

# Nucleolar Organizer Region in Renal Cell Carcinoma

## RENAL HÜCRELİ KARSİNOMDA NÜKLEOLAR ORGANİZER BÖLGE

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### Summary

The purpose of this study was to determine the number of Nucleolar Organizer Regions (NOR) in renal cell carcinoma and to investigate its correlation with the tumor grade and stage.

Data were obtained from samples of 30 patients with renal cell carcinoma. AgNOR staining was assessed in each case. Silver binding black dots per nucleus were counted in 200 tumor nuclei under  $\times 500$  magnification and the mean number of AgNORs per nucleus was calculated for each specimen.

The statistical studies showed that there was a significant difference between the indices of grades 1 and 2 ( $p < 0.05$ ) and grades 1 and 3 ( $p < 0.01$ ) and between grades 2 and 3 ( $p < 0.01$ ) renal cell carcinomas. As well there was a statistically significant relationship between stage groups and proliferation indices of renal cell carcinoma ( $p < 0.01$ ).

These findings suggested that measurement of the proliferating indices by AgNOR method in renal cell carcinoma are correlated well with the grade and stage. AgNOR count in renal cell carcinoma may prove to be an objective and quantitative assay of biological aggressiveness and provide significant prognostic information and may also have an impact on the treatment of patients.

**Key Words:** Renal cell carcinoma, Nucleolar organizer region, Prognosis, Proliferation

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Renal cell carcinoma is almost exclusively a cancer of adults occurring at rates of 5.6 and 4.1 per 100.000 among males and females, respectively. The tumor affect men 2 times more frequently than

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### Özet

Bu çalışmanın amacı renal hücreli karsinomlarda Nükleolar Organizer Bölge (NOB) sayısını saptamak ve tümör grade ve evresi ile ne şekilde ilişki gösterdiğini araştırmaktır.

Veriler renal hücreli karsinomu olan 30 hastanın örneklerinden elde edildi. Her vakaya AgNOR boyaması yapıldı. Gümüş bağlanmış siyah noktalar 200 tümör nükleusunda 1500 büyütme ile sayıldı ve nükleus başına düşen ortalama AgNOR sayısı her vaka için hesaplandı.

İstatistiksel incelemede; grade 1 ve 2 ( $p < 0.05$ ), grade 1 ve 3 ( $p < 0.01$ ), grade 2 ve 3 ( $p < 0.01$ ) renal hücreli karsinomlar arasında belirgin farklılık gözlemlendi. Aynı şekilde evre grubları ile renal hücreli karsinomların proliferasyon indeksleri arasında istatistiksel olarak belirgin ilişki ( $p < 0.01$ ) saptandı.

Bu bulgular, renal hücreli karsinomlarda AgNOR methodu ile proliferasyon indekslerinin ölçümünün grade ve evre ile iyi şekilde korelasyon gösterdiğini düşündürdü. Renal hücreli karsinomlarda AgNOR sayısının objektif olduğu, biyolojik agresifliğin bir sayısal göstergesi olarak kabul edilebileceği ve önemli prognostik bilgi verebileceği gibi, hastaların tedavisinde de etkisi olabileceği düşünüldü.

**Anahtar Kelimeler:** Renal hücreli karsinom, Nükleolar organizer bölge, Prognoz, Proliferasyon

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women and its frequency increases with advancing age, occurring generally during the fifth or sixth decades of life (1).

The overall prognosis of renal cell carcinoma depends on a comprehensive and accurate description of size, extent and microscopic characteristics of the tumor. Grade, nuclear pleomorphism, mitotic rate, pseudocapsule thickness, Uumor pattern, local extension, renal vein invasion and stage have been investigated as possible prognostic variables (2-5).

Consequently there has been an interest in the characterization of supplementary markers to improve on conventional routine histopathology. One such area under investigation is the study of nucleolar organizer regions (NORs).

Nucleolar organizer regions (NORs) are loops of ribosomal DNA coding for ribosomal RNA (6). NORs are essential part of the nucleus and are related to the proliferative activity of cells (7,8). In nucleus these regions can be demonstrated by a silver staining procedure (9).

Assessment of proliferation by silver staining of nucleolar organizer regions (AgNORs) have been reported to be of prognostic relevance in breast, colon and other tumors (10-13).

The goal of this study was to quantify NORs in a series of renal cell carcinoma and to compare mean NOR counts with grade and clinical stage.

### Materials and Methods

Thirty specimens of renal cell carcinoma were included in this study. The group was comprised of 20 men and 10 women with a mean age of  $58 \pm 9.2$  (range 38-72).

The diagnosis was established by histopathologic examination in all patients. Tumor grade based on the size, contour and conspicuousness of nucleoli which were defined by Fuhrman et al (14). Tumors were assigned to stages according to the classification of the "Union International Contre Le Cancer" (UICC) (15). We divided patients into two groups as low stage group (T1+T2) and high stage (T3+T4) group.

Tumors had been fixed in 10 % formalin and processed to paraffin wax. Sections were cut at 4 um thickness and were taken to water via xylene and graded alcohols.

The sections were submitted to the AgNOR procedure at room temperature for 30 minutes. The reaction mixture comprised 2% gelatin in 1 % aqueous formic acid. This was mixed in a proportion of 1:2 volumes with 50% aqueous silver nitrate under dark room condition (9). Counterstaining was not performed.

Sections were examined under oil immersion lens at a magnification of 1500 and 200 nuclei were studied. Discrete black dots and dispersed AgNORs

in the nuclei were counted and the mean number of AgNORs per nucleus was calculated for each specimen.

### Results

The distribution of 30 cases in grades and stage groups were summarized in Table 1. A statistically significant relationship was found between grades and stage groups ( $p < 0.001$ ) (student's t-test).

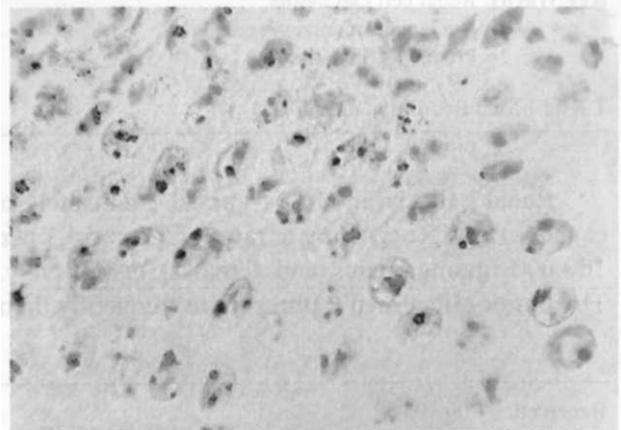
AgNORs were clearly recognized as black dots in cell nuclei (Fig 1). The average of the NOR indices of all cases was  $6.8 \pm 1.3$ . The mean and standard deviation of NOR indices seen in each grade and stage is shown in Table 2.

As shown in Table 2 the higher the grade the higher the AgNOR counts were observed ( $p < 0.001$ ) (Kruskall Wallis One way Anova). When the groups were analyzed by means of the Mann Whitney U test, it was seen that in grading system there was a highly significant difference in AgNOR counts between patients with renal cell carcinoma of grades 1 and 2 ( $p < 0.05$ ), as well as

**Table 1.** The distribution of grade and stage groups

	Grade 1	Grade 2	Grade 3	T
T1 + T2	9*	5	2	16
T3 + T4	1	5	8	14
Total	10	10	10	30

\*  $P < 0.001$  (Student's t-test)



**Figure 1.** Nucleolar organizer region, x 1500. Grade 3 renal cell carcinoma with numerous AgNORs seen within the nuclei.

**Table 2.** Silver stained nucleolar organizer regions per nucleus in grades and stage

	N	%	AgNOR
Grade 1	10	33.3	3.1 ± 0.7#
Grade 2	10	33.3	6.5 ± 1.3
Grade 3	10	33.3	9.3 ± 0.7
T1 + T2	16	53.3	5,4± 0,30
T3 + T4	14	46.7	9.1 ± 1.2

# p<0.01 (Kruskal Wallis One way Anova)  
pO.001

between tumors of grades 1 and 3 (p<0.01) and between patients with grades 2 and 3 (P<0.01).

It was apparent that irrespective of the grade the more advanced the stage the higher the AgNOR counts was observed (p<0,01).

Correlation coefficient analysis revealed that AgNOR counts was associated strongly with the tumor grade (r=0.9748 p<0.001) and pathologic stage (r=0.9582 pO.001).

### Discussion

Traditional methods of prognostication in histopathology include histologic grading and stage. Tumor grade, stage, tumor size, and tumor pattern were the most important prognostic factors in determining the progression of the renal cell carcinoma (2-5). The UICC TNM classification and tumor grade have been accepted as the most important and useful prognostic factors in recent studies (16,17). However tumor grading has the disadvantage of being subjective. Grading of renal cell carcinoma is complicated by the lack of a universally accepted grading system, and it has been suggested that grading is subjective (5). As well the prognostic value of stage is not always useful. Because of this reason various attempts have been made to identify retrospectively other parameters that could provide additional prognostic information about tumor progression. In recent years more objective and reproducible methods, based on the quantitation of cell characteristics, have been developed.

Methods directed at different parts of the cell cycle are available. Silver staining of proteins associated with nucleolar organizer regions (AgNORs) was used on conventional histological sections for assesment of proliferation rates (9-13). By using AgNOR technique, many AgNOR sites have been

observed in certain malignant and benign lesions (9-13). The use of the AgNOR technique in paraffin wax sections of minors was recently described for assesment of grades of malignancy. Initial studies have indicated that enumeration of the AgNORs can help in the identification of high grade tumors from low grade tumors (10-13,18). It is clear that an increase in AgNOR count can be seen in actively proliferating cells due to increased trancriptionally active ribosomal sites. Subsequent studies have shown that the number of AgNORs observed are closely related to the percentage of cells recognised by the antibody Ki-67 (10).

The diagnosis and grading of renal cell carcinoma using evaluation of AgNORs by light microscopy has been shown to be of great value (18). Significant differences of AgNOR counts are seen between low grade and low stage tumors with high grade and high stage tumors (11,18,19). Contradictory results have also been noted with marked variations in AgNOR scores reported from some series (20,21). This variation may be due in part to proliferative compartmentalization in renal cell carcinoma. In some studies, evaluating areas were chosen randomly, while in other studies areas for quantification were chosen from greatest proliferating activity areas. In addition, staining variability of AgNOR method and lack of agreement in counting AgNOR dots could change the results of proliferation index of the tumor.

In the present study, the results show that the mean number of AgNORs in renal cell carcinoma have a tendency to increase with increasing grade and stage. Those with high grade renal cell carcinoma with high stage were significantly shows high AgNOR counts than those in grade I tumors with low stage.

In conclusion, AgNOR counts in renal cell carcinoma were correlated to the grade and stage. It was also shown that a good linear correlation exist between AgNOR counts with grade and stage. Currently on the basis of our data, AgNOR gives the accurate cell kinetic information for more immediate application as diagnostic and prognostic tool.

### REFERENCES

1. Kantar AF. Current concepts in the epidemiology and etiology of primary renal cell carcinoma. *J Urol* 1972; 117:415.
2. Thrasher JB, Paulson DF. Prognostic factors in renal cancer. *Urol Clin North Am* 1993;20:247-62.

3. Lanigan D, Convoay R, Bavvy-Walsh C, Loftus B, Royston D, Leadev M. A comparative analysis of grading systems in renal cell adenocarcinoma. *Histopathology* 1994;24:473-6.
4. Kolbeck PC, Kaveggia FF, Johansson SL, Grune MT, Taylor RJ. The relationship among tumor-infiltrating lymphocytes, histopathologic findings and longterm clinical follow-up in renal cell carcinoma. *Mod Pathol* 1992;5: 420-5.
5. Bostwick DG, Eble J. Neoplasms of the kidney In: *Urologic Surgical Pathology*. St. Louis, Missouri: Mosby-YearBook, 1997: 84-147.
6. Buys CM, Osinga J. Selective staining of the same set of nucleolar phosphoproteins by silver and giemsa. *Chromosoma* 1984;89:387-96.
7. Alberts B, Bray J, Lewis J, Raff M, Roberts K, Watson JD. *Molecular Biology of the Cell*. Newyork Garland 1983:424-6.
8. Leek RD, Allison MR, Sarraf CE: Variations in the occurrence of silver staining nucleolar organizer regions (AgNORs) in non-proliferating and proliferating tissues. *J Pathol* 1991; 165:43-51.
9. Ploton D, Menager M, Jeannesson P et al. Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer regions at the optical level. *Histochem J* 1986; 18:5-14.
10. Gasparini G, Boracchi P, Verdcrio P et al. Cell kinetics in human breast cancer: comparison between the prognostic value of the cytofluorimetric S-phase fraction and that of the antibodies to Ki-67 and PCNA antigens detected by cytochemistry. *Int J Cancer* 1994;57:822-9.
11. Ruschoff J. Prognostic significance of nucleolar organizing regions (NORs) in carcinomas of the sigmoid colon and rectum. *Pathol Res Pract* 1990;186:85-91.
12. Scagliotti G, Miccla M, Gubelta L et al. Prognostic significance of Ki-67 labelling in resected non-small cell lung cancer. *Eur J Cancer* 1993;29 A: 363-5.
13. Kunishio K, Ohmoto T, Matsuhisa T et al. The significance of nucleolar organizer region (AgNOR) score in predicting meningioma recurrence. *Cancer* 1994;73:2200-5.
14. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol* 1982; 6:655-63.
15. Union International Contre Le Cancer. *TNM atlas third ed, second revision*. Springer-Verlag 1992.
16. Robson CJ, Churchill BM, Anderson W. The results of radical nephrectomy for renal cell carcinoma. *J Urol* 1969; 101:297.
17. Medeiros LJ, Gclb AB, Weiss LM. Renal cell carcinoma: Prognostic significance of morphologic parameters in 121 cases. *Cancer* 1988; 61:1639.
18. Delahunt B, Nacey JN, Hammett GD, Frater WJ. Nucleolar organizing regions in renal cell carcinoma, renal oncocytoma and renal adenoma. *Anal Cell Pathol* 1989;1:185-90.
19. Shimazui T, Tomobe M, Hattori K, Uchida K et al. A prognostic significance of nucleolar organizer region (AgNOR) in renal cell carcinoma. *J Urol* 1995; 154:1522-6.
20. Pich A, Valente G, Azzoni L et al. Argyrophilic nucleolar organizer region counts and Ki-67 scores in human renal cell carcinoma. *Pathol Res Pract* 1991; 187:482-6.
21. Delahunt B, Bethwaite PB, Nacey JN, Ribas JR. Variation in AgNOR scores for renal cell carcinoma. *J Pathol* 1992; 168:424-5.