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Nonhistone chromosomal proteins (NHCP) in adenocarcinoma of human endometrium

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Concentration and electrophoretic pattern of nonhistone chromosomal proteins (NHCP) in cancer and normal endometrium were examined. In normal tissues the NHCP/DNA ratio ranged from 0.6 to 0.8 whereas in malignant tissues from 1.2 to 1.7. Mean nuclear concentration of NHCP was 14.16 pg/cell nucleus of normal endometrium and 33.35 pg/cell nucleus of cancer tissue. The two tissues differed in the electrophoretic pattern. Heavy fractions (>60 kDa — 36%) predominated in normal tissue whereas light fractions (< 30 kDa — 48%) in malignant tissue samples. The percentage of intermediate fractions (30–60 kDa) was similar.

Neoplastic endometrium shows, irrespective of the patient's age, a high NHCP content. It seems that NHCP, especially the fraction extracted with 0.35 M NaCl, because of its tissue and cell specificity are involved in neoplasm formation.

Nonhistone chromosomal proteins (NHCP)[1] which are known to be species and tissue specific, are perhaps the most important factors in proliferation of cells [1]. NHCP extracted from chromatin, especially those soluble in 0.35 M NaCl, are metabolically active and undergo posttranslational modifications [2–5]. Quantitative and qualitative changes of NHCP were found to occur in the course of normal cell growth and malignant cell transformations [4, 6–8]. Kiang *et al.* [9] observed, in the estrogen dependent and estrogen positive mouse breast cancers, a high level of 31 kDa NHCP proteins. Their content was lower when no hormone dependence was detected, even when estimations of the estrogen receptor were positive. Cancer-associated NHCP in human hormone-dependent tissues were investigated in prostate and breast [10, 11]. In our previous studies differences in NHCP extracted from myomas, myometrium and endometrium were noted [12, 13].

This study was undertaken to check the differences, if any, in quantity and in electrophoretic pattern among NHCP soluble in 0.35 M NaCl isolated from adenocarcinoma of endometrium and normal proliferative endometrium in women.

MATERIAL AND METHODS

NHCP soluble in 0.35 M NaCl from cancer tissue (adenocarcinoma endometriale) of five women (mean age 59.5 years) were compared to those from normal tissue (endometrium proliferativum) of five women (mean age 43 years).

[1]Abbreviations: NHCP, nonhistone chromosomal proteins; PMSF, phenylmethylsulfonyl fluoride.

All preparative steps were carried out at 0°C. Tissue samples were homogenized in 0.33 M sucrose, 10 mM Tris/HCl buffer (pH 7.1), 5 mM $MgCl_2$ and 0.1 mM phenylmethylsufonyl fluoride (PMSF) in a polytron Ultra-Turrax homogenizer with teflon pestle. Homogenates were filtered through six layers of gauze and centrifuged twice at 10000 × *g* for 10 min. Nuclear pellets were suspended by gentle homogenization in 2.2 M buffered sucrose and ultracentrifuged at 100000 × *g* for 1 h. The number of nuclei was counted in Thom's chamber after staining with Nuclear Red. Chromatins were prepared according to Bonner *et al.* [14] in Spelsberg modification [15] but without washing with 0.35 M NaCl. The nuclei were homogenized in 10 mM Tris/HCl, 0.25 M sucrose, 0.2% Triton X-100, 0.1 mM PMSF (pH 7.5). After centrifugation the nuclear pellets were suspended in 0.8 M NaCl, 0.02 M EDTA, 0.1 mM PMSF (pH 6.3), homogenized and centrifuged twice at 10000 × *g* for 10 min. Next, a 1/100 SSC solution was used to hydratize chromatins. DNA was estimated spectrofotometrically at 260 nm in 5 M urea. According to the requirement of Pollow *et al.* [16] the purity of chromatin was considered satisfactory if the absorption ratio 320 nm/230 nm was lower than 0.1. NHCP were twice extracted with 0.35 M NaCl in 10 mM Tris/HCl, pH 7.1, in a glass homogenizer for 5 min. The supernatants after centrifugation were concentrated in dialysing membrane sacks, covered with Sephadex G-200 for 24 h. The amounts of NHCP were estimated according to Lowry *et al.* [17]. Electrophoresis was carried out by the method of Laemmli [18], using 30–50 μg of proteins per well. Electrophoretic gels were silver stained as described by Blum *et al.* [19]. The content of electrophoretic proteins fractions was evaluated by computed densitometry of gels and digitized scannig of the patterns obtained. Protein fractions were arbitrarily divided into three groups: of > 60 kDa, 30–60 kDa and < 30 kDa.

RESULTS

In normal tissue samples the NHCP/DNA ratio ranged from 0.6 up to 0.8 (mean value 0.65). In malignant tissue samples mean NHCP/DNA ratio was 1.35 (1.2–1.7). Mean nuclear concentration of NHCP varied, being 14.16 (12.11–16.32) pg/cell nucleus of normal tissue, and 33.35 (27.72–37.21) pg/cell nucleus of cancer tissue. Electrophoretic patterns were different, new protein fractions appearing in cancer tissue. These additional fractions were observed in every electrophoretic molecular mass group but they were not separately ana-

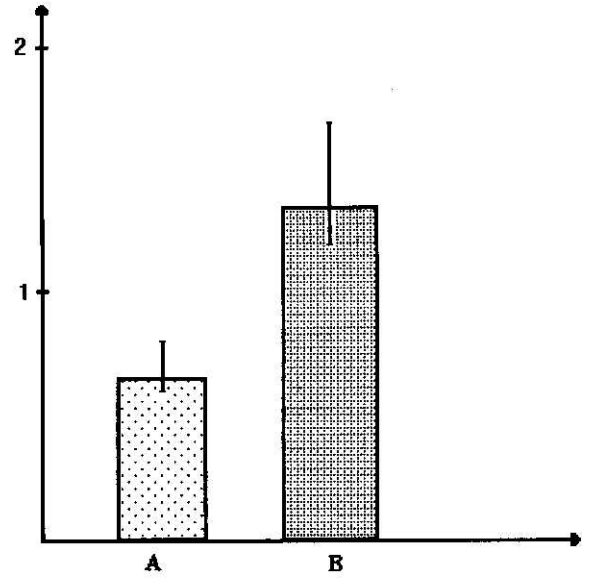

Fig. 1. The NHCP/DNA ratio in the proliferative endometrium and adenocarcinoma of women.
A, Endometrium proliferatum — 0.65, range: 0.6–0.8; B, adenocarcinoma — 1.35, range: 1.2–1.7.

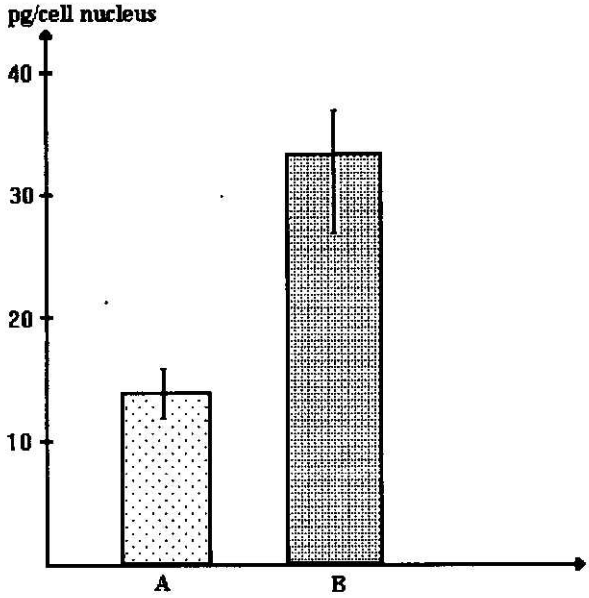

Fig. 2. Nuclear concentration of NHCP in proliferative endometrium and adenocarcinoma of women.
A, Endometrium proliferatum — 14.16 (12.11–16.32) pg/cell nucleus; B, adenocarcinoma — 33.35 (27.72–37.21) pg/cell nucleus.

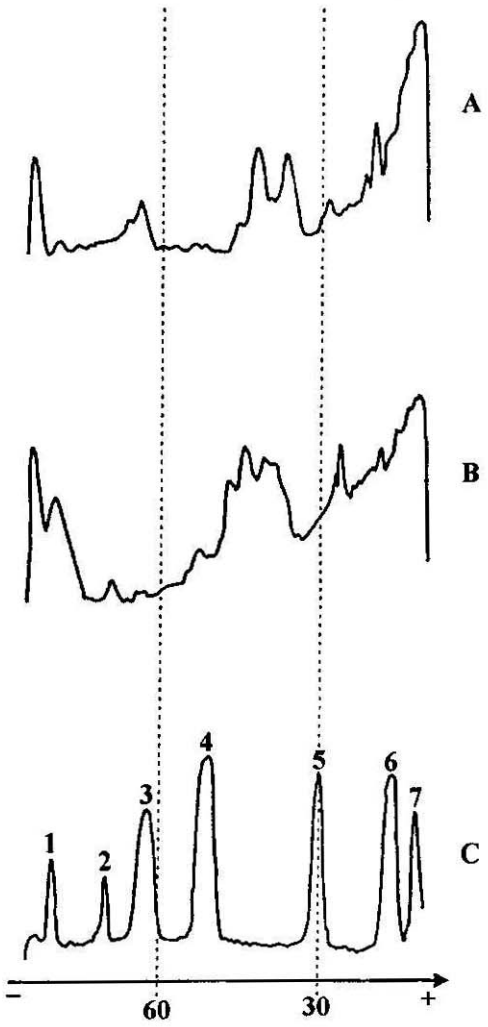

Fig. 3. Electrophoretic pattern of NHCP of proliferative endometrium and adenocarcinoma of women.

A, Endometrium proliferativum (case: T.B. 43 years); B, adenocarcinoma (case: G.C. 57 years); C, molecular mass markers: 1, myosin 200 kDa; 2, phosphorylase B 97.5 kDa; 3, bovine serum albumin 69 kDa; 4, ovalbumin 46 kDa; 5, carbonic anhydrase 30 kDa; 6, trypsin inhibitor 21.5 kDa; 7, lysozyme 14.3 kDa.

Table. 1

Mean portion of NHCP fractions in proliferative endometrium and adenocarcinoma of women

Tissue	NHCP fractions		
	> 60 kDa	30–60 kDa	< 30 kDa
Endometrium proliferativum	36 (30–40)	34 (28–36)	30 (26–34)
Adenocarcinoma	19 (17–26)	33 (28–37)	48 (38–52)

lyzed. Heavy fractions (> 60 kDa — 36%, range: 30%–40%) have dominated in normal tissue samples and light fractions (< 30 kDa — 48%, range: 38%–52%) in malignant tissue samples. The content of intermediate fractions (30–60 kDa) was similar: 34% (range: 28%–36%) for normal tissue and 33% (range: 28%–37%) for cancer tissue. In normal endometrium content of fractions < 30 kDa was 30% (range: 26%–34%) and content of fractions > 60 kDa in cancer tissue samples was 19% (range: 17%–26%).

DISCUSSION

The growth and function of endometrium are influenced by several factors, the most important of which are steroid hormones acting on receptors [20]. These receptors consist of NHCP proteins which play also a role in the cytoplasm-chromatin transport of steroid hormone [21, 22]. Cohen & Hamilton [21] found in rat uterus a 70.5 kDa protein, the concentration of which was raised when the animals were given estrogens. In chicken oviducts, Teng & Teng [23] found a tissue specific 90.5 kDa protein, the amount of which was raised in estrogenized animals. In a normal woman Pollow *et al.* [16] observed 222 fractions in the secretory and 220 in the proliferative endometrium. This difference in the number of protein fractions could be caused by hormonal, estrogen-progesterone influences occuring in the course of a normal menstrual cycle. In our NHCP electrophoretic pattern we have observed an increased percentage of low molecular mass fractions, < 30 kDa, in cancer tissue in comparison with normal endometrium where high molecular mass fractions of > 60 kDa predominated. It has been proved by others [9, 11, 24] that differences in electrophoretic fractions are characteristic of carcinogenesis and may be due either to a single fraction or a group of fractions. However, Wada *et al.* [11] noted the highest percentage of 42 kDa, 55 kDa and 190 kDa fractions in benign neoplasm of human prostate gland whereas in normal and cancer tissues the amounts of these proteins were similar. In cell lines of human colon cancer Ht-29, Hnilica *et al.* [24] have observed proteins of 67 kDa and 92 kDa not existing in normal cells. Pollow *et al.* [16] in normal endometrium found the NHCP/DNA ratio of 1.27 in the proliferative

phase and 1.26 in the secretory phase of cycle. In our study this ratio was lower almost by a half (0.65) which could have been caused by differences in the methodological approach, as Pollow *et al.* [16] extracted almost all proteins with high salt concentrations (4 M NaCl) whereas we extracted the 0.35 M NaCl soluble fraction. In an earlier study on endometrium [12] we have noticed a similar NHCP/DNA ratio (0.64) but in other tissues, e.g. in normal myometrium the NHCP/DNA ratio ranged from 0.48 to 0.83.

In cancer endometrium the NHCP/DNA ratio was higher in all the tissue samples studied and ranged from 1.2 up to 1.7 (mean 1.35) and was similar to our previous estimations (1.36) [25]. Similarly, Schieck *et al.* [26] have observed, in human lung cancer, the NHCP/DNA ratio of about 1.32. Benign neoplasms, like uterine myomas, are characterised by a lower content of NHCP, resulting in the NHCP/DNA ratio of about 0.8 [13].

We can say that the tissues growing in an uncontrolled way, like cancers, exhibit a higher content of NHCP soluble in 0.35 M NaCl as compared to normal tissues.

Estrogen dependence of adenocarcinoma of endometrium has been proved but, on the other hand, we have observed adenocarcinomas insensitive to these steroids [20]. In the present study we have observed increased amounts of low molecular mass fractions (< 30 kDa) and lowered amounts of other fractions in adenocarcinoma. This could have been caused by this same molecular mechanism due to which endometrial cancer is estrogen receptor positive but out of estrogen-progesterone growth control. The above mentioned Kiang's hypothesis [9] suggest that NHCP, especially those extracted with 0.35 M NaCl, are directly involved in cell metabolic functions and probably play an important role in endometrium cancerogenesis.

Halikowski & Liew [27] are of the opinion that some NHCP which are oncogen products showing enzymatic or acceptor functions, exist as structural proteins in nuclear matrix and influence RNA synthesis.

It seems possible that in future the NHCP pattern specific for neoplastic tissues could be helpful in diagnosis and treatment of the adenocarcinoma endometriale patients.

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