Br. J. Surg. 1991, Vol. 78, September, 1080–1083

c-*myc* protein product is a marker of DNA synthesis but not of malignancy in human gastrointestinal tissues and tumours

D. A. Rew, I. Taylor, H. Cox*, J. V. Watson* and G. D. Wilson†

*University Surgical Unit, Southampton General Hospital, Tremona Road, Southampton SO1 6HU, *Medical Research Council Laboratories, Cambridge and †The Gray Laboratory, Northwood, UK*

Correspondence to:
Mr D. A. Rew

*c-*myc *is a conserved cellular gene. The gene product is a nuclear-bound 62 000 molecular weight phosphoprotein (p62c-*myc*). Although p62c-*myc *levels have been measured in colorectal cancers, little is known about the expression of the protein in upper gastrointestinal tumours and tissues. Studies were performed on tumour and mucosal specimens from 87 patients with colorectal cancer, from two with polyposis coli, from six with squamous oesophageal carcinomas and from 18 with gastric carcinomas. The mean p62c-*myc *content was measured in units of fluorescence in the G1 diploid and G2 diploid peaks of the cell cycle by multiparameter flow cytometry using the 6E10 antibody. The nuclear p62c-*myc *content increased with DNA synthesis in tumours and mucosa. G2 levels of p62c-*myc *were higher in glandular mucosa than in adenocarcinomas. No differences in peak nuclear c-*myc *expression were found in relation to histological grade or to anatomical site of colorectal tumours. There was a broadly inverse relationship between G2 p62c-*myc *levels in tumours and mucosa and their* in vivo *5-bromo-2′-deoxyuridine labelling indices. Nuclear p62c-*myc *levels are cell cycle related but the protein has not been shown to be a marker of increased tissue proliferation or of gastrointestinal malignancy. The reduction of the nuclear p62c-*myc *content of many adenocarcinoma cells compared with glandular mucosa cells suggests that reduced synthesis or nuclear retention of the normal protein may be a factor in the development of gastrointestinal adenocarcinomas, although the mechanism by which this may occur is not clear.*

c-*myc* is a cellular gene, sited on chromosome 8 at location q24 (Reference 1). It shows differential expression between resting and proliferating cells and is highly conserved between species[2–4]. Its gene product is a 439-amino acid, 62 000 molecular weight phosphoprotein (p62c-*myc*) whose structure and function have been described[5–8]. Little is known about its expression in upper gastrointestinal tissues and tumours. The protein can be detected in fresh, ethanol, methanol or formalin fixed specimens using monoclonal antibodies such as 6E10, by multiparameter flow cytometry (MFCM), by Western blotting and by histochemistry. The type of fixative and analysis may have an important bearing on the results obtained.

There is contradictory evidence about whether the c-*myc* gene is overexpressed in colorectal cancer. Moreover, the selective expression of a gene such as c-*myc* in the cell cycle does not necessarily indicate that it has a regulatory role[9]. Deregulation and overexpression of c-*myc* may be associated with loss of allelic markers on chromosome 5q, which is also known to contain the gene 'apc' associated with familial adenomatous polyposis[10,11]. Dolcetti *et al.*[12] found by DNA hybridization that only one of 44 colorectal tumours showed overexpression of c-*myc*. Watson[13] measured the nuclear content of p62c-*myc* in colonic polyps and tumours by MFCM and found that p62c-*myc* is less well expressed in poorly differentiated colorectal tumours. This finding correlated with c-*myc* mRNA transcript levels measured by Northern blotting[14]. p62c-*myc* may have a role in the evolution of colonic neoplasia and be a prognostic indicator for colonic tumours[15,16]. Finley *et al.*[17] found that c-*myc* mRNA but not N-*myc* or L-*myc* mRNA was significantly overexpressed in colorectal adenomas and tumours compared with normal mucosa. Constantini *et al.*[18] found a wide variation in c-*myc* mRNA expression within tumours and adenomas by *in situ* hybridization.

c-*myc* is expressed in archival (formalin fixed) specimens of colorectal mucosa, but at significantly higher levels in colorectal carcinomas[19]. In both mucosa and tumour cell nuclei the level of p62c-*myc* increases through the cell cycle. The p62c-*myc* in tumour cells, unlike deep crypt mucosa, may be both nuclear and cytoplasmic in distribution[20]. The p62c-*myc* may be expressed throughout the thickness of the normal human colon epithelium and not preferentially in any one compartment[21].

To test the hypothesis that nuclear-bound p62c-*myc* may be a marker of neoplasia in the gastrointestinal tract, we have compared the expression of the nuclear protein in both tumours and mucosa by MFCM. We have also measured its cell cycle related expression in ethanol fixed material, in which the proliferation kinetics as measured by 5-bromo-2′-deoxyuridine (BrdUrd) incorporation were known.

Patients and methods

This study was performed on biopsies of colorectal, gastric and oesophageal carcinomas and tissues excised during surgery which were labelled with BrdUrd. The method of analysis to obtain an *in vivo* BrdUrd labelling index and potential doubling time (T_{pot}) by MFCM analysis has recently been reported[22].

At the time of surgery, biopsies were immediately excised from

0007–1323/91/091080–04

transplantation to prevent GVHD. Immunosuppression with cyclosporin A not uniformly successful[3,10]. Donor[2] or graft[20,25] irradiation or donor pretreatment with anti-lymphocyte serum[26] is more successful. Donor pretreatment needs to commence at least 2 days before transplantation and would not be a practical clinical option. Graft irradiation is a possible alternative, but it may cause T lymphocyte destruction and radiation damage to the mucosa may occur. Mucosal shielding could be used but the results of the current study suggest that this would not guarantee protection from GVHD.

Graft mesenteric lymphadenectomy would not be practicable in human small bowel transplantation because of the anatomical arrangement of the mesenteric nodes. Graft perfusion with a specific T lymphocyte toxin might prevent GVHD and in bone marrow transplantation such a toxin (a combination of an anti-T lymphocyte monoclonal antibody with ricin A-chain) is effective[27].

Future efforts to prevent GVHD following experimental and clinical small bowel transplantation should be directed at T lymphocytes in the graft lamina propria and Peyer's patches as well as those in the mesenteric lymph nodes. In experimental models of small bowel transplantation the intensity of GVH reactivity depends on the strain combination used.

Acknowledgements

C. L. Ingham Clark is a Wellcome Training Fellow.

References

1. Slavin RE, Santos GW. The graft *versus* host reaction in man after bone marrow transplantation: pathology, pathogenesis, clinical features and implications. *Clin Immunol Immunopathol* 1973; **1**: 472–98.
2. Monchik GJ, Russell PS. Transplantation of small bowel in the rat: technical and immunological considerations. *Surgery* 1971; **70**: 693–702.
3. Diflo T, Maki T, Balogh K, Monaco AP. Graft *versus* host disease in fully allogeneic small bowel transplantation in the rat. *Transplantation* 1989; **47**: 7–11.
4. Kirkham RL. Small bowel transplantation. *Transplantation* 1984; **37**: 429–33.
5. Cohen Z, Silverman RE, Wassef R, Levy GA, Burnstein M, Cullen J *et al.* Small intestinal transplantation using cyclosporine. *Transplantation* 1986; **42**: 613–21.
6. Grant D, Wall W, Mimeault R, Zhong R, Ghent C, Garcia B *et al.* Successful small bowel/liver transplantation. *Lancet* 1990; **i**: 181–4.
7. Starzl TE, Iwatsuki S, Shaw BW Jr, Greene DA, Van Thiel DH, Nalesnik MA *et al.* Pancreaticoduodenal transplantation in humans. *Surg Gynecol Obstet* 1984; **159**: 265–72.
8. Simonsen M. Graft *versus* host reactions. Their natural history and applicability as tools of research. *Progr Allergy* 1962; **6**: 349–467.
9. Mason DW, Pugh CW, Webb M. The rat mixed lymphocyte culture: roles of a dendritic cell in intestinal lymph and T cell subsets defined by monoclonal antibodies. *Immunology* 1981; **44**: 75–87.
10. Kirkman RL, Lear PA, Madara JL, Tilney NL. Small intestine transplantation in the rat – immunology and function. *Surgery* 1984; **96**: 280–6.
11. Schroeder P, Goulet O, Lear PA. Small bowel transplantation: the European experience. *Lancet* 1990; **ii**: 110–11.
12. Goodacre R, Davidson R, Singal D, Bienstock J. Morphology and functional characteristics of human intestinal lymphoid cells isolated by a mechanical technique. *Gastroenterology* 1979; **76**: 300–8.
13. Deeg HJ, Loughran TP Jr, Storb R, Kennedy MS, Sullivan AM, Doney K *et al.* Treatment of human acute graft *versus* host disease with antithymocyte globulin and cyclosporine ± methylprednisolone. *Transplantation* 1985; **40**: 162–6.
14. Milton AD, Fabre JW. Massive induction of donor-type class I and class II MHC antigens in rejecting cardiac allografts in the rat. *J Exp Med* 1985; **161**: 98–112.
15. Jefferies WA, Green JR, Williams AF. Authentic T helper CD4 (W3/25) antigen on rat peritoneal macrophages. *J Exp Med* 1985; **162**: 117–27.
16. Lear PA, Cunningham AJ, Crane PW, Wood RFM. Patterns of lymphocyte migration in small bowel transplants. *Transplant Proc* 1989; **21**: 2881–2.
17. Grant D, Zhong R, Gunn H, Duff J, Garcia B, Keown P *et al.* Graft *versus* host disease associated with intestinal transplantation in the rat. *Transplantation* 1989; **48**: 545–9.
18. Schraut WH, Lee KKW, Dawson PJ, Hurst RD. Graft *versus* host disease induced by small bowel allografts. *Transplantation* 1986; **41**: 286–90.
19. Ingham Clark CL, Cunningham AJ, Crane PW, Wood RFM, Lear PA. Lymphocyte infiltration patterns in rat small bowel transplants. *Transplant Proc* 1990; **22**: 2460.
20. Deltz E, Ulrichs K, Schack T, Friedrichs B, Muller-Ruchholtz W, Muller-Hermelink H-K *et al.* Graft-*versus*-host reaction in small bowel transplantation and possibilities for its circumvention. *Am J Surg* 1986; **151**: 379–86.
21. Luck R, Klempnauer J, Steiniger B. Transplantation of MHC incompatible vascularised mesenteric lymph nodes: identical pattern of lethal graft-*vs*-host disease as induced by small bowel grafts. *Transplant Proc* 1990; **22**: 2332.
22. Pirenne J, Lardinois F, D'Silva M, Fridman V, Boniver J, Mahieu P. Relevance of mesenteric lymph nodes to graft-*versus*-host disease following small bowel transplantation. *Transplantation* 1990; **50**: 711–14.
23. MacDonald TT, Philip BC. Mouse Peyer's patches contain T cells capable of inducing the graft *versus* host reaction. *Transplantation* 1978; **26**: 162–5.
24. Baca M, Mowat AMcI, Mackenzie S, Parrott DMV. Functional characteristics of intra-epithelial lymphocytes from mouse small intestine. III. Inability of IEL to induce a systemic graft *versus* host reaction is because of failure to migrate *in vivo*. *Gut* 1987; **28**: 1267–74.
25. Lee KKW, Schraut WH. *In vitro* allograft irradiation prevents graft *versus* host disease in small bowel transplantation. *J Surg Res* 1985; **38**: 364–72.
26. Shaffer D, Maki T, Demichele SJ, Karlstad MD, Bistrian BR, Balogh K *et al.* Prevention of graft *versus* host disease with preservation of allograft function by donor pretreatment with antilymphocyte serum. *Transplantation* 1988; **45**: 262–9.
27. Filipovich AH, Vallera DA, Youle RJ, Haake R, Blazar BR, Arthur D *et al.* Graft *versus* host disease prevention in allogeneic bone marrow transplantation from histocompatible siblings. A pilot study using immunotoxins for T cell depletion of donor bone marrow. *Transplantation* 1987; **44**: 62–9.

Paper accepted 3 May 1991

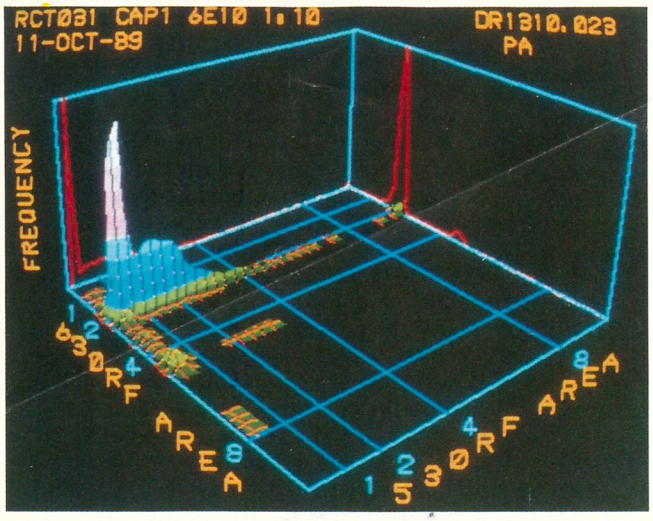

Figure 1 *Three-dimensional histogram illustrating the analysis of p62c-*myc *content by multiparameter flow cytometry. The 530 nm width plot reflects p62 c-*myc *content. The 630RF area plot is a ploidy histogram which records the DNA content of the nuclei. Gating around the G1 and G2 diploid and aneuploid populations on the 530 nm axis allows the mean protein content to be measured, after subtracting the mean fluorescence from similar analyses from specimens from which the 6E10 antibody has been omitted*

surgical resection specimens. These tissue blocks were stored in 70 per cent ethanol at −4°C. After mechanical mincing, fragments were incubated in 8 ml porcine pepsin (Sigma, Poole, UK) solution in 0·1 M HCl at a concentration of 0·1 mg/ml at 37°C, filtered and washed in phosphate buffered saline. The concentration of nuclei was adjusted to 2×10^6/ml. Each sample was divided into two aliquots in separate vials for primary antibody and fluorescence control measurements[23].

Nuclei were incubated in 20 μl 1/10 6E10 antibody (Cambridge Research Biochemicals, Cheshire, UK) for 1 h at room temperature, washed and resuspended in 20 μl of rat anti-mouse antibody-FITC conjugate (Sigma) for 1 h. Nuclei were counterstained with 0·025 mg/ml propidium iodide (Sigma) solution. The fluorescence control nuclei were resuspended in 20 μl of phosphate buffered saline.

Analyses were carried out on the Cambridge Medical Research Council custom-built dual laser flow cytometer using methodology previously described by Watson *et al.*[23]. An Innova 70-5W argon ion laser (Coherent, Palo Alto, California, USA) was used to excite at 488 nm light wavelength. Green fluorescent light was collected at 510–560 nm and red fluorescence above 630 nm in list mode. Data for 10 000 nuclei were collected from each specimen (*Figure 1*). The mean p62c-*myc* content of each cell cycle phase in each specimen was calculated by subtracting the fluorescence control values from the 'primary antibody' values. The results were expressed in units of fluorescence in the linear range 1–1024 units. The data were analysed by the non-parametric pairs method of Mann and Whitney.

Results

Tumour biopsies were studied from 87 patients with colorectal carcinomas. There were two well differentiated, 56 moderately and 29 poorly differentiated tumour specimens; 47 specimens were diploid and 40 specimens were aneuploid. There were 51 normal mucosal specimens from terminal ileum and sites throughout the colorectum, seven specimens from two patients with polyposis coli and four specimens of villous adenomas.

A further 26 biopsies were studied from 18 tumours to assess the intratumour variation in p62c-*myc* expression. Where multiple specimens were studied, the mean values of all data were used in all other calculations. All specimens of mucosa were excised from tumour resection specimens, and were histologically normal. Colorectal mucosal specimens were between 10 and 30 cm from the primary tumour.

Six oesophageal squamous carcinomas and 18 gastric adenocarcinomas were also studied. There were 19 specimens of histologically normal gastric mucosa and seven normal squamous mucosa specimens. Mucosal specimens were between 2·5 and 10 cm from the primary tumour.

*Evidence for cell cycle related p62c-*myc *expression*

Nuclear p62c-*myc* expression increased with DNA synthesis in both tumours and mucosa, such that the mean G2 fluorescence was up to twice that associated with the G1 diploid (G1D) peak. S phase nuclei expressed intermediate values between the G1D and G2D values. G1 aneuploid p62c-*myc* content in aneuploid tumours was intermediate to these values (*Table 1*). Comparisons between specimens and groups were based on the p62c-*myc* expression measured in the G2 diploid peak.

The difference between p62c-*myc* G1D and G2D content was significant in all tissues and tumours (Mann–Whitney pairs test, $P = 0{\cdot}001$). In 13 colorectal tumour specimens the mean G2D p62c-*myc* content was < 50 units (range 0–34 units) in both G1 and G2. There was no correlation between G2D p62c-*myc* content and ploidy, anatomical site (proximal or distal colon or rectum) or grade of the colorectal tumours (*Table 1*).

*p62c-*myc *content and the BrdUrd labelling index*

The BrdUrd labelling index, an *in vivo* index of proliferation, was calculated for all specimens by MFCM[22] (*Table 1*). Mucosal data tissue labelling index was obtained for both crypt and stromal cells. A broadly inverse relationship was observed between the mean tissue BrdUrd labelling index and the nuclear p62c-*myc* content in both upper and lower gastrointestinal tumours and mucosa. If p62c-*myc* were important in the regulation of proliferation, higher levels of the protein might have been expected in cells from the more rapidly proliferating tissues.

*Intratumour variation in p62c-*myc *content*

Between two and four separate biopsies from random sites in 18 colorectal tumours were analysed (*Figure 2*). The mean(range) of values is shown for each tumour. Some tumours showed a marked intersample variation in p62c-*myc*. This is

Table 1 *Mean(s.e.m.) nuclear p62c-*myc *content in units of fluorescence of the G1 and G2 diploid peaks, and the mean 5-bromo-2'-deoxyuridine labelling indices (Br LI) of oesophageal, gastric and colorectal tumours, adenomas and mucosa*

Specimens	G1D	G2D	Br LI (%)
Oesophageal (squamous)			
Carcinoma ($n=6$)	140(49)	248(76)	6·9(2·2)
Mucosa ($n=7$)	137(26)	238(49)	3·7(1·0)
Gastric (glandular)			
Carcinoma ($n=18$)	144(17)	258(24)	5·8(1·3)
Mucosa ($n=19$)	232(34)	388(36)	2·5(0·3)
Colorectal mucosa			
All ($n=51$)	227(20)	306(24)	2·1(0·2)
Ileum ($n=10$)	115(13)	201(20)	1·8(0·5)
Right colon ($n=10$)	189(22)	330(38)	2·3(0·6)
Transverse colon ($n=8$)	239(30)	369(31)	2·3(0·5)
Left colon ($n=8$)	213(45)	315(44)	2·5(0·6)
Sigmoid colon ($n=8$)	180(27)	279(48)	1·6(0·4)
Rectum ($n=7$)	252(39)	346(45)	2·1(0·4)
Polyposis coli mucosa ($n=7$)	443(93)	503(64)	2·6(0·5)
Villous adenoma ($n=4$)	96(25)	157(58)	6·0(1·1)
Colorectal tumours			
All tumours ($n=87$)	127(10)	188(14)	9·7(0·5)
Well differentiated ($n=2$)	150	203	7·5
Moderately differentiated ($n=56$)	121(9)	171(14)	9·0(0·6)
Poorly differentiated ($n=29$)	147(19)	218(29)	10·6(0·8)
Diploid ($n=47$)	131(16)	185(21)	9·8(0·6)
Aneuploid ($n=40$)	121(10)	190(17)	9·8(0·7)
Right, transverse colon ($n=23$)	119(16)	184(22)	7·6(0·8)
Left, sigmoid ($n=17$)	119(26)	196(30)	11·8(1·6)
Rectal ($n=47$)	133(13)	209(16)	9·9(0·6)

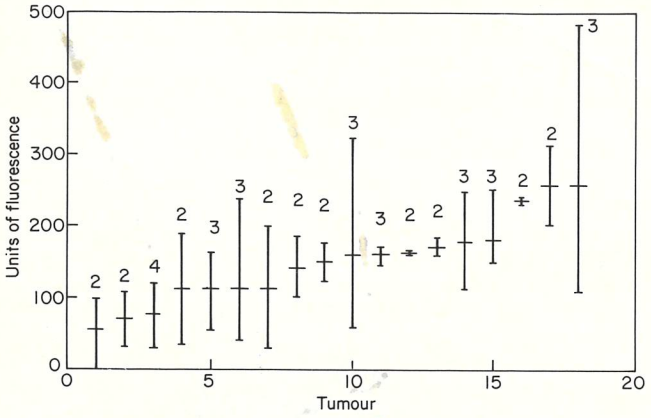

Figure 2 *Variation in the mean p62c-*myc *content of 18 colorectal tumours from which between two and four blocks were analysed. Values are mean(range). Figures above each bar represent the number of specimens analysed from that tumour*

likely to reflect tumour heterogeneity, which has also been noted for *in vivo* BrdUrd labelling in these tumours.

*Expression of c-*myc *in polyposis coli*

Seven mucosal specimens without microadenomas were studied from two patients undergoing total colectomy for polyposis coli. Nuclear p62c-*myc* content was significantly higher than the equivalent values in mucosa from colorectal tumour patients (Mann– Whitney pairs test, $P=0{\cdot}001$).

Study of paired gastric tumour to mucosa specimens

Pairs of carcinoma to mucosa specimens were analysed. In only four of 18 gastric specimens and in eight of 33 colorectal pairs was the p62c-*myc* content of the mucosa lower than the corresponding tumour block (*Figure 3*). The mean G2D content of p62c-*myc* was significantly higher in both gastric and colorectal mucosa than in the corresponding adenocarcinomas ($P=0{\cdot}001$) but there was no difference between the smaller series of oesophageal mucosa and squamous carcinomas (*Table 1*).

Discussion

Efforts to understand the role played by the c-*myc* gene and its protein product in the process of DNA synthesis during colorectal tumour proliferation have been complicated by the range of experimental techniques and cell models used. These variously have included the measurement of complementary DNA, messenger RNA and gene transcript levels by blotting techniques. The measurement of protein expression rather than gene transcript expression has theoretical and practical advantages. Protein content is the end point of gene expression, and is the mechanism by which gene function is effected. It is therefore likely that the measurement of protein expression using monoclonal antibodies such as 6E10 will provide another useful indicator of changed gene function and expression, being the end point of changes in gene regulation, transcription and translation.

The flow cytometric and histological study of nuclear p62c-*myc* content in paraffin embedded archival material is complicated by formalin fixation artefact. The use of 70 per cent ethanol or methanol as a preservative appears to reduce these problems[24]. Although the epitope recognized by 6E10 is believed to be resistant to pepsin, care must be taken using pepsin digestion[25].

It was found in this series that nuclear p62c-*myc* content of colorectal tumour cells does not appear to vary with histological grade, ploidy or anatomical site of origin. This finding is contrary to some previous reports. It was not possible to demonstrate a relationship between nuclear p62c-*myc* levels and cell proliferation in tumour cells as measured by BrdUrd incorporation.

The results described raise a number of questions about the role of the c-*myc* gene product in human gastrointestinal cancer. The c-*myc* gene product *as measured by the 6E10 antibody* in both tumour and mucosal cells is a protein whose quantity increases linearly with DNA content through the cell cycle. It has previously been shown that DNA binding may be a part of p62c-*myc* function through the leucine zipper mechanism. A simple doubling of mean protein content with chromosome duplication from G1D to G2D is not observed. This may reflect a partial degradation of nuclear p62c-*myc* during tumour disaggregation. It may be more appropriate to compare the *early* G1 protein content with the *late* G2 protein content.

The finding of high nuclear p62c-*myc* expression in normal gastrointestinal mucosa as compared with adenocarcinomas suggests that either the c-*myc* gene is down-regulated in malignant cells, or a higher proportion of the protein content is exported from the nucleus to the cytoplasm of tumour cells. It is clearly important that no gene is designated as an oncogene until the expression of its product has been assessed in both the tumour and in its tissue of origin. Transcriptional expression (messenger RNA products) may differ from translational expression (protein product). Conclusions about the nature of a gene should be based on studies from a number of techniques, including quantitative measurement of the protein product where possible. The protein content of malignant cells may be low despite a rise in the absolute number of gene and transcriptional copies caused by altered gene control, because of impaired protein synthesis.

The function of the c-*myc* gene remains unclear. The findings reported here would be compatible with a role for p62c-*myc* as a DNA binding structural protein, or as a gene regulatory or enzymatic protein. Immunohistochemical studies of the distribution of p62c-*myc* within tumour cells, stromal cells and mucosa have been reported[19,20,26]. The finding of p62c-*myc* in the cytoplasm of differentiated colonic crypt mucosal and tumour cells, for example, would be compatible with a role for p62c-*myc* as a differentiation regulator which was extruded from the nucleus as cells reached maturity. The role that p62c-*myc* might play in the putative sequence of changes from mucosa through adenomas to frank carcinoma is unclear.

It is possible that nuclear p62c-*myc* is differently expressed in mucosa of tumour-containing bowel than in mucosa of non-malignant bowel. There was no evidence from histological or BrdUrd proliferation studies on this material (C. S. Potten, personal communication) that our mucosal specimens were in any way different from 'non-malignant' mucosa.

The p62c-*myc* levels were high in polyposis coli mucosa but were low in the small number of villous adenomas studied. The observation of a high p62c-*myc* content in the mucosa of

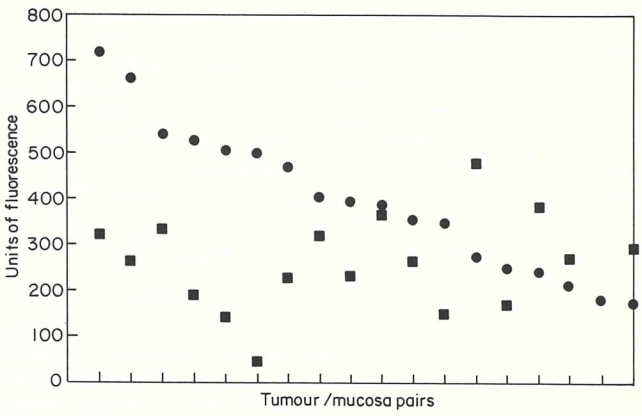

Figure 3 *Pairs of gastric mucosa and carcinoma specimens are plotted on the vertical axis to illustrate how in all but four cases the mean G2D p62c-*myc *content of the mucosa exceeds that of the carcinoma.* ●, *G2D gastric mucosa;* ■, *G2D gastric tumour*

patients with polyposis coli mandates further investigation, because it suggests that p62c-*myc* may be useful as a disease marker in polyposis coli, and may provide further clues to the role of p62c-*myc* in the process of adenoma formation in polyposis coli.

Our observations using MFCM are confined to nuclear p62c-*myc*. Cytoplasmic and cell membrane protein is lost during extraction, so it is not possible to quantify any non-nuclear protein which may be present. For this reason and because tissue architecture is destroyed by pepsin extraction, immunohistochemical studies are important. Changes in the pattern of p62c-*myc* staining in intestinal mucosa mirror the distribution of proliferating cells to some extent. Mucosal p62c-*myc* is both nuclear and cytoplasmic in distribution but becomes progressively more cytoplasmic in the zone of maturation. The changing intracellular distribution of the protein may be an important clue to function. In one model, expulsion of the protein from the nucleus may be associated with a change in the state of differentiation of the cell or tissue. Thus, surface colorectal mucosal crypt cells may have a lower nuclear to cytoplasmic p62c-*myc* ratio than do cells in the proliferation zone.

In conclusion, the studies reported in this paper support the view that the c-*myc* protein is a cellular protein of unknown function whose quantity increases with DNA synthesis, more so in mucosal than in tumour cell nuclei. The c-*myc* gene in colorectal tumour and mucosal cells may be a normal cellular gene rather than an oncogene. Multiparameter flow cytometry is a powerful tool with which to measure translational gene expression in the assay of nuclear antigens in solid tumours and tissues. The finding of high c-*myc* expression in normal gastrointestinal tissue as compared with adenocarcinomas suggests that reduced nuclear content of normal c-*myc* protein in adenocarcinomas may be an aetiological feature of gastrointestinal malignancy. The underlying mechanism and the primary function of the p62c-*myc* protein remain unclear.

Acknowledgements

We thank Mr P. C. Weaver, Mr C. D. Johnson and Mr M. R. Thompson for access to their patients. D. A. R. was funded by the Cancer Research Campaign and by the Wessex Regional Health Authority Research Fund.

References

1. Taub RM, Moulding C, Battey J *et al.* Activation and somatic mutation in the translocated c-*myc* gene in Burkitt's lymphoma. *Cell* 1984; **36**: 339–48.
2. Rabbitts PH, Watson JV, Lamond A *et al.* Metabolism of c-*myc* gene products: c-*myc* mRNA and protein expression in the cell cycle. *EMBO J* 1985; **4**: 2009–15.
3. Studzinski GP, Brelvi ZS, Feldman SC, Watts RA. Participation of c-*myc* in DNA synthesis of human cells. *Science* 1986; **234**: 467–70.
4. Burck KB, Liu ET, Larrick JW. Myc and other nuclear oncogenes. In: Burck KB, Liu ET, Larrick JW, eds. *Oncogenes – An Introduction to the Concept of Cancer Genes*. New York: Springer, 1989: 198–221.
5. Evan G, Hancock D. Nuclear structures containing p62c-*myc*. *Cell* 1985; **43**: 253–61.
6. Evan G, Lewis GK, Ramsay G, Bishop JM. Isolation of monoclonal antibodies specific for human and mouse proto-oncogene products. *Mol Cell Biol* 1985; **5**: 3610–16.
7. Dang CV, McGuire M, Buckmire M, Lee WM. Involvement of the Leucine Zipper region in the oligomerization and transforming activity of human c-*myc* protein. *Nature* 1989; **337**: 664–6.
8. Karn J, Watson JV, Lowe AD, Green SM, Vedeckis W. Regulation of the cell cycle by c-*myc* levels. *Oncogene* 1989; **4**: 773–87.
9. Calabretta B, Kaczmarek L, Ming P, Au F, Mang S. The expression of c-*myc* and other cell cycle dependent genes in human colon neoplasia. *Cancer Res* 1985; **45**: 6000–4.
10. Vogelstein B *et al.* Genetic alterations during colorectal tumour development. *N Engl J Med* 1988; **319**: 9, 525–32.
11. Erisman MD, Scott JK, Astrin SM. Evidence that the familial polyposis coli gene is involved in a subset of colon cancers with a complementable defect in c-*myc* regulation. *Proc Natl Acad Sci USA* 1989; **86**: 4264–8.
12. Dolcetti R, De-Re V, Viel A, Pistello M, Tavian M, Boiocchi M. Nuclear oncogene amplification or rearrangement is not involved in human colorectal malignancies. *Eur J Cancer Clin Oncol* 1988; **24**: 1321–8.
13. Watson JV. Oncogenes, cancer and analytical cytology. *Cytometry* 1986; **7**: 400–10.
14. Sikora K, Chan S, Evan G *et al.* c-*myc* oncogene expression in colorectal cancer. *Br J Cancer* 1987; **59**: 1289–95.
15. Stewart J, Evan G, Watson JV, Sikora K. Detection of the c-*myc* oncogene product in colonic polyps and carcinomas. *Br J Cancer* 1986; **53**: 1–6.
16. Imaseki H, Hayashi H, Taira M *et al.* Expression of c-*myc* oncogene in colorectal polyps as a biological marker for monitoring malignant potential. *Cancer* 1989; **64**: 704–9.
17. Finley GG, Schultz NT, Hill SA, Geiser JR, Pipas JM, Meisler AI. Expression of the c-*myc* gene family in different stages of human colorectal cancer. *Oncogene* 1989; **4**: 963–71.
18. Constantini CR, Theillet C, Hutzell P, Merlo G, Schlom J, Callaghan R. *In situ* detection of c-*myc* mRNA in adenocarcinomas, adenomas and mucosa of human colon. *J Histochem Cytochem* 1989; **37**: 293–8.
19. Watson JV, Stewart J, Cox C, Sikora K, Evan GI. Flow cytometric quantitation of the c-*myc* oncoprotein in archival neoplastic biopsies of the colon. *Mol Cell Probes* 1987; **1**: 151–7.
20. Williams ARW, Piris J, Wyllie AH. Immunohistochemical demonstration of altered intracellular localisation of the c-*myc* oncogene product in human colorectal neoplasms. *J Pathol* 1990; **160**: 287–93.
21. Ten-Kate J, Eidelman S, Bosman FT, Damjanov I. Expression of the c-*myc* proto-oncogene in normal human intestinal epithelium. *J Histochem Cytochem* 1989; **37**: 541–5.
22. Rew DA, Taylor I, Wilson GD, Weaver PC. The *in vivo* proliferation kinetics of human colorectal carcinomas. *Br J Surg* 1991; **78**: 60–6.
23. Watson JV, Sikora K, Evan GI. A simultaneous flow cytometric assay for c-*myc* oncoprotein and cellular DNA in nuclei from paraffin embedded material. *J Immunol Methods* 1985; **83**: 179–92.
24. Feichter GE, Goerttler K. Pitfalls in the preparation of nuclear suspension from paraffin embedded tissue for flow cytometry. *Cytometry* 1986; **7**: 616.
25. Lincoln ST, Bauer KD. Limitations in the measurement of c-*myc* oncoprotein and other nuclear antigens by flow cytometry. *Cytometry* 1989; **10**: 456–62.
26. Sundaresan V, Forgacs IC, Wight DGD, Wilson B, Evan GI, Watson JV. Abnormal distribution of c-*myc* oncogene production in familial adenomatous polyposis. *J Clin Pathol* 1987; **40**: 1274–81.

Paper accepted 13 May 1991

Br. J. Surg. 1991, Vol. 78, September, 1084–1087

Nutritional status after total and partial gastrectomy with Roux-en-Y reconstruction

C. Staël von Holstein, B. Walther, E. Ibrahimbegovic* and B. Åkesson†

*Departments of Surgery, *Clinical Nutrition and †Medical and Physiological Chemistry 3, Lund University, S-221 85 Lund, Sweden*

Correspondence to:
Dr C. Staël von Holstein

Dietary intake and nutritional status were studied in two groups of patients after total gastrectomy (n = *10*) *and after partial gastrectomy* (n = *10*). *All patients were reconstructed with a Roux-en-Y loop. Investigations were carried out 4–17 years after the operation. Eight patients in the group undergoing total gastrectomy and four patients undergoing partial gastrectomy had gastric carcinoma and were without recurrent disease 5–15 years after the operation. A clinical dietician conducted an anthropometric evaluation. All patients underwent an upper gastrointestinal endoscopy, clinical examination, [^{14}C]triolein breath test and a blood chemistry profile. The median weight loss from operation to investigation was 2·1 kg in the partial gastrectomy group and 6·7 kg in the total gastrectomy group* (*n.s.*). *After partial gastrectomy three patients had a subnormal body mass index* (*n.s.*). *This was not found in the total gastrectomy group. Energy intake was below that recommended in seven patients in the partial gastrectomy group* (*three of them had a subnormal body mass index*) *and in two in the total gastrectomy group* (*n.s.*) [^{14}C]*triolein breath testing revealed fat malabsorption in three patients after partial gastrectomy and in five patients after total gastrectomy* (*n.s.*).

Incapacitating nutritional deficiency is often regarded as an inevitable consequence in a proportion of patients after total gastric resection. Lack of gastric reservoir capacity is thought to be one of the reasons and a variety of surgical procedures have been devised to create a gastric substitute. The benefit of these pouches compared with a simple Roux-en-Y reconstruction has been hard to demonstrate.

Most patients undergoing total gastrectomy have gastric cancer. The long-term prognosis is still poor[1–3]. To justify a more complex operative procedure in this group, evidence of short-term and long-term benefits should be provided. Short-term studies in humans and animals have not revealed any benefit with pouch formation[4–6]. In short-term studies on these patients, one difficulty is to exclude recurrence as a factor contributing to a deficient nutritional status.

To assess the benefit of a gastric pouch, the nutritional status of patients subjected to total gastrectomy 4–17 years ago was compared with a similar group after partial gastrectomy and Roux-en-Y reconstruction.

Materials and methods

Patients

Since the beginning of the 1970s patients with gastric carcinoma in the Department of Surgery, Lund University have undergone total gastrectomy with Roux-en-Y oesophagojejunostomy. In 1979, a prospective study was started on all patients subjected to total gastrectomy. Reconstruction was with a 50–60 cm Roux-loop, with the oesophagojejunostomy fashioned with a circular stapler device. Patients were followed regularly by clinical examination and, in some instances, upper gastrointestinal endoscopy. Laboratory analyses of haemoglobin, serum iron, transferrin, cobalamin and folate were performed yearly and any deficiency regulated by supplementation. All patients were recommended treatment with oral iron supplementation. Vitamin B_{12} injections were given every third month unless the patient developed very high serum values. No other vitamin supplementation was routinely prescribed.

Five years elapsed after surgery before the patient was judged free of cancer. In those subjected to total gastrectomy, only eight patients fulfilled that requirement and were included in the study. Two patients were included after total gastrectomy for benign disease. The total gastrectomy group consisted of four women and six men, with a median age of 74·5 years (range 45–81 years) operated on 4–17 years previously.

Ten consecutive patients from 36 treated by partial gastric resection between 1945 and 1962 for ulcer disease and subsequently with re-resection, Roux-en-Y gastrojejunostomy and truncal vagotomy agreed to participate and constituted the partial gastrectomy group. The one woman and nine men in this group had a median age of 73 years (range 55–78 years) and had been operated on 9–16 years earlier. Patient data are shown in *Table 1*.

Clinical examination

Patients were interviewed regarding eating habits, gastrointestinal symptoms, bowel habit, substitution medication and other diseases, and all were subjected to an upper gastrointestinal endoscopy and clinical examination. Data on diagnosis, date of operation, operative procedure, preoperative weight and previous gastric surgery were retrieved from medical records.

Table 1 *Sex, age, weight development after surgery and diagnosis*

	Total gastrectomy	Partial gastrectomy
Number	10	10
Women:men	4:6	1:9
Median age (years)	74·5 (45–81)	73 (55–78)
Median age at operation	64·5 (39–71)	60·5 (45–63)
Median time from operation to investigation (years)	7·5 (4–17)*	13 (9–16)*
Median weight loss from operation to investigation (kg)	6·7 (−17·2–+2·7)	2·1 (−8·9–+9·2)
Diagnosis at operation		
Adenocarcinoma	8	4
Leiomyoma	1	–
Reflux gastritis	–	5
Stomal ulcer	–	1
Gastric retention	1	–

Values in parentheses are ranges. * $P = 0{\cdot}01$

0007–1323/91/091084–04