the degree of neoplastic cell transformation [18, 24, 26]. Elevated levels of these proteins have been linked with human cancer and with neoplastic and metastatic phenotypes in model systems [4, 23, 25]. On the other hand, heterochromatin-associated protein 1 (HP1)Hs alfa expression is down-regulated at the mRNA and protein levels in highly invasive/metastatic breast cancer cell lines compared with poorly-invasive

ones (10]. Others NHCP are widely used as proliferation markers, which display a malignancy potential and behaviour of many human tumors. Assessment of the percentage of PCNA (proliferating cell nuclear antigen) or Ki-67 positive cells in cancer tissue samples in routine histopathological examination allows to predict clinical outcome of different types of cancer [5, 7, 21]. Although a number of nuclear proteins are common for different cell types [8], others — especially some nuclear matrix (NM) proteins seem to be specific for neoplastic tissues. Significant differences in NM proteins distribution were found between cancer tissues and their normal counterparts [9, 17]. NM represents the structural component of the nucleus that determines nuclear shape and higher order DNA organisation. NM proteins vary in a cell-type specific manner, suggesting that the NM may play an important role in tissue-specific three-dimensional organisation of DNA [8] and appear to be demonstrative of cell protein alterations which occurred during the establishment of the tumor phenotype. Several NM proteins characteristic only for normal breast and cancer tissues [9], as well as for benign prostatic hyperplasia and prostate cancer have been found [17]. Furthermore, NM proteins were also detected at elevated levels in the sera of cancer patients compared with normal patients sera [15]. Thereby, the detection of cancer-specific nonhistone proteins may become an important clinical tool in the diagnosis and monitoring of cancer.

The aim of this preliminary study was to examine qualitative and quantitative changes in the content of nonhistone proteins in larynx cancer versus normal larynx mucosa.

MATERIALS AND METHODS

Squamous cell primary larynx carcinoma samples were obtained after total laryngectomy in 10 patients. 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 –80°C.

All preparative work was performed at 4°C in the presence of 1 mM phenylmethylsulfonyl fluoride (PMSF) to inhibit serine proteases. Nuclei were obtained by the sucrose method according to Blobel and Potter with final purification by ultracentrifugation at 100,000 g through 2 M sucrose (3]. The nuclear chromatin was isolated by the method of Spelsberg and Hnilica [22] with an additional treatment with 0.5% Triton X-100 applied to remove membrane ghosts and except of the 0.3 M NaCl wash. Nonhistone proteins were than divided into three fractions: extracted with 0.35 M NaCl buffer, containing proteins loosely bound to DNA — fraction 1; tightly bound to DNA proteins, soluble in 2 M NaCl buffer — fraction 2; and proteins insoluble in 2 M NaCl solution — fraction 3. Fraction 3 also included nuclear matrix proteins. Quantitative determination of proteins amount was performed according to the method of Lowry [13]. DNA concentration in chromatin was assayed spectrophotometrically at 260 nm. All NHCP fractions were separated by electrophoresis on SDS-PAGE

linear gradient gel (5–15%) according to Laemmli [12]. Gels were stained with Coomassie Brillant Blue, dried and analysed densitometrically. Statistical analysis was performed using t-student test. P value < 0.05 was considered significant.

RESULTS

Growth of cancer is connected with quantitative changes of nuclear material. It has been reported that DNA amount and aberrant DNA ploidy in larynx cancer tissue correlated significantly with the size of the tumor and presence of local metastases [5]. It is assumed that the changed chromatin structure of tumor cells is associated not only with changes in DNA content, but also in NHCP content. [20]. In the present study we have examined quantitative changes in the NHCP fractions content in relation to the nuclear DNA. The results are summarised in Table 1. The mg protein/mg DNA ratio (means ± standard deviations) shows significantly higher amount (p = 0.01) of total chromatin proteins in larynx cancer tissue, on the average, 1.5 times those in normal mucosa. The contents of proteins in NHCP fractions were calculated in relation to the total chromatin protein amount. When comparing isolated NHCP fractions, statistically significant differences (p< 0,05) in protein content between normal and neoplastic tissue were found in NHCP fraction 1 and fraction 3. Increased NHCP content originating from larynx cancer was observed in fraction 3, containing proteins insoluble in 2 M NaCl, whereas in 0.35 M NaCl extracted proteins fractions 1 NHCP content was lower than in normal larynx mucosa. The amount of proteins in NHCP fraction 2 was similar and showed almost no differences between normal and cancer tissue. Analysis of the proteins pattern in all NHCP fractions revealed that the protein bands composition of normal larynx mucosa as well as larynx cancer tissue was quite similar in both samples. Several common proteins bands could be identified in all normal and tumor samples (Fig. 1, 2, 3). However, specific qualitative and quantitative differences did exist between electrophoretic NHCP pattern derived from larynx cancer and larynx mucosa.

Table 1. Total chromatin proteins (mg) / DNA (mg) ratio in larynx cancer and normal mucosa. Comparison of protein amount in isolated NHCP fractions, calculated in relation to the total chromatin protein amount.

Total chromatin protein / DNA ratio (mg/mg)		Concentration of protein amount in isolated NHCP fractions		
		Fraction 1	Fraction 2	Fraction 3
Larynx mucosa	0.81 ± 0.13	36.88 ± 6.45	9.61 ± 2.10	53.51 ± 5.88
Larynx cancer	1.20 ± 0.36	27.02 ± 7.16	9.71 ± 2.66	66.27 ± 7.34
N = 10	P = 0.01	P < 0.05	P > 0.05	P < 0.05

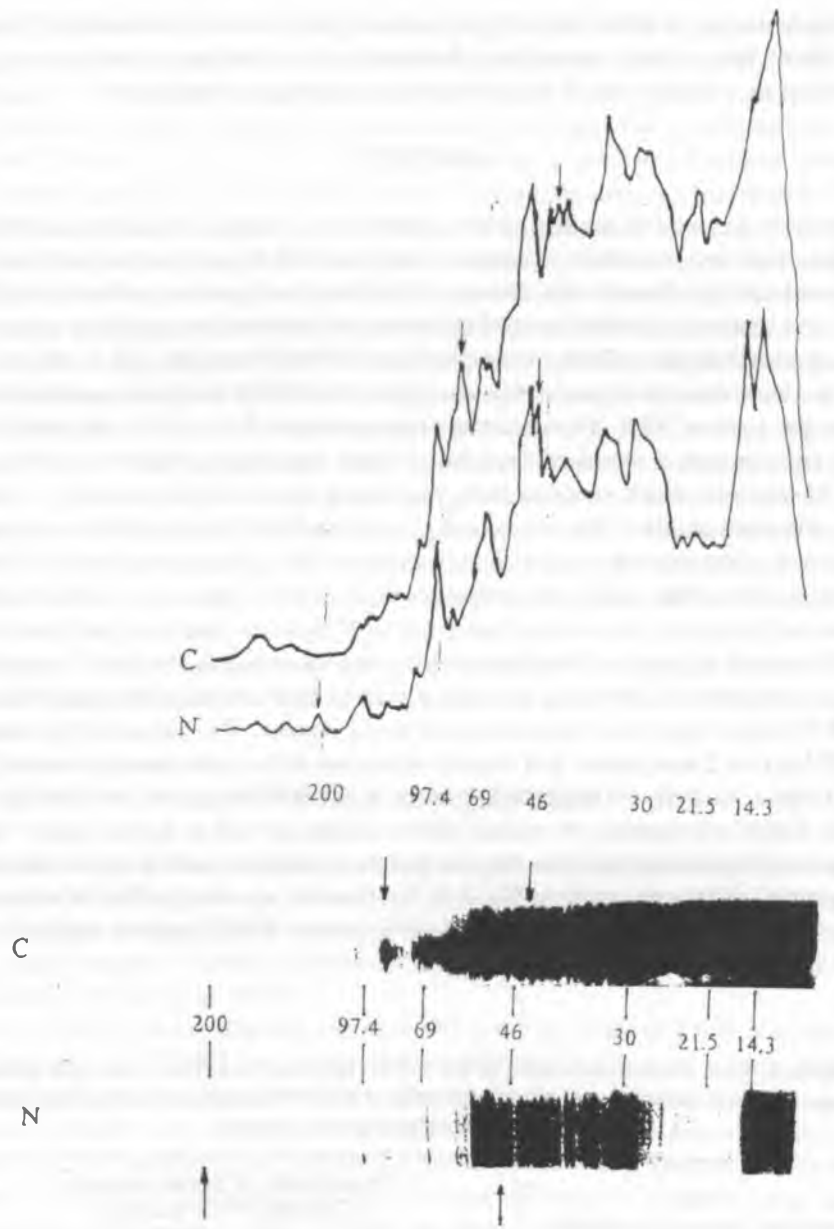

Figure 1. Electrophoretic analysis of NHCP fraction 1, soluble in 0.35 M NaCl buffer, derived from N — normal mucosa, C — larynx cancer. Densitometric scans of the stained gel. Protein bands characteristic for normal and cancer tissues are indicated by arrows. Molecular mass standards: myosin — 200 kDa, phosphorylase B — 97.4 kDa, bovine serum albumin — 69 kDa, ovoalbumin — 46 kDa, carbonic anhydarse — 30 kDa, trypsin inhibitor — 21.5 kDa, lysosyme — 14.3 kDa.

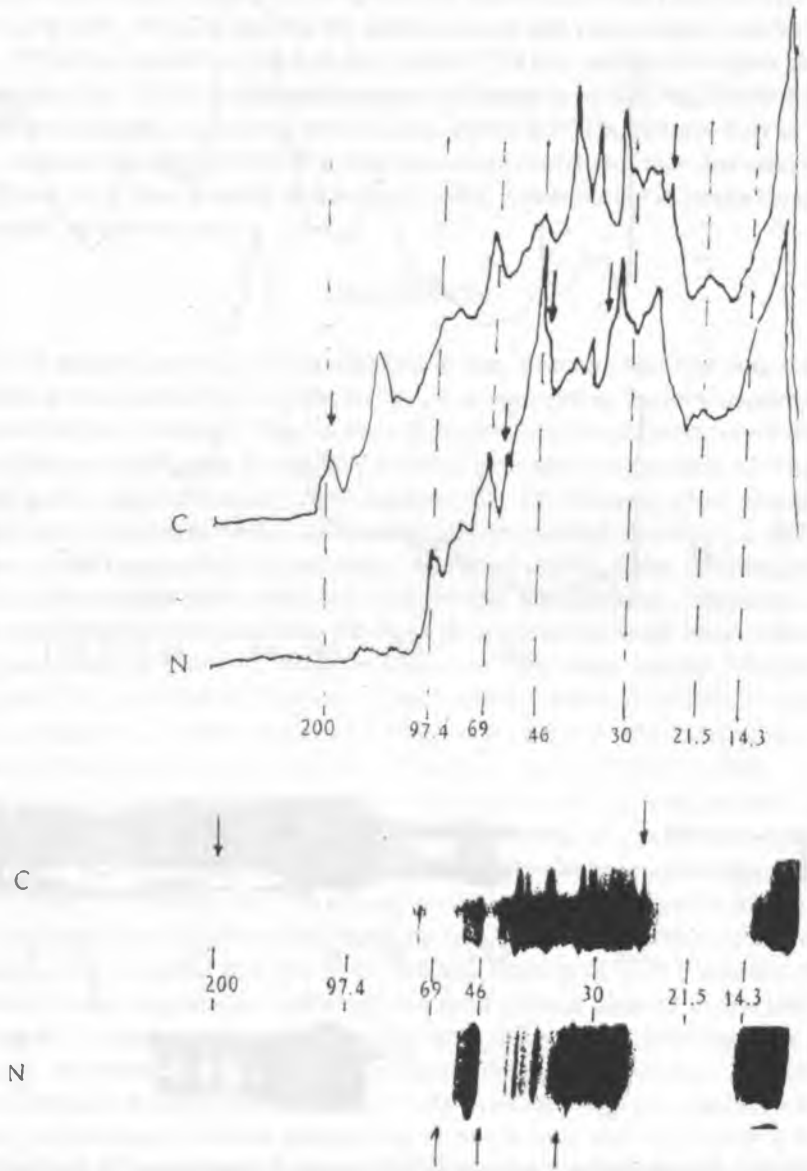

Figure 2. Electrophoretic analysis of NHCP fraction 2, soluble in 2 M NaCl buffer, derived from N — normal mucosa, C — larynx cancer. Densitometric scans of the stained gel. Protein bands characteristic for normal and cancer tissues are indicated by arrows. Molecular mass standards as in Fig. 1.

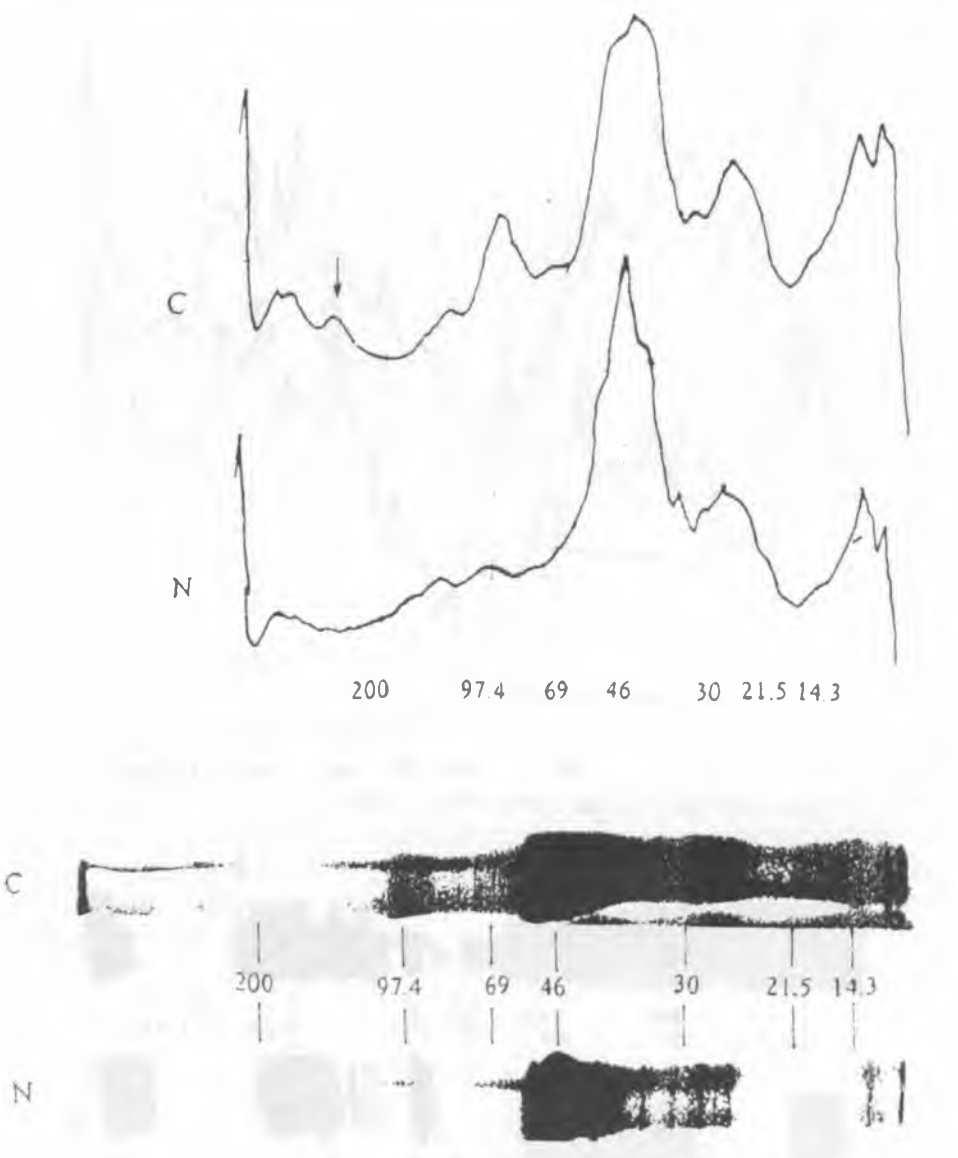

Figure 3. Electrophoretic analysis of NHCP fraction 3, insoluble in 2 M NaCl buffer, derived from N — normal mucosa, C — larynx cancer. Densitometric scans of the stained gel. Protein bands characteristic for normal and cancer tissues are indicated by arrows. Molecular mass standards as in Fig. 1.

NHCP fraction 1 loosely bound to DNA obtained from tumor tissue revealed two protein bands of m.w. about 89 kDa and 43 kDa, which seem to be absent in NHCP from control samples. On the other hand, protein bands of m.w. about 211 kDa and 50 kDa are characteristic of larynx mucosa (Fig. 1). Similarly, in fraction 2, which

includes NHCP tightly bound to DNA, we have observed protein bands of m.w. about 62 kDa, 46 kDa, 33 kDa specific for larynx cancer and two protein bands of m.w. about 200 kDa, as well as band of m.w. about 27 kDa specific for larynx mucosa, respectively (Fig. 2). Electrophoretic analysis of insoluble in 2 M NaCl buffers NHCP showed the existence of protein band of m.w. about 100 kDa, present only in cancer tissue. Most of the stained NHCP in fraction 3 were proteins of m.w. between 60 – 42 kDa. These data show marked differences in NHCP distribution in larynx cancer versus normal larynx mucosa.

DISCUSSION

NHCP include several proteins different in size, structure and function, relatively insoluble in low salt buffers with the tendency to aggregation, thereby require special methods for their isolation. One of the widely employed methods involves dissociation of chromatin proteins from DNA by using increasing concentration of high salt or urea buffers with subsequent centrifugation [22, 27]. However, other investigators use different methods to obtain and isolate nuclear proteins, thereby it is difficult to compare their results with the presented above, especially, when referring to other tissues. In the present study total chromatin protein concentration, calculated in relation to the DNA content, has been shown to be elevated in cancer tissue, when compared with control (Table 1), which is consistent with other reports. Miturski [16], Rybakova [19] and Schieck [20] have found a similar increase of NHCP content in cancer endometrium, leukemia cells and lung cancer, respectively. We have also found marked differences of protein amount in fraction 1 and 3 of NHCP (Table 1).

Especially fraction 3, which contains residual chromatin proteins insoluble in 2 M NaCl, including nuclear matrix proteins, shows a significant increase of tumor-derived NHCP amount. It has been shown that the NM protein concentration in normal tissues usually contains up to 12% of total nuclear protein. However, with the malignant transformation its content increases up to 27% at later stages as observed with hepatoma [27]. A similar increase of the residual fraction of NHCP amount was also observed in our experiments, although the total protein amount in fraction 3 was higher than in other studies, which results from different methods used for NHCP isolation. A number of studies demonstrated qualitative differences in NHCP between normal and tumor tissues [9, 11, 17, 27]. These findings are consistent with the known peculiarities of nuclear morphology of tumor cells, and may reflect a disorder of regulation of protein and nucleic acids biosynthesis underlying undifferentiated tumor growth. Cancer specific NM proteins have been detected in prostate [17], breast [9], hepatoma [11] and other cancers. We demonstrated an additional protein band of m.w. about 100 kDa in NHCP fraction 3 (Fig. 3), which seems to be absent in normal mucosa. The presence of this protein only in cancer tissue may represent a coincidence associated with cancer phenotype. It has been reported that NM proteins were detected in the sera of cancer patients [15], which can lead to detecting more specific cancer markers and applying it as a diagnostic tool. We demonstrate here that other

NHCP fractions derived from larynx cancer are qualitatively and quantitatively different from their normal counterparts, demonstrating both the loss and gain of specific protein bands (Fig. 1 and 2). Fraction 1 of NHCP, extracted with 0,35 M NaCl, containing NHCP loosely bound to DNA proteins, which include transcription factors anti- or pro-oncogens and proteins associated with cell proliferation, including HMG proteins family [25]. HMGI(C) and HMG(Y) proteins have recently been shown to be closely associated with tumorgenesis in the pancreas [1], colorectal [2], and other solid tumors [26], as well as are directly linked to metastatic breast cancer phenotype [14]. NHCP distribution changes in NHCP fraction 1, obtained from larynx cancer and normal larynx mucosa, observed in our experiments can be associated with enhanced cell proliferation of larynx tumors. These findings indicate that further isolation and characterisation of some NHCP protein bands from larynx cancer could provide new potential biomarkers for larynx cancer.

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STRESZCZENIE

Białka niehistonowe chromatyny pełnią funkcje strukturalne, enzymatyczne i regulacyjne. Wykazywano zmiany ilościowe i jakościowe białek chromatyny w różnego typu nowotworach w porównaniu z komórkami prawidłowymi. Celem prezentowanych doświadczeń była wstępna analiza składu białek niehistonowych w komórkach raka krtani i prawidłowej błony śluzowej. Stwierdzono obecność szeregu białek charakterystycznych dla komórek prawidłowych i nowotworowych w izolowanych frakcjach białek niehistonowych chromatyny.