Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 8
Sofia • 2003

Quantitative DNA Analysis in Dysplastic Changes and Carcinoma of the Cervix Uteri

Mini-review

*Y. Gluhcheva, S. Christova**

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia
**Department of Clinical Pathology in MBAL "Alexandrovska" – MU, Sofia*

It has been reported that dysplasias and neoplasias of the Cervix uteri occur as a result of changes in the genome and DNA quantity. These changes can be determined by measuring the cell's DNA quantity using two methods: Image Analysis and Flow Cytometry. The information obtained from the analysis could be used to predict the outcome of the observed genome change and it could also help in the medical treatment of patients.

Key words: DNA quantity, DNA analysis, Image Analysis, Flow Cytometry, dysplastic changes – neoplasias (Carcinoma cervix uteri).

The normal somatic cells of mammalian tissues have a big genetic stability and a conservative cell cycle. In most tissues the number of the dividing cells is less than 10% from the total cell number. The non-dividing cells have 23 pairs of chromosomes and a fixed DNA quantity that is about 7 pg. These cells are known as diploid or non-dividing cells (G_0). Their DNA index equals 1. Entering the synthesis phase (S-phase), diploid cells undergo a process of replication of the genetic information: the DNA quantity doubles, i.e., it is about 14 pg. Such cells are known as tetraploid or G_2 cells. Their DNA index is 2. During the S-phase the amount of DNA varies between 7 and 14 pg [2].

It has been established that genome changes could be different mutations – such as translocations, delisions, duplications etc., influencing the quantity of the cell's DNA. Cells registered as abnormal to their DNA quantity are known as aneuploid [2, 19].

It has been established that the biological development of the malignant cells is associated with their DNA ploidy status. The ploidy status depends on the type of genetic changes during ontogenesis and more specifically it reflects the degree and the stage of the genome changes [2, 11].

Benign tumors have the amount of DNA and the genetic stability of the euploid cells. Relative to DNA status, malignant tumors could be either euploid or

aneuploid. It's commonly accepted that euploid tumors have better prognosis than aneuploid ones. The identification of DNA aneuploidy in dysplatic squamous epithelia can increase the predictive value for malignant transformation to over 90% [3]. The registration of an aneuploid tumor branch suggests that significant changes have occured in the genome of these cells and they have an increased malignant potential [1, 12, 13, 14]. In that case multiple changes of the cell's genetic information could be provoked as well [2, 6]. The presence of aneuploidy usually signifies either recurrence or dysplasia. Polyploidy most frequently occurs in dysplastic processes, whereas diploid cells usually denote a benign disease course [4].

The results of Strang [18] show that DNA ploidy status depends on the patient's age: women at the age of 35.6 (+/- 11.7) or younger tend to develop diploid or polyploid squamous carcinoma, while the older one develop mainly aneuploid carcinoma (the data show that 80-90% of the solid tumors are aneuploid). Nasiell et al [17] established that progression of mild and moderate dysplasia (CIN I and CIN II) is due to serious genome changes. In Fu's [5] investigations all patients diagnosed as CIN III have aneuploid DNA amounts. Presented in per cent, the aneuploid DNA status in different types of dysplasia varies from 20% to 100%. That's why Hanselaar [7] considers aneuploidy as a marker for malignant progression. Kashyap et al. [12, 13] and Steinbeck [20] accept aneuploidy as a high risk factor for the development of neoplasia in women with mild and moderate dysplasia. According to Mariuzzi et al. [16] CIN I and CIN II changes could not be considered as neoplastic since aneuploidy wasn't found in the performed measurements of the DNA quantity. In CIN III, such changes could be observed, so that these lesions could be considered as neoplastic. In their investigations Shu and Gloor [18] determined that in CIN I lesions almost all cells were diploid. Most of the cells in CIN II were also diploid, but polyploid and even aneuploid cells could be found. In more than 80% of the CIN III lesions cells were aneuploid.

Leminen et al. [15] established that the patients with triploid cells had the worst prognosis for survival among those with aneuploid adenocarcinoma of the uterine cervix. The authors claimed that 69% of the tumors examined were diploid and 38% — aneuploid.

The cell fraction in S-phase is significant for the prognosis of tumor development [15]. In cases where the number of cells in S-phase is less than 14%, the survival prognosis of patients is better than in the cases with a high proliferation activity.

Using new methods such as Image Analysis and Flow Cytomerty it's possible to determine the ploidy status of the cell population and to register abnormal cells having deviations greater than 2% in their total DNA quantity [2].

DNA image cytometry, as an additional method, can be used to predict outcome in patients with CIN I and CIN II of the cervix. This is not a screening method but it can add further information for a treatment decision in doubtful cases [9].

Flow cytometry technique can examine thousands of cells within a very short time. Each cell is assessed for a variety of characteristics such as shape and size and cell compositions [8]. The principle of the flow cytometry is that the cells that will be examined are made into a cell suspension and stained by fluorescent dyes (fluorochromes). The cells are then sent into the examination region via a flowing liquid. There is a very strong light source, generally a laser beam to irradiate to the cells one by one. The irradiation will excite the fluorescently stained cells and emit a fluorescent beam, this signal is picked up and sent to the computer to process. The results will be printed out in histograms. It can measure the DNA content of different groups of the cells in a cell cycle. With the use of certain fluorescent labelled antibodies it can also measure different antigens in cells.

In this method fresh specimen could be used. The result of this measurement corresponds well with that of image analysis. E.g., Jacobsen [10] observed biopsy specimen of the pre-neoplastic lesions of the cervix by flow cytometry, and found that there are only 7% of CIN I and CIN II cases which are represented with aneuploid cells, but there are 79% of CIN III cases showing aneuploid.

The number of the samples is very big and it can yield a reliable representative results and qualified for statistics.

References

1. Костова, П. Организация, качествен контрол и ефективност на цервикалния скрининг в България. Дисерт. труд (София). 2001.
2. Auer, G. DNA — A Clinical Parameter. Denderstraat, Becton Dickinson, 1990, 1-9, 27-37.
3. Bocking, A., H. Motherby. Assessment of cervical dysplasia with DNA image cytometry. — Pathologe, **20**, 1999, No 1, 25-33.
4. Davey, D. D., S. Zaleski, M. Sattich, H. Gallion. Prognostic significance of DNA cytometry of postirradiation cervicovaginal smears. — Cancer, **84**, 1998, No 1, 1-16.
5. Fu, Y. S. DNA ploidy measurements in tissue sections. — Analyt. Quant. Cytol. Histol., **7**, 1985, 90-95.
6. Gonzalez-Oliver, A., O. M. Echeverria, R. Hernandez-Pando, G. H. Vazquez-Nin. Ultrastructural study of the nuclei of normal, dysplastic, and carcinomatous epithelial cells of the human cervix uteri. — Ultrastruct. Pathol., **21**, 1997, No 4, 379-392.
7. Hanselaar, A. G. J. M. DNA ploidy and cytometric analysis of cervical intraepithelial neoplasia grade III and invasive squamous cell carcinoma. — Cytometry, **11**, 1990, 624-629.
8. Hedley, D. W. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. — J. Histochem. Cytochem., **31**, 1983, 1333-1335.
9. Hering, B., L. C. Horn, H. Nenning, K. Kuhndel. Predictive value of DNA cytometry in CIN 1 and 2. Image analysis of 193 cases. — Analyt. Quant. Cytol. Histol., **22**, 2000, No 4, 333-337.
10. Jakobsen, A. Prognostic impact of ploidy level in carcinoma of the cervix. — Am. J. Clin. Oncol., **7**, 1984, 475-480.
11. Khaled, A., Y. Imamura, S. Noriki, M. Fukuda. Early progression stage of malignancy of uterine cervical dysplasia as revealed by immunohistochemical demonstration of increased DNA-instability. — Eur. J. Histochem., **44**, 2000, No 2, 143-156.
12. Kashyap, V., U. K. Luthra. Predictive value of morphological nuclear parameters and DNA ploidy pattern in precancerous lesions of the uterine cervix. — Indian J. Pathol. Microb., **38**, 1995, No 2, 193-197.
13. Kashyap, V., D. K. Das, U. K. Luthra. Microphotometric DNA analysis in mild and moderate dysplasia of the uterine cervix: a retrospective study. — Indian J. Pathol. Microb., **33**, 1990, No 1, 30-34.
14. Kashyap, V., D. K. Das, U.K. Luthra. Microphotometric DNA analysis in cervical dysplasia of the uterine cervix: its relation to progression to malignancy and regression to normancy. — Neoplasma, **37**, 1990, No 5, 497-500.
15. Leminen, A. Deoxyribonucleic acid flow cytometric analysis of cervical adenocarcinoma: prognostic significance of deoxyribonucleic acid ploidy and S-phase fraction. — Am. J. Obstet. Gynecol., **162**, 1990, 848.
16. Mariuzzi, G. Cytometric evidence that cervical intraepithelial neoplasia I and II are dysplasias rather than true neoplasias. — Analyt. Quant. Cytol. Histol., **2**, 1992, 137-147.
17. Nasiell, K. Cytomorphologic and cytochemical analysis in the differential diagnosis of cervical epithelial lesions. — Analyt. Quant. Cytol., **6**, 1984, 196-200.
18. Shu, Y.-J., E. Gloor. Comprehensive cancer cytopathology of the cervix uteri. — In: Color atlas of cancer cytopathology v. 4. (Ed. O.A.N. Husain). New York, McGraw-Hill Book Company, 995, 96-107, p. 413.
19. Steinbeck, R. G., G. U. Auer. Genome instability in humane tumorigenesis: microphotometry of interphase nuclei and pathologic mitoses reveals dysplasia. — Eur J. Histochem., **44**, 2000, No 2, 133-42.
20. Steinbeck, R. G. Proliferation and DNA aneuploidy in mild dysplasia imply early steps of cervical carcinogenesis. — Acta Oncol., **36**, 1997, No 1, 3-12.