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Flow cytometric DNA analysis of normal, premalignant and malignant human epidermal tissues

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The present review is based on the following papers

I **Frentz G, Møller U, Christensen I.** DNA flow cytometry on human epidermis. I. Methodological studies on normal skin. J Invest Dermatol 1980;74:119-21.

II **Frentz G, Møller U.** DNA flow cytometry on human epidermis. Interindividual and regional variations in normal skin. Clin Exp Dermatol 1983;8:19-26.

III **Frentz G, Møller U & Keiding N.** DNA flow cytometry on human epidermis. The effect of serial biopsy sampling displaced by time. Br J Dermatol 1982;107:7-14.

IV **Frentz G, Møller U.** DNA measurements by single nuclei flow cytometry in human actinic lesions. J Invest Dermatol 1981;77:431-3.

V **Frentz G, Møller U.** Clonal heterogeneity in curetted human epidermal cancers and precancers analysed by flow cytometry and compared with histology. Br J Dermatol 1983;109:173-81.

VI **Frentz G, Møller U, Larsen JK.** DNA flow cytometry on human epidermal tumours. Intra- and intertumour clonal heterogeneity and proliferative variability. Virchows Arch (Cell Pathol) 1985;48:175-183.

VII **Frentz G, Møller U.** DNA flow cytometry on the epidermis of patients with multiple epidermal carcinomas. Br J Dermatol 1984;111:271-7.

In the text the publications will be referred to by their Roman numerals.

Aims

The present study was undertaken in order to evaluate the potential usefulness of flow cytometric DNA measurements on human epidermis for clinical dermatology, focusing on normal skin and epidermal tumours. All experiments were performed on human skin samples

- to evaluate an adapted technique for skin separation, storage, epidermis disaggregation, cell dispersion and staining, based on a method developed for the hamster cheek pouch epithelium.

- to estimate the importance of age, sex, and body site as well as of the time of day and previous biopsy sampling for the DNA distribution of the normal epidermal cells

- to examine the proliferative characteristics in the uninvolved epidermis of multiple skin cancer patients

- to estimate the homogeneity/heterogeneity in the proliferative characteristics within epidermal tumours

- to study the proliferative characteristics and ploidy in epidermal tumours and their relations to clinical parameters.

Definitions and abbreviations

AK: Actinic keratosis.

Aneuploidy: Ploidy level different from both diploidy and a multiple of diploidy (2c, 4c, 8c, 16, etc.).

BCC: Basal cell carcinoma.

BO: Bowen's disease.

BSC: Basosquamous carcinoma.

CV: Coefficient of variation.

DI: DNA-index: $\frac{\text{Clonal DNA content of the sample}}{\text{Clonal CNA content of homologous normal cells}}$

Differentiating compartment: The fraction of cells which are maturing for maintenance of the tissue function and have lost the ability to divide.

DNA: Desoxyribonucleic acid.

DTT: Dithiotreitol.

EB: Ethidium bromide.

EPU: Epidermal proliferative units.

FCM: Flow cytometry.

FLM: Fraction of labelled mitoses (or PLM: percentage of labelled mitoses).

GF: Growth fraction: the fraction of potentially proliferative cells actually traversing the mitotic cell cycle.

G_1 phase: The nucleus in normal cells contains 2c DNA. The cell prepares for the forthcoming DNA synthesis.

G_0 phase: Resting phase. The nucleus contains 2c DNA, the cell is quiescent, but potentially proliferative.

G_2 phase: The nucleus contains 4c DNA. RNA and proteins are synthesized.

G_1 modal DNA content: The DNA content in the G_1 phase for the stem line in question. Normal cells contain 2c DNA. Aberration from 2c occurs especially in tumour stem cell lines, the genome of which may be altered quantitatively.

Heteroploidy: G_1 modal DNA content $\neq$ the diploid level, i.e. aneuploidy or polyploidy.

3HTdR: Thymidine labelled with tritium.

MI: The fraction of cells in mitosis (related to the total number of viable epidermal cells or basal cells).

Monoclonal: Containing only one detectable cell clone as judged from the cellular DNA content distribution.

M phase: Mitosis.

MPM: Microphotometry.

PI: Propidium iodide.

Pluriclonal: Containing more than one cell clone as judged from the cellular DNA content distribution.

Polyploidy: Ploidy level equalling a multiple of diploidy (4c, 8c, 16c, etc.).

Proliferating compartment: The fraction of cells in a population having the potential for proliferation (includes resting cells i.e. G_0 cells which may reenter the cell cycle).

SCC: Squamous cell carcinoma.

S phase: The DNA synthesis phase. The nuclear DNA content increases from 2 to 4c.

Steady state: Cell loss and cell gain equals for the entire population and for the different compartments and the different phases of the cell cycle.

Transit time: The time span from a cell enters a compartment (or the total viable epidermis) till it leaves it again.

Turn-over time: The time required for the replacement of the number of cells making out the compartment in question (or total epidermis.

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BACKGROUND

Introduction

The epidermis is a renewing cell population as opposed to static, expanding or neoplastic cell populations, i.e. the epidermal cell population maintains a constant size and the permanent physiological loss is counterbalanced by a continuous proliferation (Leblond 1964). Functionally the stratified squamous epithelium of the skin is divided into the proliferating compartment, the viable differentiating compartment and the finally differentiated compartment, the stratum corneum. In normal human epidermis the proliferating compartment is located partly in the basal, partly in the suprabasal layer(s) (Lachapelle & Gillman 1969, Penneys et al. 1970, Lavker & Sun 1982). The size of the fraction of actually proliferating cells, the growth fraction (GF), is unknown, because no appropriate measuring method has been available (Wright 1983). According to Potten's recent theories the fraction of proliferating epidermal cells consists of at least two kinetically distinct epidermal cell populations, clonogenic stem cells and transit proliferative cells. Structurally the epidermis can be considered composed of small groups of intercommunicating cells, the epidermal proliferative units (EPUs), each presumably originating from a single stem cell (Hume & Potten 1982, Potten 1983).

The renewing cell system of epidermis thus has a complex organization and the functional state of epidermis may be difficult to evaluate and describe adequately by simple methods. Physiological and pathological stimuli may also elicit diverse reactions from the continually renewing cell population.

At present the theoretical background for the use of the conventional technique is a most controversial issue (Wright 1977, Potten et al. 1982, Clausen 1983b, Wright 1983). In vivo cell kinetic investigation on human epidermis involve ethical and practical constraints because the majority of theoretically utilizable methods includes use of radioisotopes, cytostatics and/or multiple biopsies. Experimental human data are consequently sparse.

An estimate of the proliferative status of a certain tissue can be obtained by flow cytometric single cell DNA analyses. The method is rapid and does not require radioactive isotopes or drugs with potential adverse influence on the cell proliferation of the organism or tissue investigated. This makes flow cytometry (FCM) unique for use in man and the method is expected to provide a useful tool in the search for more knowledge of human epidermal cell kinetics.

In Denmark very extensive and accurate studies on FCM techniques have been performed by Vindeløv (Vindeløv 1977, Vindeløv et al. 1980, 1983a, b, c)

who has developed a useful one step method for preparing samples for FCM and evaluated the importance of both methodological and biological details. For the keratinizing squamous epithelium a separation technique based on ultrasonic disaggregation suitable for the hamster cheek pouch was elaborated by Møller and Larsen (1978, 1979), while Clausen and coworkers in Norway (1976) used a different technique for the skin of hairless mice. Using enzymatic tissue disaggregation and dithiotreitol maceration Bauer and coworkers in Holland pioneered the FCM investigations of human epidermis (Bauer & de Grood 1975, 1976, Bauer et al. 1980).

The technique of flow cytometry

Flow cytometry designates automated quantitative measurements of physical properties of single microparticles suspended in an aqueous solution, while they — in a liquid stream at high speed — individually traverse a detector system (Fig. 1). Naturally suspended cells — e.g. in blood or ascites fluid — are thus ideal objects for FCM. The method has developed rapidly in the field of cell biology and cell kinetics since 1968-69 (Dittrich & Göhde 1969, Melamed & Mullaney 1979, Gray & Coffino 1981, Kruth 1982). In its first decade FCM has mostly been used for studies on the cellular DNA content. The DNA is stained with a fluorescent dye that binds specifically to DNA, and the DNA content of each cell is measured by the fluorescence emitted from every single particle on the exciting of the dye. At the same time as the DNA fluorescence is being registered it is possible to measure additional parameters on the same particle, e.g. light absorption, light scatter or a second component labelled with a fluorescent dye. The major advantages of FCM for cell proliferation studies are accuracy and speed, and the fact that radioactive isotopes have become redundant.

The steps involved in the total procedure for DNA FCM on solid tissues, e.g. epidermis, are:

1) tissue sampling (biopsy)
2) tissue isolation
3) tissue disaggregation, i.e. preparation of a monodisperse suspensions of nuclei or cells
4) specific staining to label the component(s) of interest, i.e. the DNA
5) the measurement in the flow cytometer
6) quantitative analysis of the DNA distribution curve yielding the estimates of the fractions.

A possibility for storage of a sample at one of the steps is highly desirable for sequential and accurate studies (Vindeløv et al. 1983a).

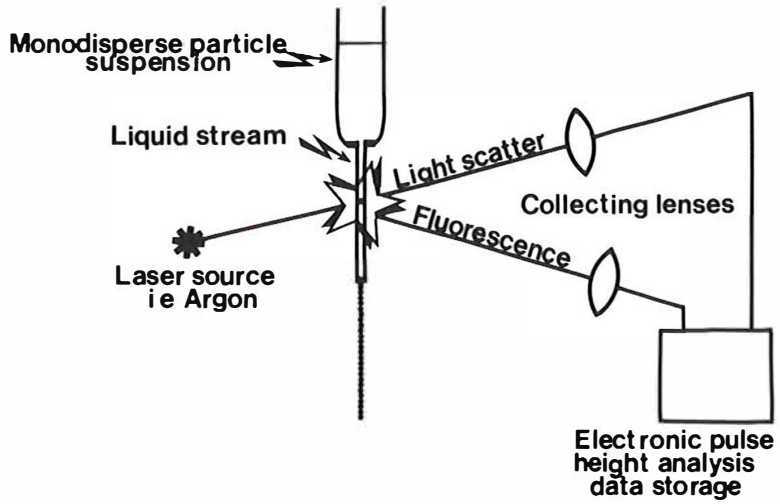


Fig. 1: Diagram illustrating the FCM procedure for quantitating the physical properties of the DNA particle. When hit by the laser beam the isolated nucleus stained with a fluorescent dye emits fluorescent light quantitatively paralleling the amount of DNA. The laser beam is scattered by the DNA particle, most pronounced scattering elicited by large particles. Also the amount of scattered light may be registered.

Tissue sampling

Ideally the tissue sampling should be performed without any risk of influencing the parameters measured. For human cell kinetic studies this might be difficult, since the parameters are apt to show a high degree of lability — e.g. by recent biopsy trauma or local injections. For human epidermis shave and punch biopsies, suction blister formation and surgical excision have been used (I, III, Bauer & de Grood 1975, 1976, Thorud & Volden 1980).

Tissue isolation

To avoid unnecessary contamination, the epidermis should be isolated from the dermis before the tissue disaggregation procedure. This can be done by the suction blister technique, by use of acetic acid, enzymes such as trypsin and collagenase, 2N sodium thiocyanate, by heat separation or by mechanical stretching (Kiistala & Mustakallio 1967, Haag, Tschahargane & Ehemann 1975, Clausen 1983a).

Preparation of the monodisperse suspension

This is a crucial step for FCM investigation on solid tissues, not least for epidermis, where the desmosomes hold the cells firmly attached. During the DNA FCM measuring procedure two adhering cells will be interpreted as one cell with a high DNA content. By the dispersion it is essential that no selective loss takes place among the particles of interest. Mitotic cells/nuclei are particularly susceptible to various interferences, e.g. treatment with enzymes (Clausen et al. 1976, Bech 1977). For disaggregation of epidermis trypsin has been used (Lærum 1969, Clausen et al. 1976), as has trypsin and dithiotreitol (DTT) (Hentzer & Kobayasi 1976, Bauer et al. 1980), homogenisation (Goerttler, Balmain & Sauerborn 1976) or ultrasonication and DTT (Møller & Larsen 1979).

Specific staining

DNA can be quantitatively stained with ethidium bromide (Göhde & Dittrich 1971); to eliminate ethidium bromide stained double stranded RNA, RNAse treatment should precede the staining. A simple proportional relationship between the fluorescence intensity — the amount of dye — and the amount of DNA is implied. For increased specificity propidium iodide (PI), mithramycin a.o. have been introduced in DNA FCM (Crissman & Tobey 1974, Krishan 1975, Clausen 1983a).

The measurement in the flow cytometer (Fig. 1)

The power of resolution for DNA measurements by FCM depends on the qualification and purity of the stained monodisperse suspension, and in particular on the physical properties of the flow cytometer used. The highly sensitive instrument is usually susceptible to technical sources of error (Vindeløv 1977, Vindeløv et al. 1980). It is vital to calibrate a flow cytometer before use and adjust it to maximal resolution by means of standard particles, e.g. polystyrene beads or trout erythrocytes. FCM provides no possibility for histolocial observation of the particles at the moment of fluorescence registration (Fig. 1). However, simultaneous measurement of an additional property of the particle e.g. forward light scattering is helpful in the evaluation of the quality — and artefacts — in the measurements performed (II, fig. 2).

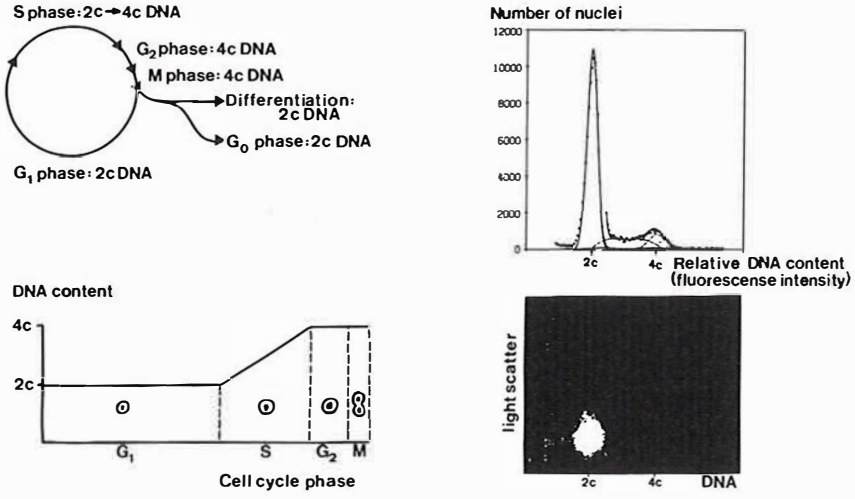


Fig. 2: Left: Graphic illustration of the cell cycle traverse. Top: The relative length of the various phases is shown approximately, and the possible fate of a postmitotic cell is indicated. Bottom: The increase of the individual cell DNA content in the course of a cell cycle traverse is shown. Right: A typical DNA distribution diagram from normal human epidermis as obtained by flow cytometry. Top: The left peak area reflects the number of cells with a 2c DNA content — i.e. G_1, G_0 and differentiated cells — and the second peak the number of cells with a 4c DNA content — i.e. G_2 cells and mitotic cells. This peak is best visualized from the upper, ten fold amplified curve. The intermediate area represents the S phase cells. Bottom: Simultaneous registration of the fluorescence emitted from and the light scatter caused by the particles sent through the flow cytometer. These diagrams can be used for estimation of clumping and debris in the sample.

Quantitative analysis

The signals from each particle are usually automatically analysed in the associated pulse height analysor and a DNA distribution diagram is obtained showing the number of cells/nuclei which contain the varying amount of DNA (Fig. 1 and 2). Relevant interpretation of the curve requires experience of the proliferative behaviour of the cells, but both simple planimetric evaluations and advanced automated deconvolution procedures are useful in the mathematical calculations (I, Clausen et al. 1976, Johnston, White & Barlogie 1978, Baisch et al. 1982).

FCM parameters

DNA FCM can yield information on the relative distribution of cells in the various cell cycle phases in a cell suspension at a given time, since the individual nuclear DNA content depends on which phase the cell is traversing (Fig. 2). The size of the various cell cycle compartments, e.g. of the S- or G_2M-fractions thus reflects the proliferative characteristics of the tissue under investigation. Additionally, the G_1 modal DNA content for a sample can be assessed as normal, i.e. diploid, or not, when also comparative measurements of standard particles are performed.

Proliferative characteristics

For a differentiating renewing cell system like epidermis the fraction of cells with a 2 c DNA content contains the differentiated cells together with the G_1 (+ G_o) cells in the proliferating compartment (Fig. 2). The fraction of cells with a DNA content between 2 c and 4 c defines the S-fraction, i.e. the cells of the DNA synthesis phase, and the fraction of cells with a 4 c DNA content the G_2 phase cells plus the cells in mitosis (Fig. 2).

Ploidy, G_1 modal DNA content, DNA-index

Normal human cells in G_1 phase have a diploid DNA content equal to 46 normal human chromosomes. Heteroploid cell clones with a G_1 phase DNA content different from that of normal diploid cells — i.e. aneuploid or polyploid clones — may arise, e.g. in neoplasia. The DNA-index (DI) for a cell population is defined by the following ratio:

$$DI = \frac{G_1 \text{ modal DNA content of the cell population}}{G_1 \text{ modal DNA content of the corresponding normal cells}}$$

(V, Vindeløv 1977, Barlogie et al. 1978, Vindeløv et al. 1980, Barlogie et al. 1983). Normal diploid cells thus have a DI of 1.00, hypodiploid cells a DI < 1.00, hyperdiploid cells DI > 1.00, tetraploid cells DI $= 2.00$, etc.

FCM versus conventional cell kinetics

FCM versus microphotometry

DNA FCM gives a relative estimate of the cellular DNA content in a single particle suspension. Microphotometry (MPM) provides a similar estimate based on histologic sections and theoretically the parameters obtained by FCM and MPM should be similar, when the cut nuclei in the latter method are disregarded (for MPM studies on human epidermis see Leuchtenberger, Leuchtenberger & Davis 1954, Kint 1963).

S-fraction versus labelling index (LI)

Most studies on the growth of human epidermis are based on light microscopical studies on mitoses and on 3HTdR autoradiographic determination of the fractions of the DNA synthesizing cells (labelling index studies). Comparing the size of the S-fraction determined by FCM and the LI — and especially studying the variability in these parameters — it is vital to realize that these parameters differ basically: The LI by nature measures cells with a certain rate of active DNA synthesis, while the S-fraction measures all cells with a DNA content between 2 c and 4 c. Thus the S-fraction generally exceeds the LI, most pronounced in slowly proliferating tissues, while the figures are more equal in hyperproliferating tissues (Clausen et al. 1979, Kruth 1982, Møller, Keiding & Engel 1982). Furthermore, several sources for methodological errors in the LI determination have to be taken into account (Maurer 1981, Potten et al. 1982, Clausen 1983 b, Wright 1983).

G_2M-fraction versus mitotic index (MI)

A simple proportional relationship should exist between the mitotic index (MI) determined by light microscopy and the G_2M-fraction determined by FCM providing steady state conditions in the cell cycle as is assumed in the classical cell kinetic concepts of Leblond (1964). The size of the G_2-fraction can be indirectly estimated from FLM studies (studies on the fraction of labelled mitoses). The reliability of mitotic countings depends on various details: difficulties in recognizing very early and very late mitotic figures by light microscopy and the fragility of the mitotic figures when exposed to a variety of factors, e.g. a prolonged fixation procedure or proteolytic enzymes (Scheving 1959, Fisher

1968b, Clausen et al. 1976, Bech 1977).

Time related parameters

FCM data yield no immediate information on the duration of the various phases of the cell cycle, but the method is useful for studies on alteration in the cell cycle distribution from one time to another (Clausen 1983 a, Lærum 1983). According to the classical steady state cell kinetic concepts the ratio S/G_2M should reflect the ratio between the duration of the S phase and the joint duration of the G_2 and M phases.

OWN INVESTIGATIONS

Methodology (I, II)

The procedure here used for DNA FCM is initially developed for use on the hamster cheek pouch epithelium and adapted for use on human epidermis (Møller, Larsen & Frentz 1978, Møller & Larsen 1978, 1979). The main differences concern the tissue sampling procedure, the acetic acid maceration time and the statistical model used for deconvolution of the DNA distribution. The modifications for human epidermis were elaborated aiming at reproducible and equal results from different samples taken from one large piece of skin.

Biopsy sampling (I, II, VI)

Normal and clinically uninvolved skin was obtained a) during universal or local anesthesia from basically healthy individuals undergoing plastic surgery for cosmetical reasons, and b) by small surgical excisions (0,5 — 1 cm^2) under local anesthesia with 2% mepivacaine 1 ml (leg ulcer patients and skin tumour patients).

Samples from tumour lesions were obtained under similar local anesthesia as 4 mm punch biopsies or the tumours were removed in toto by curettage.

Tissue isolation (I, III)

For human skin acetic acid 1% was used in 24 — 72 hours for macerating the sample to obtain complete separation of epidermis from dermis (I, Haag, Tschahargane & Ehemann 1975, Møller, Larsen & Frentz 1978, Møller & Larsen 1979).

Prolonged acetic acid maceration time diminished the cell number, but did not influence the final results significantly (III). Microscopical examination confirmed the completeness of the dermal/epidermal separation. (Møller & Larsen 1979). The epidermal appendages were left in the dermis, only the very opening adhering to the loosened epidermis with separation at the level of the dermal epidermal junction (Frentz & Møller, unpublished).

nitrogen from 5 days to 5 weeks, only exceptionally for a longer period (I, II). The storage time of the isolated epidermis did not affect the results significantly for 32 (-40) days. As a rule only biopsies treated identically were compared.

Tissue disaggregation

After thawing in a 35° C water bath the specimen was cut into pieces and treated with DDT for disrupting the desmosomes (Hentzer & Kobayasi 1976). For final disaggregation the tissue was ultrasonicated for 10 seconds in a 150 W ultrasonic disintegrator.

After filtration through gauze, aspiration by syringe and centrifugation the sediment was resuspended in a ribonuclease solution (Møller, Larsen & Frentz 1978, Møller & Larsen 1979).

That the inevitable cell loss did not tend to be selective with respect to the different layers of epidermis and the different phases in the cell cycle was shown for the hamster cheek pouch epithelium (Møller & Larsen 1978, 1979). The cell yield in human epidermis could not be increased by diminished, prolonged or repeated ultrasonication or by exchanging the gauze filter with nylon filters with several different pore sizes (Frentz, Møller & Larsen, unpublished).

Staining

After centrifugation the sediment was resuspended for staining in an isotonic solution of ethidium bromide (EB) 50 μg/l containing TRIS HC1 buffer pH 7.4.

Measurement procedure in the flow cytometer

Immediately after filtering and within 5 hours after staining the suspension of single nuclei was examined by means of a laminar sheet flow technique in the flow cytometer (Cytofluorograph Bio Physics System Inc. type 4802 and 6300 A) at a flow rate of no more than 500 cpm (counts per minute) (Fig. 1). On an average 100,000 nuclei were counted for each sample. The cytofluorograph was adjusted to maximal resolution with polystyrene microspheres and/or trout erythrocytes as standard fluorescent particles. To record possible changes in instrumental gain or resolution every third to fifth run contained standard

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Quantitative analysis

The DNA frequency distribution for each sample was recorded as a histogram The number of nuclei with 2c, 2 to 4c and 4c DNA content expressed a percentages of the total number of nuclei was calculated by a least square estimation procedure, using a 0 degree polynomial for description of the S phas (I). Because the standard calculation procedure for the deconvolution require high quality histograms for consistency in results each curve was evaluate individually before use.

Excluded were histograms with one or more of these features:

1) a cell number < 20.000
2) a poorly defined S level (also controlled by scatterograms)
3) a level at the 6c DNA point of $\geqslant$ one fourth of the S level (clumping coincidence)
4) for uninvolved epidermis: peaks with higher fluorescence intensity tha equalling 4c DNA (clumping, coincidence).

Number of cell clones and ploidy (IV, V, VI)

The number of cell clones was estimated by defining differences in clonal DNA content by

1) 2 separate 'G_1' peaks — corresponding peak fluorescence differences of mor than twice the mean value of the CV's of the peaks.
2) a CV of the G_1 peak $\geqslant 11.5\%$
3) a tetraploid fraction $\geqslant 20\%$, if also peak formation at the octoctaploid leve appeared without a hexaploid peak.

The DNA-index of a cell clone was calculated as the ratio between th fluorescence of the G_1 peak for the clone and the fluorescence of the G_1 peak o normal epidermis. Fluctuations in the fluorescence of the G_1 peak which migh occur due to instrumental instability were corrected for each sample assumin that the fluorescence of the G_1 peak was half of the fluorescence of the G_2M peak. The stability of the fluorescence signals for each series of measurement was controlled by run of standard particles. The standard reference used for th

Vindeløv et al. 1980, 1983c, the use of external standards disregarded.

Normal skin (I, II, III, IV, VII)

Reproducibility (I, II, IV)

Intra/interassay variability

The accuracy of the measurement was evaluated by the intraassay, intraindividual variability. For the S-fraction the intraassay CV approximated 15%, for the G_2M 14%, and for the DNA-index determination 3,5%. The corresponding interassay values amounted to 19, 24, and 5% (I, II, VI). The G_2M values exhibited no consistent homogeneity of variances. For approximate calculations of interassay G_2M values the pooled variance value was used.

Inter/intraindividual variability

For the proliferative characteristics in normal human epidermis for a given body site, the interindividual variation exceeded the inter- and thereby the intraassay and intraindividual variation (I, II).

Physiological variability (II, III)

Influence of age and sex (II)

For the age group 36 — 87 years (n = 20) no convincing changes by age or sex were seen in lower abdominal skin in the FCM proliferative characteristics S- and G_2M-fractions (II). It was noted that the G_2M values of the females varied more

Influence of body site (II)

Obvious topographic variations in the FCM proliferative characteristics wer seen in normal human epidermis depending on the S-fraction, but not on th G_2M-fraction when forearm skin was compared to lower abdominal and femora skin.

Influence of time of day (III)

The question of possible circadiurnal variations in human epidermal ce proliferation was approached by taking biopsies from each of 10 individual from the lower abdominal skin four times with a 6 hour interval, each patien serving as his own control.

No unequivocal or consistent circadian variation occurred in the S-fraction when the influence of the biopsy trauma was taken into consideration, while th G_2M-fraction showed peak values at noon.

Influence of trauma (III)

The disturbing influence on human epidermal FCM proliferative characteristic elicited by a standardized surgical biopsy trauma was evaluated (III). The result indicated that the biopsy trauma elicited a decrease in the S-fraction after 1 hours and a slight increase after 48 hours in the adjacent skin. However, decrease was also seen in the S-fraction in the contralateral region after 6 hours suggesting that also more complicated mechanisms than local trauma ar involved in the cell kinetic regulation in man.

Epidermal tumours

Uninvolved epidermis in multiple epidermal cancer

'Unexposed' epidermis (IV, VII)

The proliferative characteristics i.e. the S- and the G_2M-fractions of the epidermis not exposed to external carcinogens in unselected individuals with multiple malignant/premalignant epidermal tumours — i.e. basal and squamous cell carcinomas/Bowen's disease and actinic keratoses — did not differ significantly from the unexposed skin of leg ulcer patients in a comparable age group (VII). A trend for larger variability was seen in the S-fraction for the tumour patients. More detailed analyses incorporating the influence of pre/coexisting psoriasis, intolerance to sun light, previous arsenic intake, exposure to X-rays/Grenz rays, external exposure to sun/UV light and the types of tumours developed, indicated an interaction between the proliferative characteristics in the unexposed skin and the tumour type developed. Both the S- and the G_2M-fractions were higher in the unexposed skin of individuals developing SCC in addition to BCC's than of individuals developing BCC's solely. When repeated measurements were performed on the unexposed skin of one individual with presumably actinically induced epidermal cancers, the S-fraction was significantly higher than in the control group, while, due to a large G_2M range in the controls, the increase in the G_2M-fraction was not statistically significant (IV). However, there was no general trend towards an increase in S- og G_2M-fractions in the unexposed skin of actinically damaged individuals with multiple epidermal cancer.

'Exposed' epidermis (VII)

The perilesional skin and the skin exposed to external carcinogens of individuals with multiple skin cancer is supposed to be a higher risk than the above mentioned 'unexposed' skin. For practical reasons the epidermis at risk is designated exposed epidermis. By DNA FCM the exposed skin differed from the unexposed skin by having a higher S-fraction, while the increase in the G_2M-fraction was only marginally significant. In accordance with the findings in the unexposed skin the type of tumour was related to the size of the proliferative

characteristics in the exposed skin: the G_2M-fraction was far higher in individuals with both SCC and BCC than those with BCC solely. Remote exposure to X-ray/Grenz ray tended to correlate a high G_2M-fraction, while the proliferative characteristics showed no correlation to remote external exposure to sun/UV light, oral arsenic intake, intolerance to sun light or to pre/coexisting psoriasis.

Proliferative characteristics in tumours (IV, V, VI, VII)

The criteria used in these studies to define intraassay pluriclonality have previously been stated. In the present studies the proliferative characteristics in tumours are merely considered for the tumours which appeared monoclonal.

Tumour versus normal/uninvolved epidermis (V, VII)

For the epidermal tumours removed by curettage the proliferative characteristics differed significantly from those of the corresponding normal/unexposed epidermis (VII). The S- and G_2M-fractions increased stepwise from the unexposed skin over the exposed skin to the tumour tissue. The most important increase in the S-fraction appeared between unexposed and exposed skin, and in the G_2M-fraction between the exposed skin and the tumour. For a small number of tumours, primarily superficial BCC's and some SCC's the proliferative characteristics were indistinguishable from those of uninvolved epidermis, demonstrating that neoplastic growth in itself does not necessarily have a unique impact on the values (V, VI).

Intratumour variability (VI)

For multiple samples from the peripheries of 10 different epidermal tumours the variability in the S- and G_2M-fractions were larger than in normal epidermis, possibly expressing the influence from the stroma or the inflammatory cells. However, the S- and G_2M-fractions were highly characteristic for the periphery of each tumour. As a whole the variability within the different tumour peripheries was similar, even if the G_2M-fractions sometimes exhibited some fluctuation as also seen in normal epidermis.

Histological tumour type (V, VI)

Among 37 curetted epidermal malignant (BCC's, BSC's and SCC's) and premalignant tumours (BO's and AK's), and among punch biopsy sampled peripheries of 10 different epidermal tumours of the proliferative characteristics showed a wide range. The S- or G_2M-fraction levels did not separate the histological groups consistently (V, VI).

Age, sex and body site (V)

The proliferative characteristics in the tumours could not be correlated to age, sex or body site.

Clonality and ploidy in epidermal tumours (IV, V, VI)

In the present studies a total of seventy-five epidermal tumours were evaluated by DNA FCM, 65 samples were removed by curettage, 10 tumours were investigated from multiple punch biopsies. Certain evidence of more than one tumour cell clone was an exceptional feature and could only be demonstrated in two tumours, one BO and one BSC (V, VI).

Reproducibility in the DNA-index determination (VI)

The reproducibility of the DNA-index determination for punch biopsied tumour samples — determined as the variation in DNA-indices for tumour peripheries — equalled that of normal epidermis with an interassay CV amounting to 5%. Only in one out of 10 tumours examined by several biopsies taken from different locations the clonal composition differed significantly from one site to another. In this tumour — a BSC — the clonal DNA content in the centre deviated from that of the periphery.

Ploidy versus histology (V, VI)

For the curetted tumours the frequency of heteroploidy was clearly related to the histological tumour type, 24% of the BCC's showing heteroploidy against 78% of the squamous cell cancers (SCC's) and — unexpectedly — 75% of the precancerous lesions (BO's + AK's). Also the degree of heteroploidy differed

among the histological groups, the BCC's being predominantly near diploid, the SCC's near diploid and near tetraploid, the precancers exhibiting more scattered DNA-indices (V).

Ploidy versus age, sex and body site (V)

The frequency of heteroploidy showed no relationship to the patients' age, sex and body site for human epidermal tumours or to whether the patient in question had developed multiple lesions or not.

Interaction between S-fraction and DNA-index (VI)

A trend for a positive correlation was observed between the S-fraction and the DNA-index determined for ten human epidermal tumours ($p = 0.13$). For each of these tumours the mean values of multiple biopsies from the periphery were used.

Table I

Technical details, variables investigated and results obtained from four independent groups studying normal human epidermis by DNA FCM.

Group	Bauer et al.
Year of the initial report	1975
Individuals studied	healthy volunteers
Biopsy technique	shaving
Local anesthesia	-
Material used for production of the single particle suspension	total epidermis + dermis
Possibility for storage	+ (refrigerator)
Disaggregation procedure	Trypsin DTT ultrasonic vibration
Staining	propidium iodide
Mitosis preserved	-
Particles measured	cells
Cell yield	high
Type of flow cytometer	ICP 11
Procedure for deconvolution of histograms	planimetry
CV of G_1 peak (%)	n.m.
Influence of aging	decreasing G_2-fraction
Influence of sex	n.s.
Influence of season	highest S and G_2M fractions in June
Influence of body site	higher G_2M-fractions for arm than for back and leg
Influence of trauma	n.m.
S-fraction (%)	2.6 ± 0.8*
$G_2(M)$-fraction (%)	1.2 ± 0.5*

* not corrected for coincidence (Bauer et al. 1980).
n.m.: not mentioned; n.d.: not done; n.s.: not significant.

Thorud & Volden	Mansbridge	Frentz et al.
1980	1981	1978
dermatological patients	n.m.	obese women leg ulcer patients
suction	suction	excision
-	-	+
mainly proliferative compartment	epidermis	total epidermis isolated by acetid acid
n.m.	n.m.	+ (liquid nitrogen)
Trypsin vortexing pepsin	Trypsin EDTA	DTT ultrasonication
ethidium bromide (EB)	PI	EB
-	n.m.	+
nuclei	cells	nuclei
50%	n.m.	10%
ICP 11	FACS	Bio Physics cytofluorograph
planimetry	n.m.	automated least squares/max. likelihood estimation procedures
4 - 8	n.m.	8.5
n.m.	n.m.	n.s.
n.m.	n.m.	more variable G_2M in women
n.m.	n.m.	n.d.
n.m.	n.m.	higher S for arm than for thigh and lower abdomen
n.m.	n.m.	+
approx. 2.5	0.75 ± 0.09	2.6 ± 0.8
approx. 2.0	1.63 ± 0.19	2.1 ± 1.1

DISCUSSION

Normal skin

The methodological differences in the FCM techniques hitherto used for normal human epidermis can be read from table I. Both conventional cell kinetic technique studies and FCM studies on normal human epidermis confirm that the overall value of the S-fraction of 2.6 ± 0.8% from the present study provides a reasonable estimate for percentage of cells in the S phase of all the viable epidermal cells. Thus the S-fraction in the present investigation equalled the S-fraction found by Bauer et al. (1980, see Table I). Also a comparison of the reproducibility of the results is satisfactory (Epstein & Maibach 1965, Lachapelle & Gillman 1969, Hell & Maibach 1972, Allegra & Panfilis 1974, Camplejohn et al. 1984, Weinstein, McCullough & Ross 1984, Table II). The overall G_2M-fraction of 2.1 ± 1.1 is an acceptable figure compared with other FCM studies and also with MI values, when the influence of the G_2-fraction is considered (Rowe & Dixon 1972, Pullmann, Lennartz & Steigleder 1974). Yet, Camplejohn et al. (1984) found a MI of about 0.06% (related to the basal layer) (Table I and II).

Table II

MI, LI and microphotometric parameters from human epidermis.

	% of basal cells		% of viable cells		References
			0.16		Pinkus 1952
			(6.13)		Scheving 1959
			0.02 - 0.05		Thuringer & Katzberg 1959
			0.01 - 0.09		Fisher 1968b
MI	0.9		0.01 - 0.09		Weinstein & Frost 1969
	0.09 - 0.36				Rowe & Dixon 1972
	0.4 ± 0.11				Pullmann et al. 1974
	0.06				Camplejohn et al. 1984
			G_2:2.2 (MPM)		Grove et al. 1976
	in vitro	in vivo	in vitro	in vivo	
		4.9 ± 1.1			Epstein & Maibach 1965
			0.9 ± 0.6		Hell & Hodgson 1967
	4.5				Braun Falco et al. 1967
	2.3	2.2	2.2	2.1	Lachapelle & Gillmann 1969
		5.2			Weinstein & Frost 1969
LI			0.97 - 1.37	1.6 - 2.5	Hell & Maibach 1972
		3.5			Weinstein & McCullough 1973
	5.8 ± 0.6				Heenen et al. 1973
		3.8			Allegra et Panfilis 1974
	2.4 ± 0.16				Pullmann et al. 1974
	2.9 ± 0.5				Schell et al. 1977
	2.8 ± 1.0				Galosi et al. 1980
		5.5			Camplejohn et al. 1984
		2.7 ± 1.2			Weinstein et al. 1984
				7.8 (MPM)	Grove et al. 1976

In our studies on both normal epidermis and on neoplastic tissue repeated S-fraction determinations were more stable than G_2M-fraction determinations (II, VI). The interday/interassay variation was higher than the intrarassay variation as also observed by Vindeløv et al. (1983a). A considerable interindividual variability is a general feature in human cell kinetic studies (Table II).

Age and sex

The relation between age/sex and human epidermal cell growth has not been conclusively established. Studies on S- and G_2M-fractions, LI and MI have been equivocal (II, Braun-Falco, Christophers & Kurban 1967, Bauer et al. 1980). For instance the MI was reported to increase until middle age by Thuringer & Katzberg (1959), while a decrease from the age of 20 was found by Fisher (1975). Likewise an increase in stratum corneum transit/turnover time by advancing age was observed by Baker & Blair (1968), Jansen, Hoyio-Tomoko & Kligman (1974), and Roberts & Marks (1980), while, according to Epstein & Maibach (1965) the turnover time for viable epidermis will not change significantly by age. Females tend to have lower mitotic rates than males (Fisher 1968a), and the impression of a rather complicated interaction between sex and age on the growth of human keratinizing structures is reinforced from studies on nails and stratum corneum. These indicate a more rapid growth in males compared to females in the first decades of life, while the reverse pattern was seen after middle age (Hillman 1955, Ohrentreich & Sharp 1967, Baker & Blair 1968).

Also the menstrual cycle should interfere with the human epidermal cell proliferation (Fisher 1975).

Body site

Pronounced regional variations in the proliferative characteristics in two independent FCM studies (II, Bauer et al. 1980) confirm the body site dependence of human epidermal growth. This is also found by other techniques, particularly evident in studies on stratum corneum turnover time (Jansen, Hoyio-Tomoko & Kligman 1974, Galosi et al. 1980, Roberts & Marks 1980, Finlay, Marshall & Marks 1982).

Time of day and injury

In man circadian variations are observed for a variety of parameters, e.g. body temperature, excretion of cortisol, sodium, potassium and creatinine by urine. Also the different phases of hemopoiesis are known to undergo diurnal rhythmicity (Lærum 1983). Analogous circadian variations are expected to exist in human epidermal cell proliferation. However, consistent diurnal variation in adult skin growth has hitherto not been finally documented. Neither the DNA FCM studies by Thorud & Wolden 1980 nor by Frentz, Møller & Keiding (III) could show impressing circadian variation in the proliferative characteristics, but the number of individuals was small and the interpretation of the findings in the latter study was partly confounded by the influence from the biopsy trauma. On the other hand previous MI studies yield some support for the existence of diurnal G_2M variations as found by Frentz et al. (III). Thus MI studies on newborn foreskin (Cooper & Schiff 1938, Broders & Dublin 1939) and adult skin (Scheving 1959, Fisher 1968b) have suggested that MI peaks should occur during the dark period — before and around midnight and in the early hours of the day. Other MI studies have indicated higher MI's at 3 p.m. than at 9 a.m., but the findings have been equivocal (Rowe & Dixon 1972, Rowe, Dixon & Forsythe 1978, Gelfant 1982, Gelfant et al. 1982, Camplejohn et al. 1984). Most LI studies have failed to show any circadian variation (Hell & Hodgson 1966, Pullmann, Lennartz & Steigleder 1974, Camplejohn et al. 1984), but a slight trend towards an increase at midnight was seen in one study (Kahn, Weinstein & Frost 1968). Recently Schell et al. (1981a) performed longitudinal in vitro LI studies; their results indicated some circadian variation, but the rhythmicity did not appear until 12 hours after the initiation of the study. Peaks occurred at 6 a.m. and troughs at 6 p.m., and the level of the LI was inversely correlated to the serum cortisol level.

The duration of the DNA synthesis phase was by the same group reported to be almost constant during the day (Schell et al. 1981 b), in contrast to the findings in animal studies (Clausen et al. 1979, Møller et al., 1982).

Recently Gelfant (1982) questioned the theory on the existence of a consistent circadian variation in normal epidermis in vivo emphasizing the lack of circadially consistent correlation between MI and LI. He proposed that the control mechanisms for growth in human epidermis might differ from those in mice.

Pronounced circadian variations in epidermal cell kinetic parameters are well documented in both hamsters and mice (Møller, Larsen & Faber 1974, Møller, Hartmann & Faber 1979, Clausen et al. 1979). It has been debated whether these circadian variations are primarily due to the time of the day, e.g. the presence or absence of daylight, or to behaviour, e.g. food ingestion, physical activity.

The general inconsistency in the results in the studies on circadian variation in

human epidermal cell proliferation might very well be due to differences in a variety of non or hardly standardizable sources for disturbances, i.e. psychological factors, sampling procedure, perturbations, inborn or environmental conditions. Neither can methodological artefacts be ruled out.

Local injury to epidermis is well documented to disturb the epidermal cell proliferation in both man and animal (III, Pinkus 1952, Hell & Cruickshank 1963, Sullivan & Epstein 1965, Braun-Falco et al. 1967, Clausen & Lindmo 1976, Wiley & Weinstein 1979, Steigleder & Pullman 1979, Grove 1980).

Epidermal tumours

Uninvolved epidermis

Human skin carcinogenesis depends on both inborn features, e.g. skin type, and systemical and external exposures during life, e.g. arsenic preparations, X-ray and Grenz ray, tar, UV-light and chronic irritation (Everall & Dowd 1978, Pollock et al. 1982). Thus also the uninvolved skin of epidermal cancer patients might differ by proliverative characteristics from skin of normal individuals.

In the present studies a difference was observed in the proliferative characteristics of epidermis not exposed to external carcinogens between individuals developing both multiple SCC's and BCC's and those developing BCC's only (VII). This may be an expression of a cell kinetic difference between these groups paralleling the clinical observation that the tumour spectrum may be individually determined without any consistent regard to the carcinogenic exposure. For lymphocytes from a similar group of patients the UV induced DNA synthesis has recently been observed to be higher in patients with both BCC's and squamous cell differentiated tumours than in patients with BCC's only (Munch-Petersen et al. 1985).

In the present studies on multiple epidermal cancer (VII) the S- og G_2M-fractions of the epidermis of actinically damaged individuals did not exceed those of patients orally exposed to arsenic preparations; this leaves the findings in the labelling index studies by Gregg & Mansbridge (1982) on a few individuals unconfirmed.

It has been concluded from previous studies that the skin perilesional to epidermal tumours shows an increase in the proliferative parameters compared to skin from unexposed body sites (Kint 1963, Pearse & Marks 1977). This is in basic accordance with the present findings for the skin at risk in individuals with multiple epidermal tumours (VII). However, the interference of the well

established regional changes in epidermal cell proliferation has to be taken into account in the interpretation of these results. In multiple epidermal cancer the perilesional skin and the skin exposed to external carcinogens can be regarded as being primed for malignant transformation. In the present studies remote exposure to ionizing radiation only (therapeutical doses of Grenz and X-rays) was related to a marginal change in the G_2M-fraction (VII).

The proliferative characteristics of tumours

The basic significance for the tumour growth rate of the proliferative characteristics S- and G_2M-fraction is as yet uncertain. In the present studies the variability in the proliferative characteristics within and between the different histological tumour groups was high and the values partly overlapped those of normal epidermis, a feature which was also observed in MI and LI studies and studies on other tumours by FCM (Frindel, Malaise & Tubiana 1968, Weinstein & Frost 1970, Friedland & Weinstein 1972, Sealy, Immerman & Shepstone 1972, Heenen, Achten & Galand 1973, Lærum & Farsund 1981, Grässel-Pietrusky & Hornstein 1982). For a variety of different human solid tumours in 261 patients Barlogie et al. (1982) reported that the proliferative FCM parameters were related to the histological diagnosis, while in the present studies the histological classification appeared unimportant for these parameters. No intratumour heterogeneity was found in our studies along the epidermal tumour periphery. The centre and periphery, however, might be different (VI). Neither in the study of Barlogie et al. (1982) nor in the present study could the proliferative activity be related to age or sex.

The clonal DNA content

Flow cytometric evidence of intratumour pluricloniality in human tumours appears to be uncommon as judged from the present study on epidermal tumours and the studies of Barlogie et al. (1978, 1980, 1982) and Frankfurt et al. (1984). However, Søndergaard et al. found at least 8 out of 35 malignant melanomas and Taylor et al. (1983) 16% of 114 breast tumours to be pluriclonal. Mosaic composition of colo-rectal tumours was reported by Petersen, Bichel & Lorentzen (1979). Frankfurt et al. (1984) noted that mechanical disaggregation gave more accurate determination of ploidy and clonality by FCM than did enzymatic treatment. Thus both the preparation procedure, the power of resolution of the flow cytometer and the tumour type are important for the flow cytometric recognition of additional clones in a given tumour.

A discrepancy between the frequency of aneuploidy for BCC's and SCC's —

23% against 80% respectively — was observed by Schumann et al. (1979) in perfect agreement with the present studies. Also Ehlers (1968) observed trends for heteroploidy in some BCC's. On the other hand, by photometric in vivo and in vitro studies, Tilgen et al. (1980) found aneuploidy in SCC's and BO's but not in BCC's, and Manocha (1969) reported BCC's to be almost consistently diploid. Most BCC's in our studies were near diploid and artefacts can not be completely disregarded neither in the FCM nor in the MPM studies. Using homologous epidermis as internal standard we have, in very recent studies, seen quite unquestionable aneuploidy in BCC's (Frentz & Møller, unpublished). Abnormal clonal DNA content is often by itself used as a safe criterion of malignancy. From our studies on premalignant and malignant epidermal tumours and the studies of Søndergaard et al. on melanocytic tumours (1983) it is evident that heteroploidy does not equal ouvert malignancy. A pronounced heteroploidy was seen in the majority of the clinically and histologically non-aggressive epidermal precanceroses (V). Similar observations were made in MPM studies (Manocha, Steele & Stich 1969, Ehlers & Stephan 1972). Also Barlogie et al. (1980) reported aneuploidy in premalignant conditions by FCM. For certain cells, e.g. hepatocytes, polyploidy is a normal feature (Lærum & Farsund 1981).

For a variety of malignant tumours in other organ systems FCM generally failed to demonstrate an unequivocal correlation between ploidy and prognosis (Jakobsen, Bichel & Sell 1979, Dixon & Carter 1980, Thommesen et al. 1980, for review see Lærum & Farsund 1981). Frankfurt et al. (1984) reported a correlation between the degree of aneuploidy and disease stage or degree of differentiation depending on the disease category studied. According to Barlogie et al. (1983) a high DNA-index in itself might signify a poor prognosis. The present studies did not support the suggestion that increased clinical malignancy should be reflected in a high S-fraction or a high DNA-index, but a trend for a positive correlation between these latter parameters appeared. Barlogie et al. (1982, 1983) reported the size of the S-fraction and the DNA-index to be significantly correlated, the higher the values for each of these parameters the worse the prognosis, and a similar interaction between S-fraction and ploidy was observed for breast tumours by Taylor et al. (1983).

CONCLUSIONS

DNA analyses by FCM according to the method used in the present studies yield virtually reproducible values of the proliferative characteristics of normal human epidermis and human epidermal tumours and of the clonal DNA content in these tissues. Most stable are the S-fraction and DNA-index determinations. The method is rapid and safe for the individual investigated compared to the conventional cell kinetic techniques, but in the present studies the practical use was narrowed by the size of the biopsy. The findings in normal skin show that the human epidermal proliferative characteristics depend on the body site. For the age group and the number of individuals here investigated no consistent alteration by age and sex is evident. Disturbances in the cell proliferation from previous biopsy sampling are strongly suggested to complicate consecutive studies on the changes in the cell proliferation in the single individual, e.g. when investigating the natural course of skin diseases or the responses to therapy. Also the time of the day has to be taken into account.

The uninvolved skin of individuals with multiple epidermal cancer showed no immediate aberrations in the proliferative characteristics, when epidermis not exposed to external carcinogens in the past was considered. For both exposed and unexposed skin, however, the subgroup of patients who had developed SCC as well as BCC differed from those developing BCC only by both higher S- and G_2M-fractions. The exposed skin differed from the unexposed by having a higher S-fraction and from the neoplastic epidermal tissue by having a lower G_2M-fraction. Neither carcinogenic exposures, i.e. previous arsenic ingestion, UV light/heavy sun exposure and ionizing radiation nor preexisting conditions, i.e. psoriasis and intolerance to sun light statistically significant influence on the proliferative characteristics in patients with multiple epidermal cancer.

The epidermal cancers and precancers showed a wide variability in the proliferative characteristics sometimes with overlapping to normal epidermis. The histological classification was without significant importance for the level of these parameters. For the individual tumour periphery, the size of the S- and G_2M-fractions proved characteristic as did also the clonal DNA content indicating that the biopsy from the periphery in general is representative of the entire tumour periphery. The tumour centre might deviate considerably from the periphery. In the individual tumour only exceptionally more than one cell clone could be demonstrated. The empirically non aggressive BCC's differed both from the empirically more aggressive SCC's and from the total group of squamous cell differentiated tumours by a lower frequency and degree of heteroploidy. Concurrently, the ouvert malignant SCC's did not differ basically from the precancerous squamous cell differentiated tumours by ploidy, indicating that heteroploidy is not in itself a marker of manifest malignancy.

AIMS FOR FURTHER STUDIES

1) For optimal exploitation of the potentials of DNA FCM for human epidermis the technique should be further elaborated in order to obtain an increase in the cell yield without using ingredients destroying the mitoses (Larsen et al. 1985).

2) In order to understand the basic processes in the cell kinetic events accompanying the changes in the proliferative characteristics multiparameter FCM studies are required (Darzynkiewicz et al. 1976), because in humans it is impossible to imitate the successful experimental set-up used in animal studies combining several conventional techniques and FCM.

3) For future sequential investigations on the natural course of skin diseases and the influence of therapeutic modalities more extensive studies are required, taking the influence of the time of the day and previous biopsy sampling into account.

4) For evaluation of the potential predictive value of the proliferative characteristics in uninvolved skin with respect to the development of psoriasis or epidermal malignancies or for evaluation of the influence of the skin carcinogens more extensive and prospective studies are necessary.

5) Cell clones with abnormal DNA content are frequently seen in premalignant squamous cell differentiated tumours and this calls for further studies, on the mechanisms involved in skin carcinogenesis e.g. chromosomal aberrations, supplementary to the FCM DNA analysis (Boukamp et al. 1982).

DANSK RESUMÉ

Flow cytometri (FCM) er en hurtig metode til kvantitering af forskellige parametre på cellulært og subcellulært niveau. Metoden har gennemgået en rivende udvikling fra slutningen af 60'erne og et hovedområde for dets anvendelse i cellebiologi er kvantitering af DNA i de enkelte celler med fastlæggelse af 1) en cellepopulations fordeling i de forskellige faser af cellecyklus og 2) den DNA mængde, der dominerer i en cellepopulation relateret til normal-celle DNA (DNA-indeksbestemmelse, ploidistudier). Metoden kan også anvendes til identifikation af cellekloner med forskelligt DNA indhold. Metodens store fordele til DNA analyser er dens hurtighed, der tillader målinger af op til flere tusinde celler pr. sekund, og det for humane studier vigtige faktum, at celleproliferationen kan bedømmes uden anvendelse af radioaktive isotoper.

De foreliggende undersøgelser omfatter anvendelse af DNA FCM på normal og præmalignt/malignt forandret human epidermis med en tilpasset, oprindeligt dyreeksperimentelt udviklet metode. Reproducerbarheden af resultaterne er statistisk og eksperimentelt vurderet. S-fraktion og DNA-indeksbestemmelse reproduceredes bedst. S-fraktion fra normal hud var gennemsnitlig 2,6 ± 0,8 (SD) (% af total antal epidermale celler), G_2M-fraktionen 2,1 ± 1,1, hvilket er rimelige værdier sammenlignet med andre undersøgelser. Der fandtes sikre topografiske forskelle i S-fraktionen, medens regulære alders- og kønsforskelle ikke kunne påvises. I G_2M-fraktionen sås en vis døgnvariation, medens S-fraktionen responderede på forudgående biopsitagning.

Epidermale tumorer fandtes vidtgående intrinsik homogene i **randen** med hensyn til cellekloner, ploidi og fordeling af cellerne i cellecyklus, medens **centrum** kunne afvige betydeligt. Basalcellekarcinomer (BCC) fandtes væsentlig sjældnere aneuploide end spinocellulære (SCC) (24% mod 75%). Bemærkelsesværdigt fandtes ofte aneuploidi i de ikke manifest maligne aktiniske keratoser og Mb Bowen. Ved undersøgelser af uafficeret hud hos patienter med multiple epidermale maligne/præmaligne tumorer fandtes, at hud ikke eksponeret for eksterne karcinogener adskilte sig ved en lavere S-fraktion fra eksponeret eller tumornær hud, der igen adskilte sig fra tumor ved en lavere G_2M-fraktion. Ved nærmere statistisk analyse fandtes significant lavere S- og G_2M-fraktion i uafficeret hud fra patienter, der kun udviklede multiple BCC sammenlignet med patienter, der også udviklede SCC. Ved fokusering på karcinogener — arsenik, lys og røntgenbestråling — og andre faktorer, der kunne tænkes at være af betydning for karcinogenesen, som psoriasis og anamnestisk lysfølsomhed, fandtes ingen af disse enkeltfaktorer at have sikker betydning for måleresultaterne i hverken eksponeret eller ueksponeret hud.

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