Argyrophilic Proteins of Nucleolar Organizer Regions (AgNORs) in fallopian tube carcinomas

J. K. Rabczyński[1], A. T. Kochman[1], A. Karmowski[2], Z. M. Woźniak[1], A. Bronowicz[1], Ł. Terpiłowski[2]

[1]Department of Pathological Anatomy, and [2]1st Department of Obstetrics, Medical University of Wrocław (Poland)

Summary

Forty-four patients diagnosed with primary cancer of the fallopian tube (PFTC) were analyzed with regard to AgNORs expression, morphological classification, clinical stage and survival rate. Twenty-seven patients were FIGO stage I, 7 FIGO II and 10 FIGO III. Histological classification of PFTC revealed 18 endometrioid types, 9 serous, 7 undifferentiated, 6 urothelial, 2 clear cell and 2 another type of (intestinal, squamous cell) cancer. Histological grading revealed 11 G1, 16 G2 and 17 G3 tumors. The number of AgNORs per nucleus ranged from 1 to 7, mean 2.54±0.77. The smallest number of silver stained NORs was observed in the endometrioid type (mean 2.32±0.62) and the biggest number of AgNORs in undifferentiated carcinoma (mean 3.05±0.82). There was no correlation between number of AgNORs and AgNOR area/nuclear area ratio and survival (p=0.71), histological stage or histological type of PFTC. There was a correlation between the number of AgNORs among tumors with histological grade 1 and grade 3 (p=0.023), and grade 2 and grade 3 (p=0.045). However, there was no correlation between AgNOR number and survival rate in these groups.

Key words: Nucleolar Organizer Regions; AgNORs; Fallopian tube carcinoma.

Introduction

Nucleolar organizer regions (NORs) are localized in five pairs of metaphase acrocentric chromosomes [1-5] and contain multiple gene coding for ribosomal RNA (rRNA) [6, 7]. Ultrastructural studies have demonstrated that during interphase the NORs are located in the fibrillar components of the nucleolus [7, 8]. NORs can be visualized via an argyrophil technique in which silver nitrate stains NOR associated proteins [1, 2, 5-14]. Active stained interphase NORs, named AgNORs, appear as black dots in the nucleus. AgNORs proteins are involved in the regulation of transcription and post-transcriptional modification of rRNA transcript [7]. For the last 12 years the analysis of distribution of AgNOR proteins appears to reflect various aspects of cellular activity [7, 8, 13, 14]. The study of many routinely prepared histo- and cytological samples have revealed significant differences between low-grade and high-grade malignancies [1, 2, 5, 15-18]. The prognostic significance of the argyrophilic staining technique has been evaluated in various forms of neoplasms [5, 11, 12, 16-18, 20]. Primary fallopian tube cancer (PFTC) is a rare gynecologic malignancy with a poor outcome in most cases [3, 4, 9, 11, 21-24]. Only stage of disease has established prognostic value [3, 9,11, 23]. Other factors such as ploidy, grading, histological type, p53 gene mutation, overexpression of products of c-erbB-2 oncogenes have limited or controversial significance [3, 4, 9, 11, 21, 23, 24].

The aim of this work was to compare AgNOR expression in a series of 44 cases of fallopian tube carcinomas with morphological classification, clinical stage and survival rate.

Revised manuscript accepted for publication March 1, 2000

Material and Methods

From 1981 to 1997, 44 cases of primary fallopian tube carcinomas were collected in the Department of Pathology of Silesian University of Medicine in Wrocław (Poland). The histopathological diagnosis was based on routine histology and immunohistological tests. All cases were reclassified according to the common epithelial ovarian tumor WHO classification [25]. Histological grades of malignancy were estimated with the Hu *et al.* classification [21]. Clinical stage of the 44 cases of PFTC was performed according to FIGO classification [19]. Clinical data, histological classification, p53 protein accumulation and c-erbB-2 overexpression have been presented in detail in other publications [11, 23]. The AgNOR staining method has been described elsewhere [26]. Briefly, deparaffinized specimens were sectioned at 4 μm and immersed in freshly prepared staining solution of one volume of a 2% gelatin solution in 1% formic acid and two volumes of 50% aqueous silver nitrate. The slides were incubated for 35 minutes at room temperature in the dark and washed with bi-distillated deionized water. Then the slides were placed in 5% thiosulfate solution for 5 min, washed again briefly (5 sec.), counterstained with Mayer's hematoxylin, dehydrated to xylene and mounted in synthetic medium.

Two observers without knowledge of the histologic diagnosis analyzed AgNOR stained slides. Counting was performed on 100 to 215 randomly selected cancer cells at a total magnification of x 600. Quantification of AgNORs was performed with a computer-assisted image analysis MultiScan [10]. The following parameters were computed: nuclear area, number of AgNORs per nucleus, AgNOR area per nucleus and AgNOR area/nuclear area ratio. Two independent persons performed quantification. Analysis of variance and the multiple comparison Scheffe test were used to compare the number of AgNORs, AgNOR area/nuclear area ratio in histological type and stage groups. Survival estimates (Kaplan-Meier curves) were calculated using the Kaplan-Meier life table method. Differences in survival were tested with log-rank statistics. The Cox proportional hazard model was used to analyze the association of examined parameters with survival time.

Results

The group consisted of 44 patients, median age 57.5 years (range 38-70). Staging of the disease was classified according to FIGO classification [3, 9]. Twenty-seven patients were FIGO stage I, 7 FIGO II and 10 FIGO III. Histological classification of the tumors revealed: 18 endometrioid, 9 serous, 7 undifferentiated, 6 urothelial, 2 clear cell and 2 another type of (intestinal, squamous cell) carcinoma. Histological grading revealed 11 G1, 16 G2 and 17 G3 tumors. After surgical treatment, chemotherapy and radiotherapy, 17 women died of disease recurrence (mean survival 29 months). Twenty-seven patients are alive (mean 51.5 months), 24 without any symptoms of neoplastic disease and 3 with local recurrence of the tumor. The number of AgNORs per nucleus ranged from 1 to 7, mean 2.54±0.77. The lowest rate of silver stained NORs was observed in the endometrioid type (mean 2.32±0.62) and the highest one was found in undifferentiated carcinomas (mean 3.05±0.82). The silver stained dots had the smaller square area in the nuclei of clear cell types of PFTC (mean 0.26 μm^2 ± 0.1). The relation between the area of AgNORs and nucleus square area was the biggest in the cells of undifferentiated types of PFTC. Data of silver stained dots in nuclei of the cells in various histological types of PFTC are shown in Table 1.

There were no noted statistical correlations between numbers and area of AgNORs or mean square area of AgNORs/nucleus and survival time (p=0.15, p=0.41) between the groups of 27 patients who survived and 17 patients who died (Table 2).

We also did not find any positive correlation of AgNOR parameters and histological type and staging of PFTC (Table 3). In the group of 27 patients whit FIGO stage I of PFTC, 20 patients are still alive (median survival 63.5 months) and 7 patients have died (median survival 35 months) (Table 4). A positive correlation between FIGO stage and number of AgNORs was only marginally significant (p=0.059); survival time by FIGO stage is shown in Figure 1.

Statistical analysis also revealed significant differences in the number of AgNORs between tumors with histological grade 1 (n=11) and grade 3 (n=17), p=0.023, and between tumors with grade 2 (n=16) and grade 3, p=0.045. However, there was no positive correlation, between survival time and AgNOR parameters in these groups (Table 5).

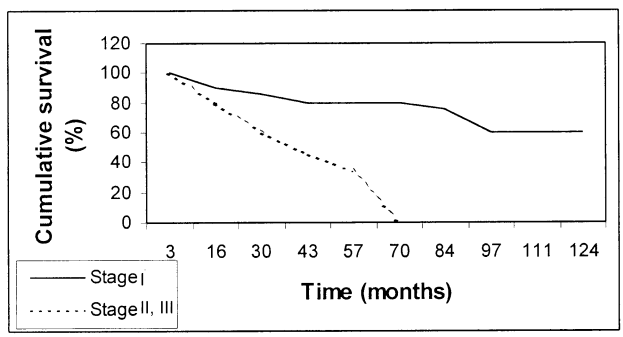

Figure 1. — Survival time by FIGO stage.

Table 1. — *Distribution of AgNORs and nuclear parameters based on histological classification of PFTC.*

	Carcinoma endomtrioid (mean) (n=18)	Carcinoma serous (mean) (n=9)	Carcinoma urothelial (mean) (n=6)	Carcinoma undifferentiated (mean) (n=7)	Carcinoma clear cell (mean) (n=2)	Another type (mean) (n=2)	Total (mean) (n=44)	*P* value
AgNOR number/nucleus	2.32±0.62	2.63±0.66	2.4±0.61	3.05±0.82	2.82	3.38	2.54±0.77	
AgNOR square of one nucleus (mean)	0.78±0.38	0.89±0.29	0.88±0.35	0.91±0.34	0.75±0.37	0.71±0.18	0.83±0.34	
One AgNOR square (mean)	0.37±0.24	0.36±0.17	0.41±0.31	0.35±0.18	0.26±0.1	0.32±0.29	0.36±0.22	NS
One nucleus square (mean)	16.95±5.7	26.55±3.77	22.01±10.22	22.73±9.85	23.8±12.9	20.27±12.32	21.03±7.9	
AgNOR square/nucleus	0.084±0.02	0.087±0.019	0.093±0.019	0.13±0.011	0.09±0.01	0.1	0.087±0.018	

Table 2. — *Relationship between number of AgNORs and AgNOR area/nuclear area ratio and survival time.*

Characteristic	Patients (mean mos alive)	AgNORs/ nucleus	AgNOR square	Nucleus square / AgNOR square	*P* value
Alive	27 (51.5 months)	2.48±0.77	0.82±0.36	0.086±0.02	NS
Dead	17 (29 months)	2.75±0.86	0.84±0.31	0.087±0.015	

Table 3. — *Distribution of AgNOR number and AgNOR area/nuclear area ratio in FIGO stage I.*

Characteristic	Alive (n=20)	Dead (n=7)	Total (n=27)	*P* value
Mean time alive (months)	63.5	35	56	
N umber AgNORs/nucleus	2.34±0.81	3.11±1.1	2.54±0.94	p=0.059
AgNOR sq/nucleus square	0.085±0.02	0.09±0.004	0.087±0.018	NS

Table 4. — *Distribution of AgNORs per nucleus regarding degree of PFTC differentiation.*

	G1 (n=11)	G2 (n=16)	G3 (n=17)	*P* value
Number of AgNORs/nucleus (mean)	2.26±0.53	2.41±0.67	2.96±0.94	G1:G3 p=0.023 G2:G3 p=0.045

Discussion

Primary carcinoma of the fallopian tube is the rarest malignancy in the female genital tract and is associated with a poor outcome [3, 4, 9, 11, 21-24]. Different studies of PFTC have been of limited value because of the difficulty in assembling long series of patients with PFTC in a short time [3, 21]. It is true that surgical treatment of PFTC is the oldest and the best method of therapy, however adjuvant therapy, i.e. radio- and chemotherapy, may distinctly prolong the life of patients with residual neoplastic disease [3]. The search for prognostic markers in this disease has brought limited results [9, 11, 22, 24]. Solely clinical staging of the disease on the day of diagnosis has praiseworthy value [9, 11, 22-24]. Other markers have rather limited or controversial usefulness [9, 11, 22-24]. The number, area and localization of NORs are indicators of proliferative activity of normal, hyperplastic and neoplastic cells [9, 11, 20, 27]. The studies of prognostic significance of NORs in carcinomas of the sigmoid colon and rectum have revealed a correlation between five-year survival rate and the mean AgNOR number per tumor cell and mean size of AgNOR [17]. In this study there was no significant relationship between AgNOR content and grade of malignancy, pT or pN categories. In renal cell carcinoma a positive correlation between tumor grade, rate of nuclear positivity for MIB-1 and PCNA and the number of AgNORs has been shown. Furthermore the number of AgNORs was significantly related to the patient's survival [18]. It is puzzling that markers of cell proliferation did not correlate with flow cytometry analysis of DNA in this study [18]. Analysis of PCNA expression and the amount of AgNORs in ovarian carcinomas indicated that their number per nucleus was of high significance with regard to histologic grade and stage of disease [16]. Both these markers may provide valuable prognostic information about the biologic potency of ovarian cancer [16]. The study of AgNOR counts in ovarian epithelial tumors with various malignancies revealed a progressive increase from adenomas to borderline tumors and to carcinomas. Unfortunately, it is not useful to anticipate the clinical behavior of borderline tumors, especially the appearance of peritoneal implants [19]. Similar results have been obtained by other authors while studying AgNORs in serous ovarian benign, borderline and malignant tumors [12]. A relationship between DNA ploidy and AgNOR number of borderline serous and mucinous ovarian tumors in other investigations has not been observed. The histological type of the tumor, flow cytometric proliferative index and AgNOR counts were not predictive of survival. The authors suggested that AgNOR number might be related to nuclear events other than proliferation and DNA ploidy [6]. On the other hand, the determination of the AgNOR number may be helpful in distinguishing between endometrial hyperplasias with and without cytological atypia [15]. Also the combination of positive staining for epithelial membrane antigen with an average area of AgNORs per cell increases sensitivity in the diagnosis of epithelial malignant mesothelioma. Determination of only AgNOR count had rather little value in distinguishing between reactive mesothelial from malignant mesothelial proliferation [12]. Investigations on AgNOR number and standard proliferation marker Ki67 in cases of vulvar carcinoma refuted the usefulness of both these parameters as predictors of inguinofemoral lymph node metastases [1]. On the other hand the results of MIB-1 and AgNOR examination proved to be useful predictors of lymph node metastases in the cases of extrahepatic bile duct carcinoma [5]. This is the first study of the evaluation of prognostic significance of AgNOR count, square area, and square of AgNOR nucleus square relation in cases of PFTC. We have not observed any correlation between these fundamental findings in AgNOR analyses and in clinical stage, histological type and survival for the whole group of PFTC cases. No relationship between AgNOR parameters in the cases of PFTC with FIGO stage II and III was found. Only examination of these relations for the group of patients with FIGO stage I revealed a correlation between number of AgNORs and survival. Between tumors of low and high degrees of histological differentiation a statistically significant difference in the number of AgNORs per nucleus was observed as well. It is true that PFTC has similar biological properties to ovarian epithelial cancer [3, 22]. In most cases, also in FIGO stage I, the microscopic examination of samples of the tubal wall revealed direct invasion and transluminal spreading which involved the serous membrane of the ovaries, uterus, intestines and opposite tube [3, 23]. The early lymphatic and blood-borne spreading is the most important factor that limits long-term disease-free survival and determines the treatment [3]. The analysis of clinical data suggests that the problem is in the late diagnosis rather than the high degree of malignancy [21]. In our opinion, AgNOR investigation in PFTC has limited value because its usefulness as an outcome predictor is limited only to early stages of

Table 5. — *Distribution of AgNOR parameters regarding FIGO stage.*

Stage	I (n=27)	II (n=7)	III (n=10)	*P* value
Number AgNORs/nucleus	2.53±0.93	2.61±0.58	2.71±0.56	
AgNOR area/nucleus (mean)	0.85±0.38	0.75±0.26	0.85±0.26	NS
AgNOR area/nuclear area ratio	0.086±0.02	0.09±0.02	0.083±0.021	

PFTC. The dramatic decrease in survival of patients with PFTC in the stages higher than FIGO stage I depended on the dissemination of neoplastic cells [3, 4]. Similarly to cancer in the other sites, the proliferation markers are not important to anticipate the progress of diesease [2, 12, 19, 20]. Our study proved that evaluation of AgNORs in early stages of PFTC has prognostic value. It is possible that the quantitation of AgNORs together with other cell proliferation markers might be of higher value as a prognostic factor in PTFC. In advanced stages of PFTC prognosis is mostly dependent on surgical and chemical treatment modalities rather than on histological type and degree of histological malignancy [11].

References

[1] Dong H., Bertler C., Schneider E. *et al.*: "Assessment of cell proliferation by AgNOR scores and Ki-67 labeling indices and a comprison with potential doubling times. *Cytometry*, 1997, *28*, 280.

[2] Griffiths A. P., Cross D., Kingstone R. E. *et al.*: "Flow cytometry and AgNORs in benign, borderline and malignant mucinous and serous tumours of the ovary". *Int J. Gynecol. Pathol.*, 1993, *12*, 307.

[3] Nordin A. J.: "Primary carcinoma of the fallopian tube: a 20-year literature review". *Obst. Gynecol. Surv.*, 1994, *49*, 349.

[4] Novak E. R., Woodruff J. D.: "Gynecologic and Obstetric Pathology". W. B. Saunders Co., Philadelphia, London, Toronto, 1979.

[5] Suto T., Sugai T., Nakamura S. *et al.*: "Assessment of the expression of p53, MIB-1 (Ki-67 antigen), and argyrophilic nucleolar regions in carcinoma of the extrahepatic bile duct". *Cancer*, 1998, *82*, 86.

[6] Henderson A. A., Warburton D., Atwood K. C.: "Localization of ribosomal DNA in the human chromosome complement". *Proc. Natl. Acad. Sci*, USA 1972, *69*, 3394.

[7] Hernandez-Verdum D.: "The nucleus today". *J. Cell. Sci*, 1991, *99*, 461.

[8] Derenzini M., Pession A., Fraegoli F., *et al.*: "Relationship between interphasic nucleolar organiser regions and growth rate in two neuroblastoma cell lines". *Am. J. Pathol.*, 1989, *134*, 925.

[9] Hellstrom A. C., Hue J., Silfversward C. *et al.*: "DNA-ploidy and mutant overexpression in primary fallopian tube cancer". *Int. J. Gynecol. Cancer*, 1994, *4*, 408.

[10] Piasecki T., Jeleń M., Kurzyński M. *et al.*: "Use of the computer program for histopathological image analysis in assessment of AgNORs expression". *Image Process Communicat*, 1998, *4*, 81.

[11] Rabczyński J., Kochman A., Kowalski P. *et al.*: "c-erbB-2 oncogene and mutant p53 overexpression in primary fallopian tube cancer: A clinicopathologic analysis of 41 cases". *Cent. Europ. J. Immunol.*, 1998, *23*, 99.

[12] Trabucco S., Varcaccio-Garofalo G., Botticella M. A. *et al.*: "Expression of AgNORs in serous ovarian tumors". *Eur. J. Gynaecol. Oncol.*, 1994, *15*, 222.

[13] Woźniak Z. M., Usson Y., Parazza F. *et al.*: "Three-dimensional distribution of the AgNORs protein during interphase in leukemic cells studied by confocal microscopy". *Cytometry*, 1996, *24*, 14.

[14] Woźniak Z. M., Bonnefoix T., Zheng X. *et al.*: "Interest of angyrophilic proteins nucleolar organizer regions (AyNOR) to estimate the reactivity of T cell clones against autologous malignant B-NHL cells". *Anal. Cell. Pathol.*, 1995, *9*, 123.

[15] Coumbe A., Mills B. P., Brown C. L.: "Nucleolar organiser regions in endometrial hyperplasia and neoplasia". *Path. Res. Pract.*, 1990, *186*, 254.

[16] Ghazizadeh M., Sasaki Y., Araki T. *et al.*: "Prognostic value of proliferative activity of ovarian carcinoma as revealed by PCNA and AgNOR analyses". *Am. J. Clin. Path.*, 1997, *107*, 451.

[17] Ruschoff J., Bittinger A., Neumann K. *et al.*: "Prognostic significance of nucleolar organizing region (NORs) in carcinomas of the sigmoid colon and rectum". *Path. Res. Pract.*, 1990, *186*, 85.

[18] Tannapfel A., Hahn H. A., Katalinic A. *et al.*: "Prognostic value of ploidy and proliferation markers in renal cell carcinoma". *Cancer*, 1996, *77*, 164.

[19] Emanuels A. G., Burger M. P., Hollema H. *et al.*: "Quantitation of proliferation-associated markers Ag-NOR and Ki-67 does not contribute to the prediction of lymph node metastases in squamous cell carcinoma of the vulva". *Hum. Pathol.*, 1996, *27*, 807.

[20] Khattech A., Spatz A., Prade M. *et al.*: "Nucleolar organizer regions in ovarian tumors: discrimination between carcinoma and borderline tumor". *Int. J. Gynecol. Pathol.*, 1992, *11*, 11.

[21] Hu C. Y., Taymor M. L., Hertig A. T.: "Primary carcinoma of the Fallopian tube". *Am. J. Obstet. Gynec.*, 1950, *59*, 58.

[22] Lacy M. Q., Hartmann L. C., Keeney G. L. *et al.*: "c-erbB-2 and p53 expression in Fallopian tube carcinoma". *Cancer*, 1995, *75*, 2891.

[23] Rabczyński J., Ziółkowski P., Kowalski P. *et al.*: "Primary carcinoma of the fallopian tube – clinico-morphological review of 41 cases". *Med. Sci. Monit.* (in press).

[24] Zeng W., Sung C. J., Cao P. *et al.*: "Early occurrence and prognostic significance of p53 alteration in primary carcinoma of the fallopian tube". *Gynecol. Oncol.*, 1997, *64*, 38.

[25] Serov S. F., Scully R. E., Sobin L. H.: "International Histological Classification of Tumors, No. 9: Histological Typing of Ovarian Tumors". WHO Genova, 1973.

[26] Guillaud Ph., Woźniak Z., Seigneurin D.: "Simultaneous quantitation of DNA and nuclear organizer regions by image cytometry". *Anal. Quant. Cytol. Histol.*, 1993, *15*, 351.

[27] Wolanski K. D., Whitaker D., Shilkin K. B. *et al.*: "The use of epithelial membrane antigen and silver stained nucleolar organizer regions testing in the differential diagnosis of mesothelioma from benign reactive mesothelioses". *Cancer*, 1998, *82*, 583.

Address reprint requests to:
JERZY RABCZYNSKI
Department of Pathological Anatomy
Medical University of Wrocław, ul.
Marcinkowskiego 1,
50-368 Wrocław (Poland)