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# CHANGES OF AN ANDROGEN-DEPENDENT NUCLEAR PROTEIN DURING FUNCTIONAL DIFFERENTIATION AND BY DEDIFFERENTIATION OF THE DORSOLATERAL PROSTATE OF RATS

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SUMMARY: Nuclei of the dorsolateral prostate of rats contain a large amount of androgen-dependent non-histone protein (20K-NHP)(mol. wt. ≒ 20,000; pI ≒ 11.5) (Matuo et al. (1)). Its content in the nuclei increased most markedly during 4-8 weeks of age, when functional differentiation of the prostate was most active on the basis of the changes of major cytosol proteins and zinc. Nuclei of the Dunning tumors originating in the dorsolateral prostate were found to lack 20K-NHP regardless of androgen dependency, indicating the disappearance of the 20K-NHP from the nuclei by dedifferentiation. These suggest that the 20K-NHP is an important nuclear protein for differentiation of the dorsolateral prostate cells.

The prostate glands depend on the level of androgen for differentiation and maintenance of their structures and functional activities. Recently we have been studying the differences between androgen-dependent subcellular proteins in the ventral and dorsolateral prostates of rats (2-5). We have found that nuclei of the dorsolateral prostate contain a novel androgen-dependent non-histone protein having a molecular weight of about 20,000 and pI of about 11.5 (20K-NHP) (1, 2). A spontaneous adenocarcinoma originating in the dorsolateral prostate was discovered by Dunning in 1961 (6). The parent tumor (R 3327-H) is the slow-growing and androgen-sensitive tumor. The R 3327-HI-S tumor which was derived from the parent tumor is the androgen-insensitive tumor (7-10). The present paper deals with studies on (i) the change of the 20K-NHP in nuclei during functional differentiation of the dorsolateral prostate of rats, and (ii) the disappearance of the 20K-NHP in the dedifferentiated prostate, i.e. the Dunning prostatic adenocarcinoma.

## MATERIALS AND METHODS

Male Sprague-Dawley rats (4-16 weeks of age) were used (1-5). Frozen tumors of R 3327H and its sublines were kindly donated by Dr. J. Isaacs, Medical

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School, Johns Hopkins University, Baltimore. Normal Copenhagen/Fischer rats of 10 weeks of age were gifts from Dr. N. H. Altman, Papanicolaou Cancer Research Institute, Miami. Preparation of cytosol and nuclei (1-5), and SDS (sodium dodecyl sulfate)-polyacrylamide slab gel electrophoresis (3) were carried out by the methods described previously. Protein species of the cytosol fractions separated in the gel are expressed as (apparent molecular weight x $10^{-3}$)K. The content of a protein species was measured from the densitograms by planimeter. "Relative content" of a species in the cytosol fractions was calculated from the equation: (stained area of a species)/(stained area of all the species) x 100(%). To minimize experimental errors, the content of 20K-NHP (non-histone protein having a molecular weight of about 20,000) was calculated from the equation: (stained area of 20K-NHP)/(total stained area of core histones). Zinc was determined by atomic absorption spectroscopy, using a Shimadzu AA-630-01 spectrophotometer in the flame mode. The contents of protein and DNA were determined by the methods of Lowry et al. (11) and Schneider (12), using bovine serum albumin and calf thymus DNA as standards, respectively.

## RESULTS

Changes of the 20K-NHP content in nuclei during functional differentiation of the dorsolateral prostate of rats In order to clarify the criteria of functional differentiation of the dorsolateral prostate, the cytosol proteins were analyzed by SDS-electrophoresis and the Zn content in the cytosol fractions was measured, with the prostate of rats from 4 to 16 weeks of age. Of the proteins separated in the gel, 67K was the most abundant species in the prostate of all the rats tested. Its content increased during 4-8 weeks of age and then remained constant until week 16 (Fig. 1). The content of 120K was low during 4-5 weeks of age, and increased after week 6; it then remained constant from week 10 until week 16. The two species were the major secretory proteins in the dorsolateral prostate (data not shown). The content of 41K, which was not secretory protein, was high during 4-6 weeks of age, and then decreased; it remained constant from 10 until 16 weeks of age. However, the content of secretory 30K protein was hardly changed from 4 to 16 weeks of age. The contents of these major protein species (41K, 67K and 120K) in addition to minor species became constant during 8-11 weeks of age. On the other hand, the Zn content in the cytosol fractions increased markedly between 4 and 8 weeks of age and then increased gradually until week 16 (Fig. 2). Accordingly, functional differentiation of the dorsolateral prostate of rats was found to be most active before 8 weeks of age. On the densitograms of nuclear proteins, the content of core histones/DNA was not influenced, whereas the contents of non-histone proteins having a molecular weight higher than 30,000 increased

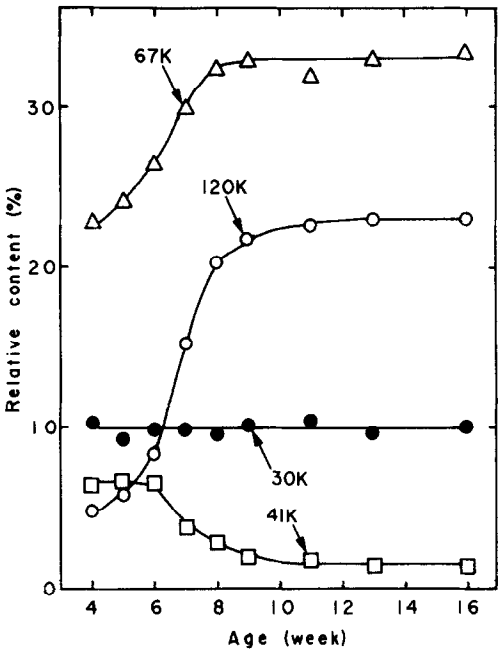


Fig. 1. Changes of major cytosol proteins in the dorsolateral prostate of rats from 4 to 16 weeks of age. Relative contents of the cytosol proteins (30K, 41K, 67K and 120K) were measured from the densitograms of SDS-polyacrylamide slab gel electrophoresis of the cytosol proteins (0.1 mg).

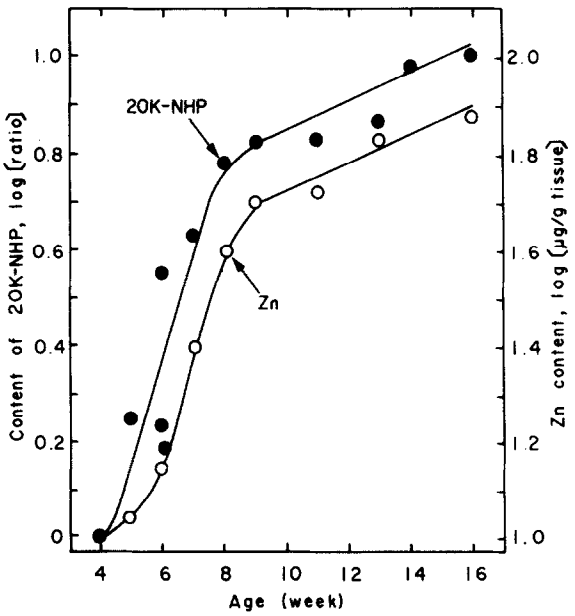


Fig. 2. Changes of the Zn content in the cytosols and the 20K-NHP content in the nuclei of the dorsolateral prostate of rats from 4 to 16 weeks of age. The 20K-NHP content (per core histones) was shown as log(relative value), where the content at week 4 was taken as log 1 = 0. The data for 20K-NHP contents except 4-, 5-, 7- and 9-week-old-rats were taken from our report (13). The Zn content was shown as log(μg/g tissue).

maximumly 2 times, by aging from 4 to 16 weeks of age, in accord with our recent findings (13). Of non-histone proteins in the dorsolateral prostate, the 20K-NHP content was highest regardless of age during 4-16 weeks of age. Its content increased markedly between 4 and 8 weeks of age and then increased gradually until week 16 (Fig. 2). The change of the 20K-NHP content in the nuclei during 4-16 weeks of age was strikingly similar to that of the Zn content in the cytosol, indicating the close relation between the 20K-NHP content in the nuclei and the Zn content in the cytosol. We emphasize, therefore, that the change of the 20K-NHP content in nuclei is closely implicated for functional differentiation of the prostate. These results suggest the low contents of 20K-NHP and Zn in the dedifferentiated prostate.

Disappearance of 20K-NHP in nuclei of the Dunning prostatic adenocarcinoma (R 3327) The Dunning tumors contained Zn in the cytosol fractions as low as the ventral prostate (Table I). SDS-electrophoretic patterns of the cytosol

Table I Protein and DNA contents of nuclei and Zn content in cytosols from normal prostates and various sublines of the Dunning prostatic adenocarcinoma and characteristics of the tumors.

| Tissue | Androgen sensitivity[a] | Growth rate[a] | Zn content (μg/g tissue) | Content (mg/g tissue) Protein | DNA | Protein/ DNA ratio |
|---|---|---|---|---|---|---|
| Normal: | | | | | | |
| VP[b] | yes | | 6.0 | 2.1 | 0.83 | 2.5 |
| DLP[b] | yes | | 50 | 3.5 | 0.88 | 4.0 |
| R 3327 tumor: | | | | | | |
| H tumor | yes | slow | 4.2 | 8.3 | 2.83 | 2.9 |
| H tumor derived: | | | | | | |
| MAT-LyLu | no | fast | 7.0 | 6.2 | 2.84 | 2.2 |
| MAT-Lu | no | fast | 6.0 | 3.8 | 2.22 | 1.7 |
| AT-2 | no | fast | 5.0 | 5.0 | 1.99 | 2.5 |
| HI tumor | no | slow | 6.2 | 10.1 | 4.27 | 2.4 |

Yields of DNA in the nuclear fraction were about 60% with either prostate and about 80% with the Dunning tumors. a) Taken from the data by Isaacs et al. (9). b) Protein and DNA contents of nuclei from Copenhagen/Fischer rats of 10 weeks of age were shown. These values were essentially the same as those from Sprague-Dawley rats of 10 weeks of age. VP, ventral prostate; DLP, dorsolateral prostate.

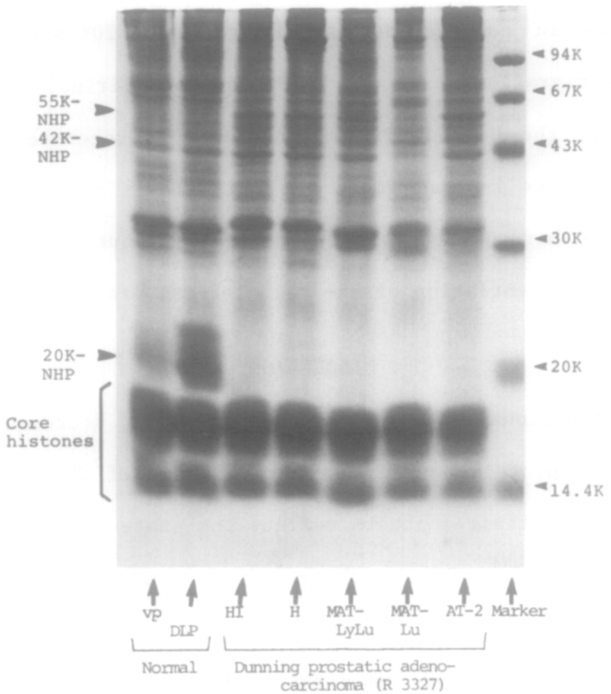


Fig. 3. **SDS-electrophoretic patterns of proteins in nuclei from the prostates of Copenhagen/Fischer rats and the Dunning tumors. Each sample contained 50 μg DNA. VP, ventral prostate; DLP, dorsolateral prostate. Characteristics of the Dunning tumors are shown in Table I.**

proteins in the Dunning tumors revealed the disappearance of 120K besides 67K and the appearance of 41K (data not shown). These indicate that all the Dunning tumors are completely dedifferentiated. The Dunning tumors showed higher DNA content than the normal prostates did (Table I). There were marked similarities in the number and density of proteins including 20K-NHP on the electrophoretic patterns of nuclear proteins in the prostate of Copenhagen/Fischer rats and Sprague-Dawley rats. As shown in Fig. 3, the core histones (H2A, H2B, H3 and H4)/DNA contents were almost equal among normal prostate and Dunning tumors, whereas the contents of non-histone protein were distinctly different; the 42K-NHP content, which was high in the normal dorsolateral prostate, was low in the metastatic subline of MAT-Lu, and the 55K-NHP content, which was low in the normal prostate, was high in all the sublines except for MAT-Lu. The R 3327-H tumor was characterized by the high content of non-histone proteins having a molecular weight higher than 94,000. Special

growth-related non-histone protein was not detected. We emphasize that besides these differences in non-histone protein regions, 20K-NHP in addition to several proteins migrating at around 23,000-daltons virtually disappears from the nuclei of all the Dunning tumors including androgen-sensitive tumor (R 3327-H). These results indicate that the 20K-NHP was related neither to the growth rate nor to hormone sensitivity of the tumor but to functional differentiation and dedifferentiation of the dorsolateral prostate of rats.

## DISCUSSION

Because the functionally differentiated prostate secretes a variety of substances such as proteins and Zn, a large amount of which is derived from the cytosol fraction, their contents reflect the state of functional differentiation of the epithelium. Of the changes in the cytosol proteins (30K, 41K, 67K and 120K) analyzed by SDS-electrophoresis, the increase of 67K by functional differentiation and its disappearance by dedifferentiation are in accord with the finding of Wilson and French (14). Timms and Chandler (15) showed that the Zn content in the cytosol of the dorsolateral prostate was dependent on serum androgen level, and that testosterone propionate was most effective in elevating Zn in the dorsolateral prostate of castrated rats. Gunn et al. (16) demonstrated that the selective uptake of $^{65}Zn$ occurred in the dorsolateral prostate but hardly in the ventral prostate of rats. We found that the contents of major cytosol proteins and 20K-NHP, in addition to the Zn content in the cytosol, changed age-dependently, and that all of these levels changed most markedly before 8 weeks of age. Moreover, we recently reported that serum testosterone concentration increased most remarkably at around 7 weeks of age (13). We emphasize, therefore, that the 20K-NHP together with the Zn content is closely implicated in functional differentiation and probably for aging of the prostate. Although there were similarities in the increasing patterns between the 20K-NHP content and the Zn content during the functional differentiation, 20K-NHP is not a metallothionein because of extremely low content of cysteine (1). On the other hand, almost all of a protein having a molecular weight of about 20,000 and pI of about 11.5 in the prostate are

localized in the nuclei. Major protein bands migrating at around 20,000-daltons were hardly recognizable in the cytosol fraction of the Dunning tumors by SDS-electrophoresis. The disappearance of 120K besides 67K, low content of Zn and appearance of 41K in the cytosol fractions of the Dunning tumors suggest that the Dunning tumors are completely dedifferentiated (to be published elsewhere). In addition, 20K-NHP was not found in nuclei of all the sublines of the Dunning tumor regardless of the difference in androgen dependency. These findings provide evidence that 20K-NHP could not be a necessary factor to androgen dependency, if the control system to androgen dependency in the normal prostate is identical to that in the Dunning tumors, but it may be an important nuclear protein for differentiation of the prostate. As far as we know, there is no report that an abundant non-histone protein such as 20K-NHP disappears from the nuclei by dedifferentiation. Further studies will be needed for the intranuclear localization of 20K-NHP and relationships between the 20K-NHP content and the Zn content in the cytosol.

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