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Review

Significance of Cell Proliferation Measurement in Gastric Cancer

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Cell kinetic data may be important indicators of clinical behaviour in many types of cancer. Recently, several antibodies to cell-cycle associated antigens have been characterised. This overview summarises the advantages and disadvantages of different methods for the assessment of cell proliferation. Moreover, the prognostic value of proliferative activity in gastric cancer is discussed and suggestions for future research are given. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

THE BASIC concept of the cell cycle is demonstrated in Figure 1. In a replicating eukaryotic cell the life cycle can be divided into four main phases, and the transition from one phase to another in the cell cycle is probably tightly controlled. DNA synthesis and doubling of the genome take place during the synthetic or S-phase. This is preceded by a period of variable duration known as the first gap (G_1) which separates the S-phase from the previous mitosis (M-phase). The S-phase is followed by a period of apparent inactivity known as the second gap (G_2) which comes before the next mitosis. Another phase can be discerned, being the G_0 or resting phase, in which cells are not in the cell cycle but, after suitable stimuli, may rejoin the cycling population [1].

In normal tissue, and probably also to some extent in tumours, there is a balance between cell birth and cell loss. Differentiation and apoptosis lead to cell death and are important for the balance between cell growth and cell death. In any tissue there are also cells that, for whatever reason, are no longer able to divide and so any cell population can be divided into a cycling and a non-cycling compartment. This leads to the definition of the proliferative fraction of any cellular population as the ratio of cycling to cycling plus non-cycling cells (cycling cells/total cells) [2].

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METHODS FOR ASSESSING CELL PROLIFERATION

There is a correlation between the proliferation rates (the ratio of cycling cells/total cells in the population) of certain tumours and their biological behaviour. Various methods are widely used to measure the percentage of proliferating cells (Table 1). In gastric cancer, as well as in other cancers, measurements of proliferative activity have been performed using different methods. However, each method has some practical problems for routine pathology. The most important methods for determination of proliferative activity are described here.

Mitotic count

The mitotic count is perhaps the most convenient and, therefore, the most widely used method for assessment of cellular proliferation. It is defined as the number of mitoses per 10 high-power fields (HPFs). It is important to distinguish this from the mitotic index, which is the fraction of mitoses expressed as a percentage. The mitotic count has long been employed by histopathologists as a diagnostic aid and a prognostic indicator in the study of tumour pathology. In spite of this, the validity of the mitotic count as a marker of tumour proliferative activity remains controversial. It is not a standardised method [3, 4]; it takes no account of cell size [5, 6] and the area of a single HPF may vary up to 6-fold between different microscopes [7]. The mitotic count is also subject to interobserver variation [8].

In spite of these obvious limitations it is widely used in clinical practice, and it can give a first impression of proliferative activity.

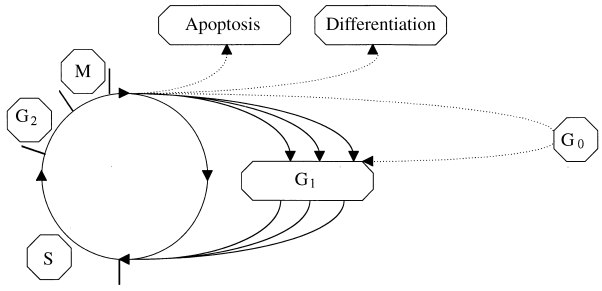

Figure 1. The concept of the cell cycle.

Thymidine labelling

The first wave of cell kinetic investigations in man started after the introduction of techniques using the incorporation of tritiated thymidine into cellular DNA. Only cells that are actively synthesising DNA (S-phase cells) will incorporate tritiated thymidine during a short exposure (pulse-labelling). These cells can be detected using autoradiography. Therefore, pulse-labelling with tritiated thymidine is regarded as a functional marker of proliferative activity, reflecting the fraction of S-phase cells. Determination of thymidine labelling requires the *in vivo* administration of radiolabelled thymidine which can rarely be justified in clinical practice. Alternatively, tissue must be incubated with tritiated thymidine for some time *in vitro* before fixation. Both of these restraints militate against thymidine labelling as a practical routine procedure for histopathologists. The use of the thymidine labelling index as an indicator of tumour proliferative activity has several other limitations. Firstly, it documents the number of cells in the S-phase, but does not measure the duration of the S-phase. It is thus possible for a tumour to have a slow rate of cell proliferation and a high thymidine labelling index [9]. Secondly, tumours may display heterogeneous growth patterns and it is argued that a method which relies upon only a sample of the tumour can be, at best, only a crude index of the proliferative capacity of that tumour [10]. Thirdly, as with any technique which involves cell counting, human interobserver variation, reproducibility error and sample size may

Table 1. *Methods for detecting proliferating cells*

Mitotic count	
Material	Fixed
Phase	M-phase
Advantage	Simple
Disadvantage	Identification of mitosis is difficult; method is not standardised; interobserver variation
Thymidine labelling	
Material	Fresh
Phase	S-phase
Advantage	None
Disadvantage	*In vivo* administration of radiolabelled thymidine (or alternatively specimens must be incubated with thymidine *in vitro* before fixation); time-consuming
Flow cytometry	
Material	Fresh or fixed
Phase	S, G_2 and M-phase
Advantage	Large number of cells can be analysed
Disadvantage	Expensive equipment; need to disaggregate tissues into a suspension of single cells
Bromodeoxyuridine or iododeoxyuridine labelling	
Material	Fresh or fixed
Phase	S-phase
Advantage	Information about cell kinetics
Disadvantage	*In vivo* administration of bromodeoxyuridine or iododeoxyuridine (or alternatively specimens must be incubated with bromodeoxyuridine or iododeoxyuridine *in vitro* before fixation)
PCNA	
Material	Fixed
Phase	G_1, G_2, S and M-phase
Advantage	Simple
Disadvantage	Expression is dependent of kind and duration of fixation
Ki-67	
Material	Fresh
Phase	G_1, G_2, S and M-phase
Advantage	Simple
Disadvantage	Need for fresh or snap-frozen material
MIB-1	
Material	Fixed
Phase	G_1, G_2, S and M-phase
Advantage	Simple
Disadvantage	More extensive research is needed to evaluate the role of this relatively new antibody
AgNOR	
Material	Fixed
Phase	Unknown
Advantage	Simple
Disadvantage	AgNOR counts are influenced by other factors such as variations in ploidy

produce misleading results. Finally, measurement of the thymidine labelling index requires fresh tissue, is time-consuming, necessitates autoradiography and is not a readily available technique for routine laboratory services.

Nevertheless, for many years this was the only reliable method available for assessing cell proliferation and the data based on this method and reported in many studies have been pivotal for the development of our understanding of tumour growth [11].

DNA flow cytometry

Flow cytometry is an automated technique which quantifies cellular DNA content and analyses cell cycle distribution [12]. A cell suspension, prepared from a tissue sample, is stained with DNA-specific fluorochrome dyes. The suspension then flows through a light source with stationary fluorescence detectors, at a rate of approximately 5000 cells/sec. The light scattered by the cells is registered by the detectors and converted into electronic signals which are stored and analysed by computer. The computer produces a DNA histogram from which a number of data can be derived. The cellular DNA content, known as its ploidy status, is expressed as the DNA index. This is the ratio of the G_0/G_1 cells of the test population to that of an internal or external standard diploid cell population. A diploid cell has a DNA index of 1.00. Cells with an index of 2.00 are tetraploid and greater than 2.00 are aneuploid or polyploid. The S-phase content and the proliferative activity ($S+G_2M$ phases) can also be determined by deconvoluting the DNA histogram. The technique can be applied to paraffin-embedded material as well as to fresh tissue [13].

Flow cytometry has the advantage of speed and statistical precision. Typically 10 000–100 000 cells or nuclei can be scanned in a few minutes or less. Multiple parameters can be measured simultaneously on individual cells which can be a useful attribute of the technique. An important disadvantage of flow cytometry is that very expensive apparatus is required. In addition, when studying solid tissues, the need to disaggregate the tissue into a suspension of single cells or nuclei can be a problem. Some solid tissues are difficult to disaggregate and in all cases tissue morphology is lost.

Iododeoxyuridine or bromodeoxyuridine labelling

Measurement of a kinetic cell profile (including data on the duration of cell cycle phases) has become feasible through the introduction of techniques using *in vivo* incorporation of iododeoxyuridine (IdU) or bromodeoxyurine (BrdU). IdU and BrdU are pyrimidine analogues which are incorporated by DNA-synthesising nuclei. IdU- or BrdU-containing cells are detected with monoclonal antibodies [14–16]. After staining the total cell population with propidium iodide (which stoichiometrically intercalates into DNA), the proportion of BrdU/IdU-labelled cells and their total DNA content can be simultaneously measured by flow cytometry [17]. From these measurements, both the labelling index and the DNA synthesis time can be determined on a single tumour sample. Other cell kinetic parameters, such as the potential tumour doubling time, can then be mathematically derived.

Flow cytometric analysis of IdU or BrdU incorporation provides cell kinetic information on human tumours, which cannot readily be obtained by other methods. However, it provides 'averaged' values and sacrifices information on the tissue spatial distribution of proliferation as a consequence of the prerequisite of a single cell or nuclei suspension. Nevertheless, because IdU or BrdU are administered *in vivo*, the application of immunoperoxidase techniques can be applied to study proliferation at the microscopic level. Immunohistochemical determination of IdU or BrdU provides information on heterogeneity and structural organisation of proliferation.

Proliferating cell nuclear antigen

Proliferating cell nuclear antigen (PCNA) is a 36 kDa nuclear protein which functions as an auxiliary protein for DNA polymerase δ and is an absolute requirement for DNA synthesis [18–23]. It is a stable cell-cycle regulated nuclear protein that is expressed differentially during the cell cycle and whose rate of synthesis is correlated directly with the proliferative rate of cells. During the cell cycle, two populations of cells with differing PCNA can be distinguished. Using immunofluorescent methods, Bravo and MacDonald-Bravo [24] showed that one population is nucleoplasmic, corresponding to PCNA present at low levels, as seen in quiescent cells capable of cell division. The second group has PCNA associated with specific nuclear structures, tightly associated to sites of DNA replication, and is thought to play a fundamental role in eukaryotic DNA synthesis. Expression of this form is closely related to the cell cycle; the levels of PCNA increase in the nucleus during the late G_1-phase, immediately before the onset of DNA synthesis, become maximal during the S-phase, decline during the G_2-phase and reach a low level in the M-phase and quiescent cells [23]. A wide range of monoclonal antibodies to PCNA are now available, including PC10, 19A2 and 19F4. The epitopes recognised by these antibodies are different [25] and there are differences in the effects of fixation and processing on the detectability of the epitopes [26], indicating that the effect of technical factors on PCNA staining should not be underestimated. Furthermore, since PCNA is a necessary but not sufficient requirement of DNA synthesis, it may be expressed by cells that are not cycling. So, the use of PCNA antibodies is not simple and straightforward but requires careful analysis and consideration of all these caveats.

Ki-67

Ki-67 is a mouse monoclonal antibody that identifies a nuclear antigen associated with the cell cycle [27]. Detailed cell cycle analysis has demonstrated that the Ki-67 antigen is expressed in all phases except G_0 and early G_1 [28]. Thus, Ki-67 immunostaining provides a measure of the tumour proliferative fraction.

The epitope recognised by Ki-67 does not survive formalin fixation and paraffin embedding. So, the requirement of fresh tissue for cryostat sections is a major obstacle to the routine use of Ki-67.

MIB-1

MIB-1 is a relatively new, but promising, antibody for the determination of the proliferative fraction of tumour cell populations. It was developed using a recombinant partial structure of the Ki-67 protein as immunogen. MIB-1 exhibits an identical immunostaining pattern to that of Ki-67 in fresh material and, furthermore, reacts with the native Ki-67 protein as well as with recombinant parts of the Ki-67 antigen [29]. MIB-1 can detect the Ki-67 protein in routinely formalin-fixed paraffin-embedded material when using an antigen retrieval method, based on microwave treatment [30, 31].

The use of MIB-1 for the determination of cell proliferation has several obvious advantages. First, the combination of strong immunoreactivity with an optimal preserved morphology allows good recognition of cellular details and, therefore, a better identification of positive cellular subsets. Second, the neat and clear immunoreaction products allow a clear-cut distinction between positive and negative cells, even if only minute amounts of antigen are stained. A third advantage of this method is that it can be applied in virtually every histopathological laboratory all over the world, since no sophisticated technical skill is needed.

Nucleolar organiser regions

Nucleolar organiser regions (NORs) are DNA segments encoding for ribosomal RNA [32]. They can be visualised because each NOR is associated with argyrophilic proteins; the silver-stained structures thus demonstrated are called AgNORs. Several studies show that there is a correlation between AgNOR counts and other cell proliferation indices. However, AgNOR counts may not be a perfectly reliable paradigm for other measures of proliferative activity because the counts are influenced by other factors, such as variations in ploidy and transcriptional activity.

COMPARISON OF DIFFERENT METHODS FOR MEASUREMENT OF CELL PROLIFERATION

Several studies have compared different methods for the determination of proliferation: a summary is given in Table 2.

In gastric cancer, a significant correlation has been found between the *in vitro* BrdU labelling index (LI) and the PCNA LI [38–40], between the *in vivo* BrdU LI and DNA ploidy [34], whereas no significant correlation was found between the PCNA LI and S+G_2M-phase fraction measured by flow cytometric analysis [47]. Lynch and associates [41] described a significant correlation between the *in vitro* BrdU LI and the MIB-1 LI in normal gastric tissue, but not between the *in vitro* BrdU LI and the PCNA LI.

Although many proliferation markers have been compared, both in gastric carcinomas and in other tumours, results from comparative studies are often contradictory. However, it must be borne in mind that these studies are not completely comparable, because of differences in the fixation method, fixation time or the use of different antibodies.

CELL PROLIFERATION IN GASTRIC TISSUE

Cell proliferation in normal gastric mucosa

In normal gastric mucosa the proliferative zone is located in the neck of the gastric gland [56]. From this region newborn cells migrate towards the surface and, to a lesser extent, towards the bottom of the gland. The downward migration seems to be more complex than that towards the surface [57]. During their migration, the cells lose their proliferative activity and become mature elements: mucous cells if they go upwards or parietal cells if they go downwards. Peptic zymogen cells seem to have an autonomous replication cycle [58].

Cell proliferation abnormalities in premalignant gastric conditions

In animal studies, it has been shown that chemical carcinogens stimulate cell renewal [59]. In man, certain conditions at risk for gastric cancer show an increased cell proliferation rate. In chronic atrophic gastritis, the number of proliferating cells is increased as compared to controls [60–63]. Higher proliferation rates have also been observed in patients with intestinal metaplasia [62, 63], *Helicobacter pylori* associated gastritis [62, 64, 65], gastric dysplasia [66] and in patients with gastric remnant resections [65].

CELL PROLIFERATION AS A PROGNOSTIC MARKER IN GASTRIC CANCER

Information on cell proliferation may be a useful adjunct to histologically based tumour classifications in the understanding of tumour behaviour. In a variety of malignant neoplasms, significant correlations have been found between proliferative activity and metastatic potential, recurrence or overall prognosis [22]. In particular, during the last few years, several studies consistently investigated the prognostic relevance of cell proliferative activity in gastric cancer. The results of these studies are summarised in Table 3. In evaluating these studies one should keep in mind that differences in numbers of patients included, presentation of the results, and most importantly, the way in which the patients were treated, make direct comparisons of these studies difficult.

Studies evaluating the prognostic significance of thymidine labelling in gastric cancer have shown contradictory results. Amadori and associates [67] determined the *in vitro* thymidine LI in endoscopic biopsies of 28 patients with gastric cancer. In this small study, the 3-year survival was significantly higher in patients with slowly proliferating tumours. However, in a second study [68], the same authors studied the prognostic significance of *c-myc*, *c-K-ras*, *hst*, *c-erb B-2* gene amplification and of the thymidine LI in gastric cancer. The oncogenes were determined in 124 tumour samples from patients who had received radical or palliative surgery for gastric cancer, whereas the thymidine LI was determined in 70 tumour specimens. They found that amplification of the examined oncogenes and determination of the thymidine LI did not reveal a new independent prognostic factor. In a study by Tanigawa and colleagues [69], the prognostic value of tritiated thymidine uptake in 173 patients with gastric cancer was evaluated. In this prospective study, the thymidine uptake was found to be an independent prognostic parameter.

The prognostic value of DNA flow cytometry in gastric cancer has been determined in seven studies. In three retrospective studies, flow cytometric analyses of DNA ploidy patterns were performed in deparaffinised formalin-fixed gastric cancer tissue. Tosi and associates [70] examined 133 samples and, in accordance with the results of Danova and colleagues [71] (42 patients), an aneuploid DNA pattern was associated with poorer prognosis. In contrast, Ballantyne and associates [72] failed to find any significant correlation between DNA ploidy, tumour type or survival after an investigation of 77 samples.

In a prospective study, Sasaki and associates [73] investigated the relationship of DNA ploidy (determined in fresh material) to clinical, pathological and prognostic data. In their study of 70 patients, patients with diploid tumours tended to do better than those with aneuploid tumours. However, the difference was not statistically significant. The prognostic value of DNA ploidy pattern and proliferative activity after *in vivo* administration of bromodeoxyuridine was examined in large groups of patients by Ohyama and associates [74] and Yonemura and colleagues [75], showing that patients with aneuploid tumours, or with tumours which showed greater proliferative activity, had a poor prognosis. In a second study, Ohyama and associates [76] examined the

prognostic values of the BrdU LI and DNA index and found that the ratio of the BrdU LI to DNA index was the most accurate reflection of the proliferation rate. Again, patients with a high proliferation rate had a worse prognosis.

In conclusion, five out of the seven studies found a significant correlation between DNA ploidy and survival, indicating that this method provides prognostic information for gastric cancer. Yonemura and colleagues [77] also studied the prognostic significance of proliferative activity, as measured by immunohistochemistry after *in vitro* BrdU labelling. In this study the BrdU LI was an independent prognostic parameter.

Table 2. Comparison between different proliferation markers

Comparison	Tumour/tissue	Significant correlation	[Ref.]
In vivo BrdU versus			
Thymidine	Colorectal tumours	Yes	[33]
DNA ploidy	Gastric cancer	Yes	[34]
Ki-67	Cerebral gliomas	Yes	[35]
	Cerebral gliomas	Yes	[36]
	Brain tumours	Yes	[37]
MIB-1	Cerebral gliomas	Yes	[35]
	Cerebral gliomas	Yes	[36]
AgNOR	Cerebral gliomas	No	[36]
	Brain tumours	Yes	[37]
In vitro BrdU versus			
PCNA	Gastric cancer	Yes	[38]
	Gastric cancer	Yes	[39]
	Gastric cancer	Yes	[40]
	Gastric mucosa	No	[41]
	Brain tumours	Yes	[42]
	Bladder carcinoma	Yes	[43]
Ki-67	Brain tumours	Yes	[42]
MIB-1	Gastric mucosa	Yes	[41]
	Brain tumours	Yes	[42]
PCNA versus			
S-phase fraction (flow cytometry)	Breast carcinoma	No	[44]
	Astrocytoma	No	[45]
	Non-Hodgkin's lymphoma	Yes	[46]
$S+G_2M$ phase fraction (flow cytometry)	Gastric cancer	No	[47]
DNA ploidy (flow cytometry)	Lung cancer	No	[48]
Ki-67	Brain tumours	Yes	[42]
	Non-Hodgkin's lymphoma	Yes	[22]
	Breast carcinoma	No	[44]
	Breast carcinoma	No	[49]
	Prostatic carcinoma	Yes	[50]
MIB-1	Brain tumours	Yes	[42]
	Astrocytoma	No	[45]
	Non-Hodgkin's lymphoma	Yes	[46]
	Breast carcinoma	Yes	[44]
	Prostatic carcinoma	Yes	[50]
	Testis carcinoma	Yes	[51]
	Normal bone marrow	Yes	[52]
AgNOR	Gastric cancer	Yes	[53]
Ki-67 versus			
S-phase fraction (flow cytometry)	Non-Hodgkin's lymphoma	Yes	[46]
	Breast carcinoma	Yes	[44]
MIB-1	Brain tumours	Yes	[42]
	Cerebral gliomas	Yes	[35]
	Cerebral gliomas	Yes	[37]
	Non-Hodgkin's lymphoma	Yes	[46]
	Breast carcinoma	No	[44]
	Breast carcinoma	Yes	[54]
	Prostatic carcinoma	Yes	[50]
AgNOR	Brain tumours	Yes	[37]
MIB-1 versus			
S-phase fraction (flow cytometry)	Astrocytoma	No	[45]
	Non-Hodgkin's lymphoma	Yes	[46]
	Breast carcinoma	Yes	[54]
	Breast carcinoma	Yes	[44]
$S+G_2M$ phase fraction (flow cytometry)	Astrocytoma	No	[45]
	Breast carcinoma	Yes	[55]

Table 3. *Correlation between patient's survival and cell proliferation rate in gastric cancer*

Thymidine labelling	
Amadori and colleagues [67]	
Aim of the study	To investigate the prognostic value of the tritiated thymidine LI
No. of evaluable patients	28
Type of study	Prospective
Follow-up time	1–59 months (median 34 months)
Results	3-year survival significantly higher in patients with slowly proliferating tumours
Amadori and colleagues [68]	
Aim of the study	To investigate the prognostic value of *c-myc*, *c-K-ras*, *hst*, *c-erb B*-2 gene amplification and of the thymidine LI in gastric cancer
No. of evaluable patients	124 for gene amplification; 70 for thymidine labelling
Type of study	Prospective
Follow-up time	1–100 months (median 61 months)
Results	No correlation between the thymidine LI and survival
Tanigawa and colleagues [69]	
Aim of the study	To investigate the prognostic value of the tritiated thymidine LI
No. of evaluable patients	173
Type of study	Prospective
Follow-up time	Not available
Results	Thymidine LI was an independent prognostic variable
DNA flow cytometry	
Tosi and colleagues [70]	
Aim of the study	To correlate DNA ploidy pattern and behaviour of the tumour
No. of evaluable patients	133
Type of study	Retrospective
Follow-up time	6 years
Results	Significant correlation between DNA ploidy and survival
Danova and colleagues [71]	
Aim of the study	To correlate DNA ploidy pattern and S-phase fraction with behaviour of the tumour
No. of evaluable patients	42
Type of study	Retrospective
Follow-up time	1 year
Results	Significant correlation between DNA ploidy and survival; no significant correlation between S-phase fraction and survival
Ballantyne and colleagues [72]	
Aim of the study	To correlate DNA ploidy pattern and behaviour of the tumour
No. of evaluable patients	77
Type of study	Retrospective
Follow-up time	3 years
Results	No significant correlation between DNA ploidy and survival
Sasaki and colleagues [73]	
Aim of the study	To correlate DNA ploidy pattern and behaviour of the tumour
No. of evaluable patients	70
Type of study	Prospective
Follow-up time	4 years
Results	No significant correlation between DNA ploidy and survival
Ohyama and colleagues [74]	
Aim of the study	To investigate the prognostic value of DNA ploidy pattern and BrdU LI
No. of evaluable patients	117
Type of study	Prospective
Follow-up time	Not available
Results	DNA ploidy and BrdU LI were independent prognostic parameters
Yonemura and colleagues [75]	
Aim of the study	To correlate DNA ploidy pattern and BrdU LI with behaviour of the tumour
No. of evaluable patients	493
Type of study	Retrospective
Follow-up time	Not available
Results	Significant correlation between DNA ploidy and survival
Ohyama and colleagues [76]	
Aim of the study	To correlate proliferation rate with behaviour of the tumour
No. of evaluable patients	172
Type of study	Prospective
Follow-up time	4 years
Results	Significant correlation between proliferative activity (BrdU LI/DNA index) and survival
Bromodeoxyuridine/iododeoxyuridine	
Ohyama and colleagues [74]	
Aim of the study	To investigate the prognostic value of DNA ploidy pattern and BrdU LI
No. of evaluable patients	117
Type of study	Prospective
Follow-up time	Not available
Results	DNA ploidy and BrdU LI were independent prognostic parameters

continued

Table 3. *contd.*

Yonemura and colleagues [75]	
Aim of the study	To correlate DNA ploidy pattern and BrdU LI with behaviour of the tumour
No. of evaluable patients	493
Type of study	Retrospective
Follow-up time	Not available
Results	Significant correlation between DNA ploidy and survival
Ohyama and colleagues [76]	
Aim of the study	To correlate proliferation rate with behaviour of the tumour
No. of evaluable patients	172
Type of study	Prospective
Follow-up time	4 years
Results	Significant correlation between proliferative activity (BrdU LI/DNA index) and survival
Yonemura and colleagues [77]	
Aim of the study	To study the prognostic significance of BrdU LI
No. of evaluable patients	98
Type of study	Prospective
Follow-up time	4 years
Results	BrdU LI was an independent prognostic factor
PCNA	
Yonemura and colleagues [38]	
Aim of the study	To correlate PCNA LI with behaviour of the tumour
No. of evaluable patients	121
Type of study	Retrospective
Follow-up time	5 years
Results	PCNA LI was an independent prognostic factor
Yonemura and colleagues [40]	
Aim of the study	To correlate PCNA LI with ploidy and with behaviour of the tumour
No. of evaluable patients	174
Type of study	Retrospective
Follow-up time	5 years
Results	PCNA LI and DNA ploidy were independent prognostic factors
Jain and colleagues [47]	
Aim of the study	To correlate PCNA LI with survival
No. of evaluable patients	91
Type of study	Retrospective
Follow-up time	68 (73%) of the patients had been followed up for a minimum of 5 years
Results	PCNA LI was not an independent prognostic factor; semiquantitative PCNA grading was of independent prognostic value
Kakeji and colleagues [53]	
Aim of the study	To correlate PCNA LI and AgNOR count with survival
No. of evaluable patients	181
Type of study	Retrospective
Follow-up time	Not available
Results	PCNA LI was an independent prognostic factor, but AgNOR count was not
Mori and colleagues [78]	
Aim of study	To correlate PCNA LI with survival
No. of evaluable patients	138
Type of study	Retrospective
Follow-up time	5 years
Results	PCNA LI was an independent prognostic factor
MIB-1	
Mueller and colleagues [79]	
Aim of the study	To correlate MIB-1 LI with survival
No. of evaluable patients	418
Type of study	Retrospective
Follow-up time	5 years
Results	No significant correlation between MIB-1 LI and survival
AgNOR	
Kakeji and colleagues [53]	
Aim of the study	To correlate PCNA LI and AgNOR count with survival
No. of evaluable patients	181
Type of study	Retrospective
Follow-up time	Not available
Results	PCNA LI was an independent prognostic factor, but AgNOR count was not

An analysis of proliferative activity using PCNA was performed in five studies. All used the PC10 antibody. In four studies [38, 40, 53, 78], the PCNA LI emerged as an independent prognostic factor, whereas a relatively small study by Jain and associates [47] failed to show a significant correlation between the PCNA LI and survival. However, unlike the PCNA LI, semiquantitative PCNA grading seemed to be a good predictor of prognosis with significant differences

between low and high PCNA grade groups. In the study of Kakeji and colleagues [53], proliferative activity was investigated using PCNA and AgNOR count. A significant correlation was found between the PCNA LI and AgNOR count. However, in a multivariate regression analysis, only the PCNA LI was an independent prognostic parameter. Therefore, the present data clearly suggest that the PCNA LI represents a useful prognostic factor in gastric cancer.

In just one study [79], the prognostic role of the MIB-1 LI in gastric cancer has been examined. No significant correlation could be demonstrated between the MIB-1 LI and survival.

The results of the above-mentioned studies assessing cellular proliferation in gastric cancer are sometimes contradictory, and a variety of methods have been used to measure the proliferative fraction. However, there is considerable evidence that assessment of proliferation provides useful information with respect to prognosis for gastric cancer; in general, high proliferation rates are correlated with a poor prognosis. Multivariate analyses should be performed to establish which method for determining proliferative activity is best.

CONCLUSIONS

There is considerable evidence that high cellular proliferation rates in a variety of tumours, including gastric cancer, are correlated with a poor prognosis. In the future, multivariate analyses should be performed to establish which method should preferably be applied in order to obtain accurate data on cell proliferation in gastric cancer.

In this review a variety of methods for the determination of cellular proliferation are described. Each of the described techniques has limitations, and currently it is not completely clear which is the best method for the assessment of proliferation. Ideally such a method should be simple, inexpensive and reproducible. For widespread use it is necessary that it is applicable to conventionally processed histological and cytological preparations. An important advantage of the use of MIB-1 for the determination of cell proliferation is that this method is very simple and can be applied on routinely processed tissue in virtually every histopathological laboratory all over the world. However, its role as a prognostic factor in gastric cancer is still unclear. More research is needed to evaluate the role of MIB-1 in gastric cancer and to unravel the interrelationships between MIB-1 and other methods of determining cellular proliferation.

Until now, little attention has been paid to the relationship between tumour proliferation and response to treatment. The hypothesis that assessment of cellular proliferation during and after treatment may give clinically useful information about response should be considered, especially in patients treated with cytostatic drugs. In this respect, it should be examined whether there are differences between fast and slow proliferating tumours, and whether cytostatic drugs can induce changes in the proliferative activity. Another main area of future research is to investigate the value of cellular proliferation in premalignant conditions.

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