Correlation Between AgNOR, MIB-1 and Radiotherapy Response in Lung Cancer

Conference Paper · February 2011

DOI: 10.13140/2.1.1194.9120

7 authors, including:

Iin Kurnia
National Nuclear Energy Agency, Jakarta, Indonesia
17 PUBLICATIONS **7** CITATIONS

SEE PROFILE

Correlation Between AgNOR, MIB-1 and Radiotherapy Response in Lung Cancer

Iin Kurnia [1], Heriawati [2], Juniarti [3], Ruth ES [2], Zubaidah Alatas [1], Elisna Syahruddin [4], Made Widhyasmara [4]

(1) Department of Biomedical, Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency, Jakarta, Indonesia
(2) Department of Pathology Anatomy, Jakarta, Indonesia
(3) Department of Radiotherapy, Jakarta, Indonesia
(4) Department of Lung, Persahabatan Hospital, Jakarta, Indonesia
(5) Study program of Pharmacy, Faculty of Mathematical and Natural Science, ISTN, Jakarta, Indonesia.

Abstract

Level radiosensitivity is highly correlated with the level of cell division that can be predicted by neither detection of NOR (Nucleolar Organizer Region), Ki-67 index and others. NOR is a segment of DNA (nucleic acid) associated with nucleus that contain genes encode ribosomal. From a number of papers found in the NOR positively correlated with Ki-67 index. The different of sensitivity of cancer cells to radiation will affect the success of radiotherapy in cancer control. The aim of the research is detection of NOR and MIB-1 (Ki-67) index as a marker tumor cell radiosensitivity in order to optimize the lung cancer patients treatment with radiotherapy or chemoradiotherapy. AgNOR staining has been performed and MIB-1 by double staining of 20 lung cancer cell smear results of fine needle biopsy from Department of Pathology Anatomy of Persahabatan Hospital, Jakarta before treated and observed the radiation response after 2 weeks of radiation. Radiotherapy response was classified by the good and poor response. Tend of a weak correlation between MIB-1 and AgNOR (r = 0.23) in the smear cells of lung cancer before treatment, there were no statistically significant difference in AgNOR value between good response and poor response after 2 weeks of radiation, the value of MIB-1 index poor response after 2 weeks of action more than the value of MIB-1 in good response (p = 0.12). Although statistically there is no difference, the higher value of MIB-1 before treatment is tend give poor response than in good response after 2 weeks irradiation.

Keywords: *AgNOR, MIB-1, Lung Cancer, Radiotherapy.*

1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for 75–80% of primary lung cancer. Tumour node metastasis (TNM) factors are generally used in the evaluation of tumour progression, and nodal involvement (N-factor) as well as distant metastasis (M-factor) is the critical factor to determine the prognosis of NSCLC [1]. Lung cancer represents a great challenge for the oncologist because only one third of cases can be treated with surgery. In cases not eligible for surgery, chemotherapy or radiotherapy is commonly used. Despite these treatments, it gives local recurrence or distant metastases in most cases [2].

Nucleolar organizer regions (NORs) are chromosomal loops of DNA involved in ribosomal synthesis [3]. The silver staining technique can easily detect NORs in formalin fixed, paraffin embedded tissues and NORs can be identified as black dots in the nucleolus (AgNOR) [4]. This method permits the rapid evaluation of morphology and tumor cell kinetics even using small biopsies. Evaluation of AgNORs parameters (number, size, and distribution) has been applied in tumor pathology both for diagnostic and prognostic purposes [5]. Their size and number have been reported to reflect the proliferative activity of tumor cells [6].

The malignant grades of tumor cell and proliferative activity have been assessed using immunohisto-chemical staining with several cell cycle related parameters [7,8]. MIB-1, also known as Ki-67, is expressed in all cell cycle stages except for G0 and early G1 phase. This antigen is thought to be associated with a nuclear antigen protein-DNA replicase complex, similar to DNA topoisomerase II [9].

Generally, a higher MIB-1 labeling index (LI) correlates with worse prognosis; however, tumors with higher MIB-1 LI are often radiosensitive.

Although many investigators have demonstrated a relationship between the number or mean area of AgNORs and MIB-1 expression in solid tumors [10-14], we have not known previous studies have simultaneously assessed AgNOR staining and Ki-67 expression in smear of lung cancer from fine needle biopsy tissue before treatment and observed radiotherapy response after 2 weeks irradiation.

This research propose detection of NOR and MIB-1 (Ki-67) index with double staining method as a marker tumor cell radiosensitivity and their correlate with response of radiotherapy behalf on to optimize treatment of lung cancer patients with radiotherapy or chemoradiotherapy.

2. Experimental Detail

Patient Characteristic

A total 20 cell smear from 20 patients with non small cell carcinoma of the lung from fine needle biopsy specimens taken before treatment with radiotherapy alone or combined with chemotherapy at Department of Pathology Anatomy, Persahabatan Hospital (Jakarta, Indonesia) from 2007-2009. The response of radiotherapy was observed two weeks after the initiation of radiotherapy. Their ages ranged from 28-73 years old (mean and median: 56.1 and 57.5 years old, respectively).

Destained, MIB-1, AgNOR Staining

Some modification method from originally method [15,16] was made. First the cover glass from stained slide was removed by incubation in warm xylene with temperature about 60°C 18–24 hours. Then the slides were processed through xylene and rehydration with grades of alcohol. Before incubation with Antigen Retrieval in Mircowave 2 x 10 minute in temperature 94°C, they were incubation in PBS 2 x 5 minutes on room temperature, then staining with MIB-1 followed with AgNOR staining as in incubated with 1 part 2% gelatin in 1% aqueous formic acid with 2 parts 50% aqueous silver nitrate at room temperature for exactly 30 min. The slides were then rinsed with distilled, de-ionized water, taken through alcohols to xylene, and mounted with an entellan. No counter staining was performed. This process was done on Department Biomedical, The Center for Technology of Radiation Safety and Metrology National Nuclear Energy, Jakarta

Assesment AgNOR, MIB-1, Radiotherapy Response

AgNOR score (the number of NORs in a tumor cell nucleoli) were counting in cell nuclei using a light microscope (magnification ×400) with the immersion oil method of Crocker *et al* [16] MIB-1 are the percentages of MIB-1 positive tumor cells in colored print photograph, (original magnification ×400) to minimize variations originating from within sections. One of the authors (MW) blindly performed cell counting [16]. We use Correlation Regression to correlate between AgNOR value and MIB-1 index, Student T test to analyze the different between AgNOR and MIB-1 among good and poor response after 2 weeks irradiation with *Medcalc* Software Program. Radiotherapy response is observed by senior Radiotherapist (YN) in Department of Radiotherapy Persahabatan Hospital, Jakarta.

3. Result and Discussion

Expression double staining AgNOR and MIB-1 in smear of lung cancer cell taken before treatment by radiotherapy or chemoradiotherapy and correlate between AgNOR value and MIB-1 index can be seen in (Figure 1 and 2). Average of AgNOR and MIB-1 index in different response after 2 weeks irradiation are given in Figure 3a and Figure 3b, respectively.

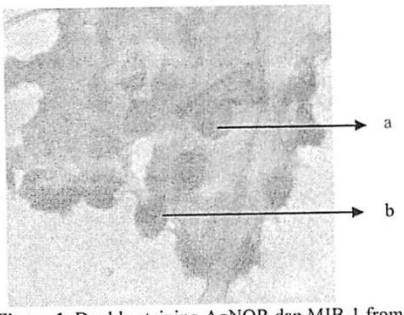

Figure 1. Double staining AgNOR dan MIB-1 from destained smear cell from fine needle biopsy lung cancer before treatment, negative MIB-1 (a) dan positive MIB-1 (b)

Cancer cell with positive MIB-1 (Ki-67) can be seen in cell nucleus with dark brown color, contrast in negative the color is light brown. The number of AgNOR in MIB-1 positive cell is higher than negative cell. It means that the positive correlation between AgNOR and MIB-1 index. In this study we found only weak correlation between AgNOR and MIB-1 ($r = 0.23$) (Figure 2).

This result is the similar with previous report, with double staining in biopsy of breast cancer. In a study using double staining with AgNOR and MIB-1 (the latter being a marker for the number of cells in the cycle) demonstrated that the number of AgNORs were ranged from 2–12 in the MIB-1 positive cells and 1–3 in negative cells [17].

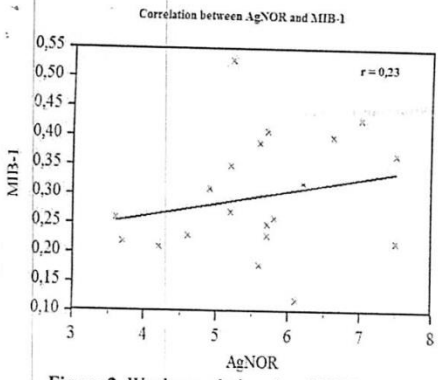

Figure 2. Weak correlation (r = 0.23) between AgNOR and MIB-1 from destained smear cell from fine needle biopsy lung cancer before treatment.

In our previous report [16] we found the strong positive correlation between AgNOR and MIB-1 in cervical cancer biopsy before treatment with chemoradiotherapy, but in this study the tissue was taken from biopsy and staining separated. Staining tissue from biopsy specimen may make more cell could be stained compare the cell from fine needle biopsy that only can get a little cancer. Another report found strong correlation between AgNOR and MIB-1 in vulva epithel [18], gastric cancer [19], oral lesion [20], and cervical epidermoid carcinoma cell line [21].

In this present study there is no statistically significant difference in AgNOR value between good response and poor response after 2 weeks of radiation. The value of MIB-1 index poor response after 2 weeks of action more than the value of MIB-1 in good response, almost border line with statistically significant (p = 0.12).

NOR that observed by AgNOR staining can be detected in phase in G1, S and G2. MIB-1 can be detected in all mitotic cells. Some report said expression of MIB-1 in was related with growth fraction of cancer cell [7,8,16]. Higher of growth fraction of lung cancer maybe also related with the resistant to radiation treatment after 2 weeks irradiation or maybe the radiation is not enough to stop of growth or proliferation of the cancer cell in the lung. High proliferation also related with angiogenesis of the lung cancer. Increased tumor cell proliferation is a contributing factor to tumor size and metabolic burden, and might be expected to be associated with angiogenesis [22]. Proliferation also essential to cancer cell invade and disseminate [23].

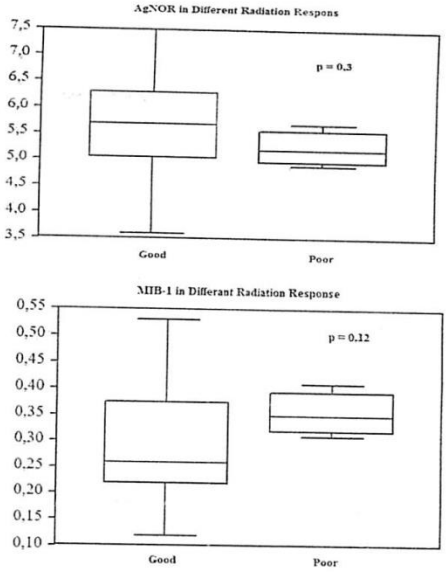

Figure 3. AgNOR (a) dan MIB-1 (b) in good and poor response, p = 0.3 (b) p = 0.12 respectively from destained smear cell from fine needle biopsy lung cancer before treatment

The number and size of NORs are highly variable within the nucleolus according to rRNA transcriptional activity. Sirri et al [24], reported that the accumulation of nucleolin and B23 (two major NOR protein) in G2 was larger than in G1 in the cell cycle. Hence, the numbers of NORs are larger in the phases of the cell cycle from S to G2[25] MIB-1 was present in all cell cycle stages except for G0 and early G1.36, [26] Dong et al[.27] reported that the AgNOR score in human cervical squamous cell carcinoma cell line showed a strong correlation with MIB-1-LI. They also reported that both AgNOR score and MIB-1-LI showed inverse correlations with potential doubling time and the length of S phase.

Different of statistical significant between MIB-1 and AgNOR in different a response after 2 weeks irradiation proved that MIB-1 MIB-1 also showed of growth fraction of lung cancer and AgNOR showed of condition of cell cycle as a part of cell mitotic or as one of parameter related to cell proliferative activity. Even though the sample of patient is include of this research was small, we think MIB-1 index is more realize than AgNOR used as prediction of radiation response of lung cancer treated with radiotherapy or chemoradiotherapy.

4. Conclusion

Although statistically there is no difference, the higher value of MIB-1 before treatment is tend give poor response than in good response after 2 weeks irradiation.

5. Acknowledgements

This research was supported by a Research Grant from Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency in Indonesia FY 2009. This research was approved by the Committee for Medical Research Ethics, Hospital of Persahabatan, Jakarta. The authors thank to Dr. Susilo Widodo, Head of The Center for Technology Radiation Safety and Metrology, National Nuclear Energy Agency, Jakarta and all of technician with their assistance of this research both in Department of Biomedical, The Center for Technology Radiation Safety and Metrology, National Nuclear Energy Agency, and Persahabatan Hospital, Jakarta.

6. References

[1]. Mountain C.F. (1997), Revisions in the international system for lung cancer, *Chest*; 111: 1710–1717

[2]. Viberti L, Papotti M, Abbona GC, Celano, A, Filosso PL, Bussoliti, G. (1997), Value of Ki-67 Immunostaining in Preoperative Biopsies of Carcinomas of the Lung, *Human Pathology*; 28.2:189-192

[3]. Gall J. G, Pardue M. L. (1969), Molecular hybridization of radioactive DNA to the DNA of cytological preparation, *Proc Natl Acad Sci* 64:600-604.

[4]. Derenzini M, Ploton D. (1991), Interphase nucleolar organizer regions in cancer cell, *Int Rev Exp Pathol.* 32:150-192.

[5]. Trere D. (2000), AgNOR. Staining and Quantification, *Micron.* 31:127–131.

[6]. Derenzini M, Pession A, Trere D. (1990), The quantity of nucleolar silver-stained protein is related to proliferating activity in cancer cell, *Lab Invest* ;63:137-140.

[7]. Nakano T, Oka K. (1991), Transition of Ki-67 index of uterine cervical tumors during radiation therapy.,*Cancer*; 68:517-523.

[8]. Nakano T, Oka K. (1993), Differential values of Ki-67 index and mitotic index of proliferating cell population, *Cancer*; 72:2401-8.

[9]. Gerdes J, Schwab U, Lemke H, Stein H (1983), Production of a mousemonoclonal antibody reactive with a human nuclear antigen associated with cell proliferation, *Int. J Cancer* ;31:13-20.

[10]. Kakeji Y,Korenaga D, Tsujitani S, Haraguchi, Maehara Y, Sugimachi. (1991), Predictive value of Ki-67 and argyrophilic nucleolar organizer region staining for lymph node metastasis in gastric cancer. *Cancer Res.* 51:3503

[11]. Kuratsu S, Aozasa K, Tsujimoto M, Ueda T, Uchida A, Hamada H, Ono K, Matsumoto K. (1991), Prognostic significance of argyrophilic nucleolar organizer staining in soft-tissue sarcomas. *Int J. Cancer.* 48:211

[12]. Plate KH, Riischoff J. Behnke J, Mennel HD. (1990), Proliferative potential of human brain tumours as assessed by nucleolar organizer regions (Ag-NORs) and Ki-67-immunoreactivity, *Acta Neurochir Wien* .104:103

[13]. Raymond WA, Leong ASY. (1989), Nucleolar organizer regions relate to growth fractions in human breast carcinoma, *Hum Pathol.* 20:741

[14]. Trere D, Farabegoli F, Cancellieri A, Ceccarelli C, Eusebi V, Derenzini M. (1991), AgNOR area in interphase nuclei of human tumours correlates with the proliferative activity evaluated by bromodeoxyuridine labeling and Ki-67 immunostaining. *J Pathol.* 165:53

[15]. Munakata S,Hendricks JB. (1994), A Multilabeling Technique for Simultaneous Demonstration and Quantitation of Ki-67 and Nucleolar Organizer Regions (AgNORs) in Paraffin-embedded Tissue, *Journal of Histochemistry and Cytochemistry*. 42;6:789-793

[16]. Kurnia I, Yoshiyuki S, Budiningsih S,Andri A,Irwan R, Cholid B,,Yukari Y Takashi N (2009), Nucleolar Organizer Regions in Squamous Cell Carcinomas of the Uterine Cervix Treated with Chemoradiotherapy. Austral - *Asian Journal of Cancer* ISSN-0972-2556, Vol. 8, No. 2, April :93-102

[17]. Mourad WA, Sneige N, Katz RL, Ordonez NG (1994), Correlation of two AgNOR counts

with Ki-67 labelling index: a study in fine-needle aspirates of lymphoproliferative disorders and breast carcinoma. *Diagn Cytopathol.* 10: 113–9.

[18]. Hermann B, Susanna N (2002), Expression of Topoisomerase II, Ki-67, Proliferating Cell Nuclear Antigen, p53, and Argyrophilic Nucleolar Organizer Regions in Vulvar Squamous Lesions, *Gynecol Oncology*, 86, 192–199.

[19]. Kakeji Y, Daisuke K, Shunichi T, Musarti H, Yoshihiko M, and Keizo S. (1991), Predictive Value of Ki-67 and Argyrophilic Nucleolar Organizer Region Staining for Lymph Node Metastasis in Gastric Cancer, *Cancer Research*; 51:3503-3506.

[20]. Teresa DB, Karina AN, Carlos B N,Paula AGF, Maria RBO, Jose´ Antonio S. Z. (2007), Computer-assisted analysis of cell proliferation markers in oral lesions, *Acta Histochemica* 109 :377—387.

[21]. Dong H, Carey B, Eric S, and Mark A.R. (1997), Assessment of Cell Proliferation by AgNOR Scores and Ki-67 Labeling Indices and a Comparison With Potential Doubling Times, *Cytometry*. 28:280-288.

[22]. Brockenbrough, JS, Janice KM, Stephen EH, Joshua ES, Janet SR (2009), Thymidine Kinase 1 and Thymidine Phosphorylase Expression n Non–Small-cell Lung Carcinoma in Relation to Angiogenesis and Proliferation, *Journal of Histochemistry & Cytochemistry*, Volume 57(11): 1087–1097.

[23]. Yamazaki D, Kurisu S, Takenawa T. (2005), Regulation of cancer cell motility through actin reorganization, *Cancer Sci*, 96:379-386.

[24]. Derenzini M. (2000), The AgNORs. *Micron* 31:117–120.

[25]. Sirri V, Pascal R, Marie C G, and Hernandez VD. (1997), Amount of the Two Major Ag-NOR Proteins, Nucleolin, and Protein B23Is Cell-Cycle Dependent. *Cytometry* 28:147-56.

[26]. Kidogawa, H, Nanashima A, Yano, H, Matsumoto, M. Yasutake,T. Nagayasu T. (2005), Clinical Significance, double staining MIB-1and AgNORs in primary breast carcinoma. *Anticancer Res*. 25:3957-62.

[27]. Dong AT, Shuqing L, Dan H. Moore II, and Edgerton SM. (1999), Comparison of Mitotic Index, In Vitro Bromodeoxyuridine Labeling, and MIB-1 Assays to Quantitate Proliferation in Breast Cancer. *J Clin Oncol*. 17:470-476.