ELSEVIER

European Journal of Cancer 40 (2004) 1941–1944

European Journal of Cancer

www.ejconline.com

DNA content of tumours ☆ Cytophotometric measurements

Walter Sandritter

Department of Pathology, Justus Liebig University, Giessen, Germany

Available online 27 July 2004

Abstract

A short account of cytophotometric investigations on malignant and on early cancerous lesions is given. It was possible to show, that human tumours exhibit an atypical DNA distribution pattern (DNA stem line). The carcinoma *in situ* of the exocervix shows similar as in malignant tumours a DNA stem line. This phenomenon can be traced also in the stage of invasion. The atypical course of Feulgen hydrolysis in tumour cells is related to a differential sensivitity towards acids of eu- and heterochromatin.

Résumé

L'auteur donne un bref aperçu de ses travaux sur la cytophotométrie des tumeurs malignes, notamment á la phase précoce de Í affection. II fut possible de montrer que les tumeurs humaines présentent une distribution anormale de l'ADN.

Le carcinome in situ du col utérin montre la même distribution de l'ADN que dans la tumeur invasive.

Ce phénomène a aussi pu être suivi aux différents stades d'invasion des tumeurs. Le pro-cessus atypique de l'hydrolyse, dans la réaction de Feulgen, au niveau des cellules tumorales, peut être mis en relation avec une sensibilité diférentielle, a l'égard des acides, de l'reuchromatin et de l'hétérochromatin.

Zusammenfassung

Es wird ein kurzer Uberblick der eigenen cytophotometrischen Messungen an malignen Tumoren und Krebsvorstadien gegeben. Es wurde gezeigt, daß die meisten menschlichen Tumoren ein atypisches DNS-Verteilungsmuster aufweisen (DNS-Stammlinie). Das Carcinoma in situ der Portio zeigt ebenfalls DNS-Stammlinien, die auch bei der Invasion nachzuweisen sind. Atypische Feulgenhydrolysekurven bei Tumorzellen werden auf eine unterschiedliche Säureempfaidlichkeit von Eu und Heterochromatin bezogen.

Cytophotometric measurements of normal cells reveal a regular distribution of DNA values which corresponds to the chromosome number [1]. In human tumours, aneuploid DNA distribution patterns were often found that deviated from the normal [2]. Our own measurements [3] demonstrated that the dry weight (interference microscopy) as well as the DNA and histone protein content showed values that deviated from the normal. Measurements on an oat cell carcinoma of the lung serve as an example. The dry weight of the cell nuclei amounted to $20–30 \times 10^{-12}$ g (normal cell nuclei of the bronchial tree $40–50 \times 10^{-12}$ g). The histone protein content (fastgreen staining pH 8.2) as well as the DNA content corresponded to a hypodiploid DNA content (Fig. 1). Investigations on thirty human malignant tumours (Fig. 2) showed that most of the tumours have a DNA "stem line" lying between diploid and tetraploid or octoploid values. However, normal DNA values were also found. These measurements of smears do not indicate whether this atypical DNA distribution occurs only in the peripheral zone or as well in the centre of the carcinoma cell cord. Fig. 3 shows measurements made from tissue sections. It is clearly seen that the

☆ Supported by the Deutsche Forschungermeinschaft.

0959-8049/$ - see front matter © 1965 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.04.012

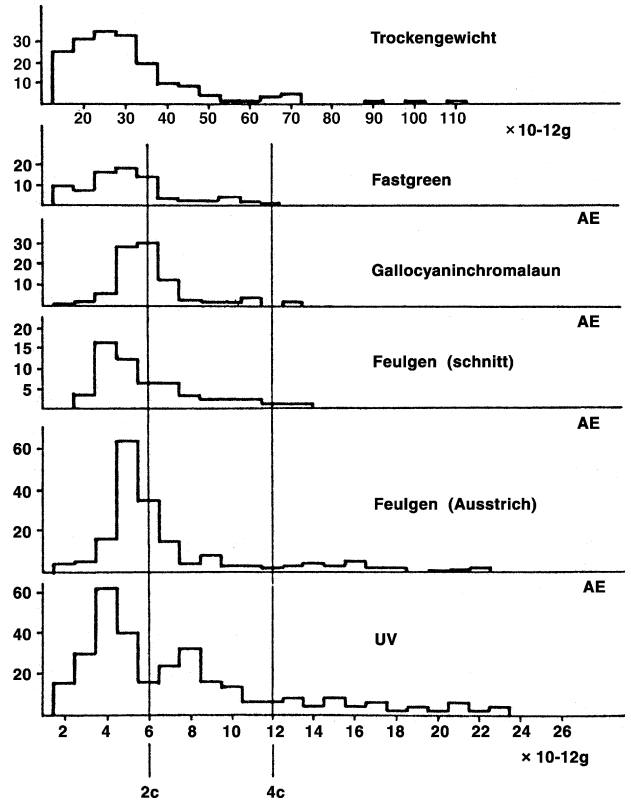

Fig. 1. Dry weight, histone protein content (Fastgreen pH 8.2) and DNA distribution patterns of an oat cell carcinoma of the bronchus.

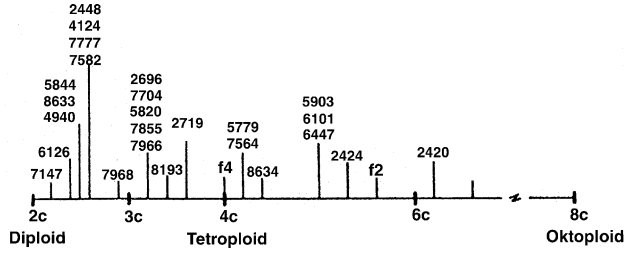

Fig. 2. Appearance of DNA stem lines from thirty human malignant tumours.

outer and inner zones of the carcinoma cell cord had cells with an atypical DNA content and that the DNA stem line is expressed in the same way in both zones.

In experimental animal tumours in contrast to the situation in humans, a diploid DNA content is usually found [4].

What do early cancerous lesions show in the human being? Carcinoma *in situ* of the exocerxvix exhibited, contrary to simple atypical epithelium (dysplasia) (Fig. 4), an aneuploid DNA stem line as seen in a total of eight cases. Fig. 5 shows that this stem line was found in all layers of the carcinoma *in situ.* The stem line persisted throughout the different steps of the invasion (Fig. 6) up to the micro-carcinoma [5]. This could also be found in a case of malignant lung adenomatosis

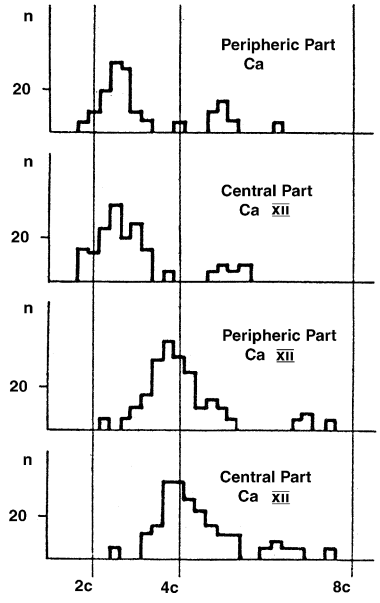

Fig. 3. DNA distribution pattern in the peripheric and central region from two carcinomatous cell cords (squamous cell carcinoma).

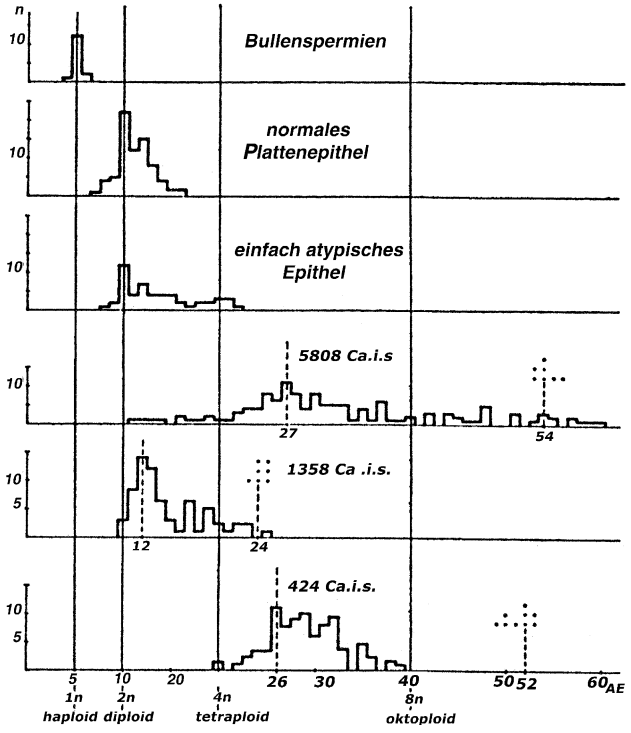

Fig. 4. DNA distribution patterns of normal portio epithelium, atypical epithelium and three cases of carcinoma *in situ* of the exocervix.

(Fig. 7). The DNA distribution pattern represents a nearly triploid stem line, independent of localisation of tumour cell population (intra-alveolar, lymphangiosis carcinomatosa and lymph node metastasis) [6].

During cancerisation *in vitro* [7], usually after one year a new DNA stem line develops that persists

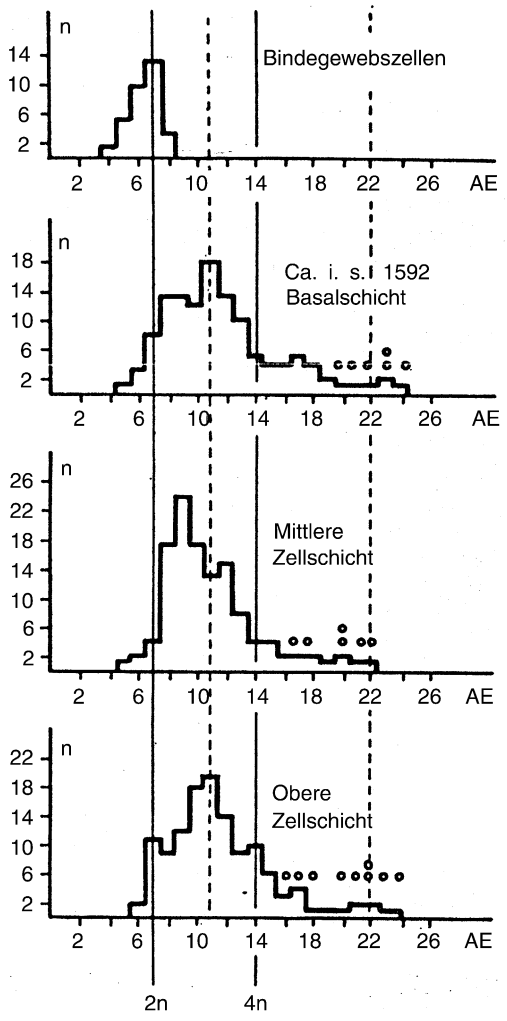

Fig. 5. DNA distributions in the different layers of a carcinoma *in situ* (basal, middle and surface).

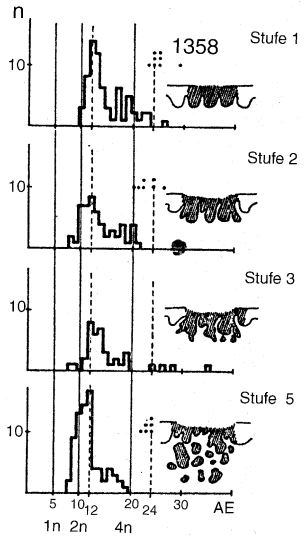

Fig. 6. Different stages of progression in a carcinoma *in situ* and the DNA stem line.

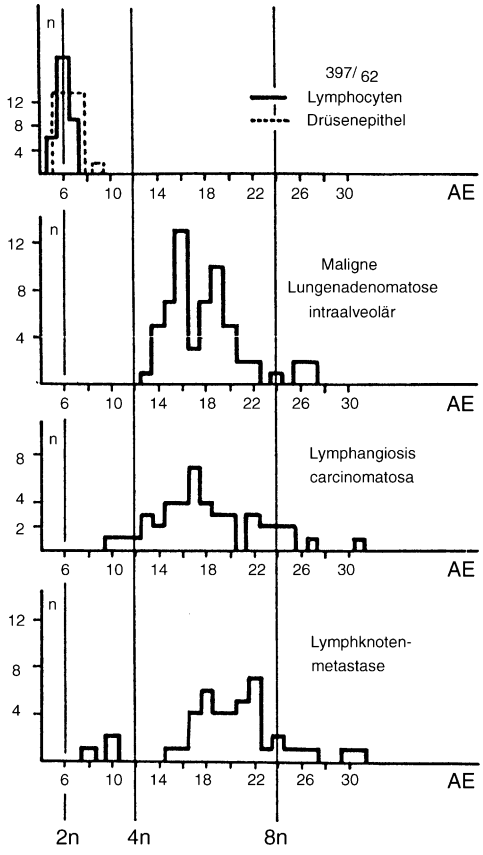

Fig. 7. Malignant lung adenomatosis (alveolar cell carcinoma) lymphangiosis carcinomatosa, and lymph node metastases, DNA distribution patterns.

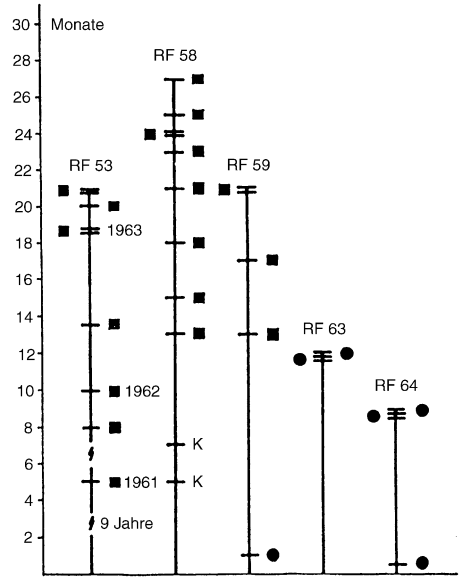

Fig. 8. Scheme of the culture times of the cell stems, giving the DNA measuring time (single cross-line) and the time the chromosomes were counted (double cross-line). The symbols on the left side of the line stand for the chromosome counts and those on the right of the line for DNA measurements. Round spot = diploid, square area = hypotetraploid, K = no stem line.

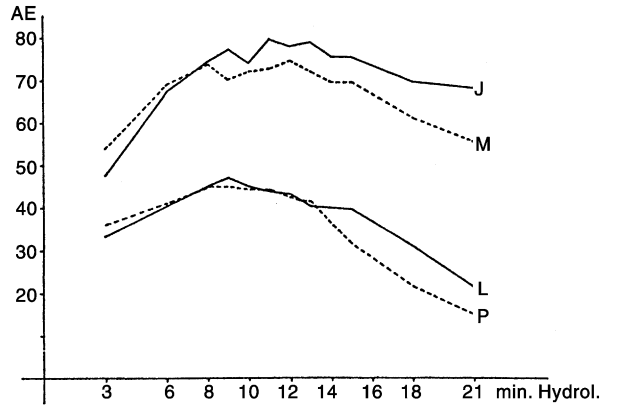

Fig. 9. Hydrolysis time curve of Feulgen reaction. I = interphase cell of mouse acites tumour cells M = mitosis of mouse ascites tumour cells L = lymphocytes P = mesothelial cells from peritoneum.

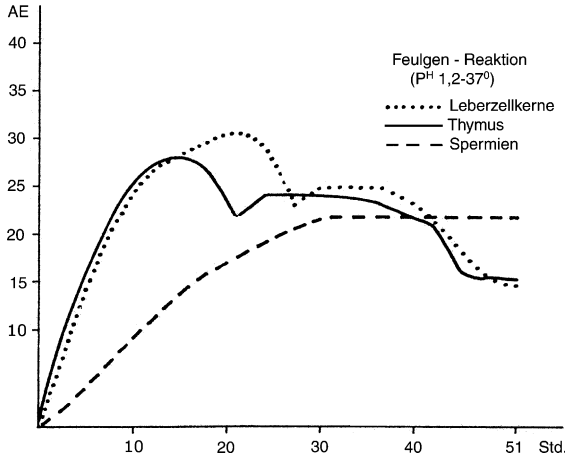

Fig. 10. Extended Feulgen hydrolysis curve of liver cell nuclei, thymus lymphocytes and sperm.

unchanged over a long period of time (Fig. 8). After a stem line has developed the rat fibroblasts can be transplanted back into rats. These transplanted tumour cells show another stem line than the original cells. Upon retransplantation of the animal tumours in tissue culture the stem line changes often again.

These investigations show that malignant tumours are often distinguished from normal cell populations by an aneuploid DNA stem line and a wide scattering of DNA values. However, tumours are also found having a regular DNA distribution. The question arises whether these are pseudodiploid tumours. Measurements of early cancerous lesions (e.g. carcinoma *in situ*) show that an abnormal DNA distribution pattern is already found with intra-epithelial atypical cell formations and that the DNA distribution remains the same during the invasive phase (realisation of the carcinoma). This leads to the conclusion that with regard to the DNA, the carcinoma *in situ* already represents a carcinoma and that the invasion depends on other factors than the atypical DNA distribution.

The amount of DNA by itself presents interesting information. However, there is the question to what extent there are also qualitative differences in the nucleoproteins of normal and tumour cells. Measurements made during the Feulgen hydrolysis (time curve) have shown that contrary to normal cells, mouse ascites tumour cells exhibit an atypical hydrolysis curve with an inflection at 9 min [8] (see Fig. 9). We believe that this atypical curve results from a differential acid sensitivity of the eu- and heterochromatin. When the hydrolysis time (HC1, pH 1.2, 37°) is extended, we also see in the case of normal cell nuclei a curve [9] with an inflection at about 20–22 h (Fig. 10). Recent data suggest that in the first part of hydrolysis (up to 20 h) the aldehyde groups of the euchromatin are set free, while the heterochromatin is less acid sensitive.

Further investigations still need to be made to resolve this relationship.

References

1. Sandritter W, Müller D, Gensecke O. UV-mikrospektrophotometrische messungen des nukleinsäuregehaltes von spermien und diploiden zellen. *Acta Histochem* 1960, **10**, 139–154.
2. Atkin NB. Nuclear size on carcinoma of the cervix: its relation to DNA content and to prognosis. *Cancer* 1964, **17**, 1391–1399.
3. Sandritter W, Kleinhans D. Uber das trockengewicht, den DNS- und histonproteingehalt von menschlichen tumoren. *Z Krebsforsch* 1964, **66**, 333–348.
4. Sandritter W, Seidel A, Kleinhans D, Paddags I, Dontenwill W. Zytophotometrische Messungen des DNS-Gehaltes an menschlichen und tierexperimentellen Bronchialmetaplasien.*Z. Krebsforsch* [in press].
5. Sandritter W. Cytophotometrische Untersuchungen am Portiocarinom und seinen Vorstufen. *Verh. Dtsch. Ges. Path.* 48. Tgg. Salzburg (1964) 34–43.
6. Seidel A, Sandritter W. Cytophotometrische messungen des DNS-gehaltes eines lungenadenomas und einer malignen lungenadenomatose. *Z Krebsforsch* 1963, **65**, 555–559.
7. Sandritter W, Hillwig I, Engelbart K, Kiefer G, Kiefer R. Versuche zur Carcinogenese *in vitro*. Chromosomenanalysen und Cytophotometrische Messungen des DNS-Gehaltes *Z. Krebsforsch*. [in press].
8. Sandritter W, Böhm N. Atypische Hydrolysekurven bei der feulgenreaktion von mäuseascitestumorzellen. *Naturwissenschaften* 1964, **51**, 273.
9. Sandritter W, Jobst K, Rakow L, Bosselmann K. Zur kinetik der feulgenreaktion bei verlangerter hydrolsezeit cytophotometrishce messungen im sichtbaren und ultravioletten licht. *Histochemie* 1965, **4**, 420–437.