SIGNIFICANCE OF NUCLEOLAR ORGANIZER REGIONS IN PROSTATE ADENOCARCINOMAS

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Abstract: *The aim of this study was to define the relation between silver stained nucleolar organizer regions (AgNORs) count and histological grade, and clinical stage in needle biopsy specimens of 65 prostatic adenocarcinomas. Histological grade was determined according to the modified Gleason system. AgNORs were evaluated by the counting method on routine histological sections stained with silver. There was a significantly increase of AgNOR count in high versus low grade prostate carcinomas. AgNORs increased significantly with clinical stage. Mean AgNOR count was significantly higher in lymph node metastases than in primary tumours. These data demonstrate that AgNOR counts reflects the proliferative activity of prostate adenocarcinomas and could be useful as prognostic factor in prostatic needle biopsy specimens.*

Key words*: AgNORs; Gleason; prognosis; prostate cancer.*

1. Introduction

Prostatic carcinoma has become the most common malignant tumor among men in the USA and the second leading cause of death from cancer [1]. The criteria available for the diagnosis and prognosis of prostate cancer, such as histological grade, clinical stage, prostate specific antigen (PSA) levels, and DNA content [2], often do not sufficiently predict the outcome of this disease [3].

The nucleolar organizer regions (NORs) are defined nucleolar components where ribosomal genes are complexed with non-histone proteins characterized by a high affinity for silver (AgNOR proteins) [4]. NORs can be selectively visualised by silver staining in routinely processed histological samples [5]. Extensive evidence shows that the quantity of AgNOR protein reflects the state of cell proliferation [6] and represents an independent marker with highly significant predictive value in numerous human tumours [7]-[14].

There are two methods for the quantitative analysis of AgNOR proteins: the counting method and the morphometric method. The counting method consists in enumeration of each silver stained dot per cell directly at the microscope by carefully focusing at very high magnification (1000x) [15].

In the present study we have analysed cell AgNOR quantity in 65 needle biopsy specimens from prostate adenocarcinomas with or without lymph node metastases.

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We have also correlated the AgNOR count with histological grade and clinical stage and compared AgNOR count in primary carcinomas and metastases.

2. Material and Method

2.1. Patients and specimens

In the present study we have analysed needle core biopsy specimens obtained from 65 patients with prostatic adenocarcinoma diagnosed between 2009 and 2010 at the Department of Urology, Clinical County Hospital of Braşov, Romania. The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Two serial sections were cut from each paraffin wax block: the first (5 μm thick) was stained with haematoxylin and eosin for histological diagnosis and tumour grading and the second (3 μm thick) with silver to visualise the NORs.

The modified Gleason system [16], [17] was used to grade the tumours. 19 (29.23%) tumours were low grade or Gleason score 2 to 5, 41 (63.08%) intermediate grade or Gleason score 6 and 7, and 5 (7.69%) high grade or Gleason score 8 to 10.
Clinical stage was assessed according to the American Joint Committee on Cancer (AJCC) system [18]. 23 (35.38%) were clinically localised tumours (stage T1 and T2), and 43 (66.15%) were advanced tumours (stage T3 and T4).

2.2. NOR silver staining and AgNOR quantification

For the determination of the AgNOR numbers, a method adapted from Ploton *et al*. [5] was used. Briefly, tissue sections were deparaffinized, rehydratated and then soaked in a silver nitrate in acid gelatine aqueous medium solution.

Quantitative analyses of tissue sections for AgNOR staining were performed using a Olympus BH50 microscope. The AgNOR granules were clearly visible in the nuclei of tumor cells (figure 1 and figure 2).
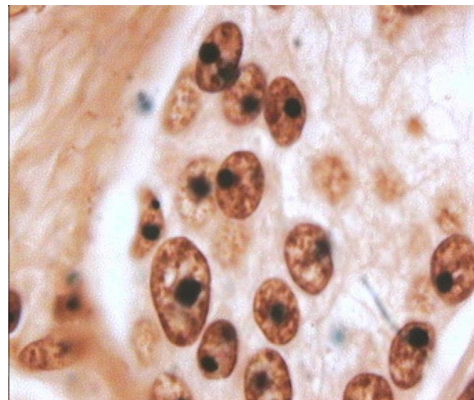

Fig. 1. *Histological section of a needle biopsy specimen of a low grade prostate carcinoma showing one or two AgNOR dots in most nuclei. Staining for AgNOR proteins (x1000)*
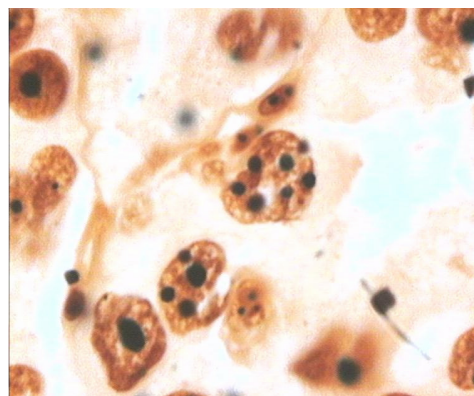

Fig. 2. *Histological section of a needle biopsy specimen of a high grade prostate carcinoma showing numerous AgNOR dots in most nuclei. Staining for AgNOR proteins (x1000)*

For each primary tumor and each lymph node metastasis, at least 100 nuclei were randomly selected for AgNOR count directly at the light microscope at a final magnification of 1000. Nuclei were counted systematically clockwise in each gland to avoid recounting. The mean of AgNOR content was calculated using the following equation:

$$\text{Mean AgNOR} = \Sigma \text{AgNOR}/100 \qquad (1)$$

The mean values were correlated with histological grade and clinical stage. Mean AgNOR counts were compared in primary tumors and their lymph node metastases.

2.3. Statistical analysis

Statistical analysis was carried out using the Statistica package (StatSoft Inc.). Correlations between quantitative proliferation marker (AgNOR) and already known prognostic factors (clinical stage, Gleason score) were analysed using the Student t test. A p-value < 0.05 (two-tailed) was considered to be statistically significant.

3. Results

Needle core prostate biopsy specimens were available from 65 patients with prostate adenocarcinomas aged 59 – 86 years (mean 72 years). Clinical follow-up was available for all 65 patients.

The mean AgNOR number ranged from 3.83 to 6.17, with mean (SD) values of 4.88 (0.53) positively stained granules per nucleus per patient specimen.

A significant difference was found in the AgNOR values between low and high grade tumors (table 1).

Table 1

AgNOR count according to Gleason score

	n	Mean (SD) AgNOR number	p value
Gleason score			
2-5	19	4.85 (0.48)	0.03
6,7	41	4.83 (0.53)	0.02
8-10	5	5.40 (0.50)	

Tumors of high-grade malignancy had smaller, more numerous AgNORs than those that are less malignant (fig. 2).

Patients with clinically localized tumor (stages T1 and T2) had lower AgNOR values than patients with advanced disease (stages T3 and T4) (table 2).

Table 2

AgNOR count according to clinical stage

	n	Mean (SD) AgNOR number	p value
Clinical stage			
T1 and T2	23	4.77 (0.28)	0.04
T3 and T4	43	5.06 (0.25)	

The mean AgNOR count was 4.66 (0.41) in primary tumours and 4.96 (0.49) in lymph node metastases. Statistical analysis of AgNOR values using linear regression method revealed a strong correlation in the two series (figure 3).
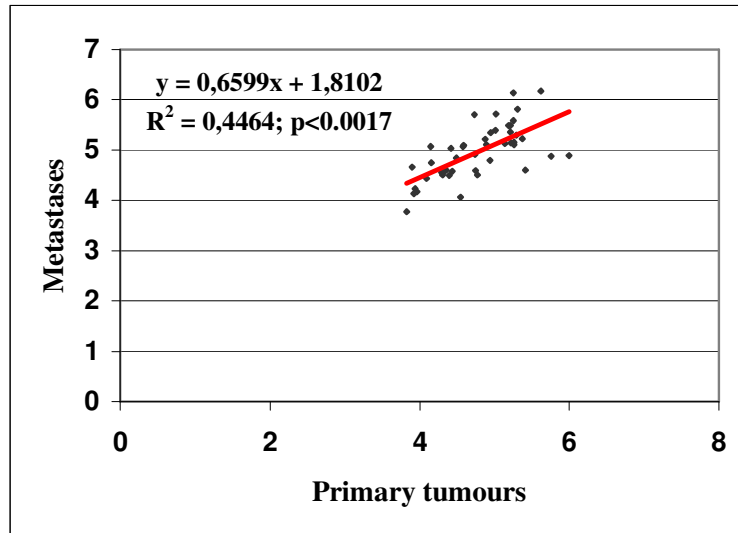
Fig. 3. *Regression analysis of AgNOR values in primary and metastatic prostate adenocarcinomas*

4. Discussion

Histological grade is the most important independent parameter for predicting prognosis of patients with prostatic cancer [19]. Of the different methods proposed for grading prostate tumours, the Gleason system is the most frequently used [20]. However, the criteria used for the histological grading are subjective. In our study the modified Gleason system [16], [17] was used to grade the tumours.

In the present study we investigated the significance of AgNOR count in prostatic adenocarcinomas. The significance of NORs is not well understood. Their count depends on the stage of cell cycle. In tumors, malignant transformation, the degree of differentiation, and the proliferation rate of cells may be reflected in the AgNORs [21].

AgNOR count has been proved to be a significant predictor in several human tumors [7]-[14].

It is well known that nucleoli increase in size and number in prostate carcinoma [22]. Thus, it seems reasonable to expect that AgNOR numbers might increase with more aggressive, higher-grade prostatic lesions.

A study by Sakr *et al*. [23] using 20 specimens showed a significant correlation between AgNOR expression and Gleason score, and concluded that AgNOR could be a marker of tumour differentiation. In our study, a significant difference was found in the AgNOR count between low grade (Gleason score 2-5) and high grade (Gleason score) tumours. Low grade and intermediate (Gleason score 6 and 7) showed similar AgNOR values.

Mundai *et al*. [24] found that AgNOR staining only showed a correlation with Gleason score, AJCC stage and PSA level when used in the mathematical model in combination with p53 and Bcl-2 but, as a stand-alone marker, AgNOR staining did not show any significant correlation with any clinical data. In the present study

patients with clinically localised tumour (stages T1 and T2) had significant lower AgNOR values than patients with advanced disease (stages T3 and T4).

Goel *et al.* [25] study showed a significant increase in AgNOR counts between localized and metastatic carcinoma. In our study differences in AgNOR dot number in metastatic versus non-metastatic carcinomas were also significant.

The advantage of the counting method is that it is easy to execute and inexpensive. Even if this method is more frequently employed, it becomes subjective and poor reproducible, especially when interphase AgNORs are clustered together or partially overlap, as often happens in malignant lesions [15].

5. Conclusion

All these data have lead to the conclusion that interphase AgNOR quantity does represent a good marker for the cell proliferation rate.

In the present study AgNOR counts, evaluated by the counting method, showed a good correlation with Gleason score and clinical stage. A significant increase in AgNOR counts between localized and metastatic carcinoma was also demonstrated.

On the basis of these results, it may be concluded that silver-stained nucleolar organizer regions (AgNORs) quantity is related to the aggressiveness of prostatic carcinomas and may provide a reliable assessment of the malignant potential of these cancers.

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