

# Argyrophilic nucleolar organizing regions associated proteins in oncocytology

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Nucleolar organizing regions (NORs) are genetic loci on chromosomes that are composed of ribosomal DNA (rDNA) and proteins, some of which are argyrophilic characteristic. So, silver binds with the rDNA sites that are transcriptionally active or previously have been transcribed, but retain residual rRNA nonhistone-associated proteins. Thus silver-stained NORs and argyrophilic NOR-associated proteins are called AgNORs [1]. Mass spectrometric studies show up more than 700 nucleolar proteins, some of which are not associated with ribosome biogenesis [2]. As a cytochemical, AgNOR technique is not specific for one protein. Alternately, it shows various silver affinity proteins belonging to ribosome assembly machinery [3]. These proteins are used as markers for proliferative and metabolic activities of cells. Thus, AgNORs are researched in different organs and disease [4–15].

There are a lot of studies to evaluate the importance of the interphase AgNOR quantity in tumor pathology, for diagnostic and prognostic characterization of various cancer types [9–11,15–25]. The various quantitative distribution of interphase AgNORs in slowly and rapidly proliferating cells can be elucidated with the function of these structures and the AgNOR proteins in rRNA synthesis [26]. Rapidly dividing cells must accumulate their ribosomal biogenesis in a shorter time than slowly dividing cells because of the correct cellular activities. This can be performed with activating a greater number of rDNA sequences for transcription. Therefore, a greater amount of AgNOR proteins must be synthesized that will

increase the count of interphase AgNORs, which have the structural–functional units for rRNA synthesis [27]. The number, distribution, and shape of AgNOR reactive sites that are counted in the cells provide significant information about the behavior of those cells. In tumor cells, the relations between cell proliferation and AgNOR amounts have been researched greatly by comparing AgNOR values with kinetic data obtained by applying a panel of proliferation markers [9–11,15]. Several studies were performed using the AgNOR method [4–15], which supply an index of cellular activity in connection with proliferation, differentiation, protective activities to dangerous agents and secretory activities.

In a study, the proliferation activity was evaluated in multicystic ameloblastoma (MA), unicystic ameloblastoma (UA) and keratocystic odontogenic tumor (KCOT) via AgNOR staining methods. The AgNOR number was higher in KCOT than UA and MA. When the distribution of AgNORs was taken into consideration, it was higher in basal layer than parabasal layer in KCOT. Also, irregular clusters in UA, whereas few clusters in MA were seen. It was reported that the AgNOR staining reflects the high proliferation rate and a more aggressive behavior of KCOT than MA and UA [16]. As possible as early diagnosis of the diseases via the most reliable and effective methods have crucial important for human health. AgNOR, Papanicolaou (PAP), and morphometric tests were performed to detect the malignant changes in exfoliative cytology samples of patients with oral cancer. A significant relation was found between the AgNOR protein amounts and diagnosis of malignancy. It was reported that the AgNOR staining method, the cheap, non-invasive and simple technique, helps to early detection of oral cancer [28]. Additionally, PAP and AgNOR staining were performed to detect the diagnostic purpose in brush biopsies obtained from suspected oral lesions for early detection of oral cancer. Sensitivity and specificity values were 91.176%, 100% for PAP and 100% for AgNOR analysis for discrimination of oral cancer and normal cells in the oral smears. It was reported that AgNOR staining can be used as an additional to other routine cytological diagnoses for the early detection of oral cancer in brush smears [17]. In a study, The

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AgNOR was evaluated in oral leukoplakia with epithelial dysplasia (ED), oral squamous cell carcinoma (OSCC) and normal oral mucosa (NOM). Increased AgNOR count was found from NOM to ED to OSCC. Higher number, smaller size and wider scatter AgNOR were seen in oral leukoplakia with ED and OSCC cases. It was suggested that the evaluation of AgNOR may place in distinguishing between NOM and oral leukoplakia with ED and OSCC [18]. In another study, it was researched that whether AgNOR can show the progression of cervical intraepithelial neoplasia grade 1 (CIN1) of the uterine cervix. It was offered that AgNOR may be a better marker of proliferation of CIN1 than p16INK4a [19]. A long-term analysis of AgNOR was performed to prove tumor grading in cases with breast carcinoma. A strong correlation was detected between survival and AgNOR. It was offered that these analyses could be used as standard measure for mitosis rate combined with the level of pleomorphism [20]. Also, the AgNOR was evaluated in cervical smears samples to evaluate the whether there is or not a relation between AgNOR count and lesion severity. A progressive increase was detected between AgNOR number and the lesions severity [normal cervix < chronic cervicitis < low squamous intraepithelial lesion < high squamous intraepithelial lesion carcinoma cervix]. The authors offered that the AgNOR number may be greatly used as a cell proliferative marker and understanding of the cervical lesion stage [21]. In addition to this, the AgNOR values were evaluated in ovarian serous epithelial tumors. AgNOR values were not associated with the prediction of disease-free survival and overall survival. AgNOR values have not a prognostic significance in cases with serous ovarian cancer [29]. The morphometric characteristics of nuclei and AgNORs were evaluated in differential cytodiagnosis of benign, atypical proliferative (borderline) and malignant ovarian mucinous tumors. AgNOR quantification consists of seven variables related to the number and area of single, cluster, total and relative AgNOR constitution per nucleus. Higher nuclear area was found in benign tumors than that in borderline tumors; malignant tumors had the highest values. Single and cluster AgNORs were significantly different in borderline tumors than malignant tumors. The total AgNOR area, number and relative area significantly increased from benign through malignant tumors. Bringing together nuclear morphometry and AgNOR analysis could be used as diagnostic tool in the evaluation of mucinous ovarian tumors [22].

Mesotheliomas are the most frequent primary malignant tumors of serosal cavities. An early diagnosis on effusion samples is important for the management of the disease. The cytological diagnoses were assisted by DNA-cytometry, immunocytochemistry and AgNOR analysis. Supported routine cytology helped to correctly detect the diagnosis [23]. Cytological examination of effusions helps to discriminate benign from malignant effusions, but fails an absolute diagnosis in a number of cases. The main problem here is to differentiate reactive mesothelial cells

from neoplastic cells. AgNOR staining method was used for discrimination of benign from malignant effusions. The effusion samples of cases were stained with AgNOR for discrimination of benign from malignant and atypical cases. For this purpose, the dispersion and shapes were compared for malignant, benign and atypical cases. While the cells in benign group include 1 to 2 dots of regular size and shape, the cells in malignant group include 3 to 5 dots of different size and irregular shape. The reactive mesothelial cells and malignant cells include 1 to 2 and 3 to 4 irregular cells in atypical group. Discrimination was detected between malignant cells and activated mesothelial cells, which was not possible in Leishman, and hematoxylin and eosin stained smears alone. Thus AgNOR can be used as an extremely useful diagnostic tool for cytodiagnosis of effusions [30]. Additionally, AgNOR staining was performed for fine-needle aspiration cytology of patients with squamous cell cancers of the head and neck region who received radiotherapy to a dose of 30 Gy. The patients exposed a pre-radiotherapy whose AgNOR score greater than 2.5 were related with disease progression and metastasis. The nuclear morphometry combined with AgNOR score could be used for prediction of radiation response in head and neck cancers [24].

The evaluation of AgNOR and image cytometry (ICM) in various tumor such as peripheral blood (PB), bone marrow (BM) and lymph nodes (LN) from cases with chronic leukemic lymphoproliferative disorders was performed. Correlation between morphometric, AgNOR and ICM characteristics showed that the cells with low proliferative activity had small, homogeneous AgNOR and the peak of DNA histogram. Conversely, the cells that have high proliferative activity acquire inhomogeneous AgNOR, frequently containing higher DNA composition than peak cells, pathologic mitoses, or the majority of cells appeared in the S-phase of the cell cycle. Cells which had medium proliferative activity and annular AgNOR were in-between. The lymphatic cells in BM regularly showed low proliferative activity but the cells in LN showed the proliferative cells activity. While a reverse pattern was seen between PB and LN, the cells did not considerably differ as stated in size and proliferative activity in BM and BP. While the PB cells are largest and mostly inactive, LN cells were smallest and most active [25]. For thyroid cytology, the mean AgNOR number and Total AgNOR area/nuclear area (TAA/NA) were found as significantly greater in malign than in benign thyroid nodules. The detection of TAA/NA is definitive for evaluating the proliferation activity of cells in benign and malignant thyroid lesions and it may assist to routine cytopathology [9–11, 15].

Description of new biological markers for discriminating malignant and benign thyroid lesions is significant for improvement diagnostic accuracy. The evaluation of AgNOR proteins' amount (especially the detection of TAA/TNA ratio) gives information about the proliferation rate and response to dangerous agents. This evaluation method has advantages with its simplicity

and cost-effectiveness. In cancer cells, not only gene expression and its products but also cell morphology was converted, too. In addition to detection of AgNOR number, size, shape and scatter; the detection of the both NOR area and nuclear area used for calculation of TNA/NA ratio is important, too. Calculation of TNA/NA values gave information about the protein synthesis capacity. Both the number of biomolecules and the size of cells and their nuclei alter depending on cellular metabolic activity. Thus, better and more confirmable knowledge about the metabolic and proliferative activity of the each cell may be obtained via detection of TAA/NA proportion.

**Keywords:** Keratocystic odontogenic tumor (KCOT), Nucleolar organizing regions (NORs), rDNA, Proteins in oncology, Tumor cytology

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#### Guarantor

The corresponding author is the guarantor of submission.

#### Conflict of Interest

Authors declare no conflict of interest.

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