26. O'Connell MJ, Colgan JP, Oken MM, Ritts RE, Kay NE, Itri LM. Clinical trial of recombinant leucocyte A Interferon as initial therapy for favorable histology non-Hodgkin's lymphomas and chronic lymphocytic leukemia. An Eastern Cooperative Oncology Group Pilot Study. *J Clin Oncol* 1986, 4, 128–136.

27. Matis LA, Young RC, Longo DL. Nodular lymphomas: current concepts. *CRC Crit Rev Oncol Haematol* 1986, 5, 171–175.

Acknowledgements—This work was supported by AREMAS and EUROCETUS.

Eur J Cancer, Vol. 27, No. 12, pp. 1680–1684, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00
Pergamon Press plc

Growth Factor Requirements of Human Colorectal Tumour Cells: Relations to Cellular Differentiation

Lily Huschtscha, Enrique Rozengurt and Walter F. Bodmer

Human colorectal tumour lines that exhibit different degrees of differentiation were studied to define their growth requirements. The poorly differentiated cell lines SW620, SW480, SW48 and SW837 proliferated in Dulbecco's modified Eagle's medium without exogenously added growth factors. In contrast, the moderately differentiated cell lines SW1222, HT29, PC/JW and LS174T proliferated only in medium supplemented with growth factor. SW1222 and HT29 cells required transferrin for growth, which was improved by other growth-promoting factors including epidermal growth factor (SW1222) and sodium selenite (HT29). PC/JW and LS174T required both insulin and transferrin for optimal growth. The tumour cell lines could be passaged continuously in serum-free medium supplemented with growth factor and in some cases they grew better than in serum-supplemented medium. The serum-free growth conditions should prove useful for studies on differentiation in colorectal cell lines and their interactions with growth factors.
Eur J Cancer, Vol. 27, No. 12, pp. 1680–1684, 1991.

INTRODUCTION

THE PROLIFERATION of cells is regulated by a complex interplay of growth-stimulating and inhibitory factors, including polypeptide growth factors and extracellular matrix proteins [1–6]. Many tumour cells in culture exhibit a marked reduction in their requirement for exogenous growth factors and an increased ability to produce growth factors that act in an autocrine or paracrine fashion [1–3, 7]. Hence, defining the particular growth factor requirements of different cell types may help understanding the normal pattern of growth control, differentiation and subsequent changes during tumour progression.

Colorectal cancer is, overall, the second most frequent cancer in the developed world [8]. New approaches are needed to improve its prevention and treatment and these are most likely to come from a better understanding of the fundamental cell and molecular biology of normal and abnormal colorectal epithelium [9]. As it is difficult to grow normal colorectal epithelium in culture [10], long-term cultures of tumour cells provide a model system to study their dependence upon exogenously added growth-promoting factors.

The study presented here defines the growth factor requirements of several human colorectal tumour cell lines of different degrees of differentiation within a particular range of passages (Table 1). The results show that poorly differentiated human colorectal cell lines can be distinguished from moderately differentiated cell lines in their ability to proliferate in serum-free medium.

Correspondence to W.F. Bodmer.
The authors are at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, U.K. L. Huschtscha is presently at the Department of Cancer Medicine, Blackburn Building, University of Sydney, N.S.W. 2006, Australia.
Revised 19 Aug. 1991; accepted 18 Sep. 1991.

MATERIALS AND METHODS

Cells

Cell lines used for these experiments were SW620, SW837, SW480, SW48, SW1222 [11]; HT29 [12]; LS174T [13] and PC/JW [14]. Cell lines SW480 and SW620 were derived from the same patient; SW480 was derived from a colon adenocarcinoma whereas SW620 was from a lymph node metastasis. For particular cell characteristics and passages used for this study, see Table 1.

Media

The cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Gibco, Europe), glutamine 2 mmol/l, penicillin 100 U/ml, streptomycin 100 μg/ml (DMEM-10). Several media, namely Ham's F12, RPMI-1640, McCoy's 5A, MCDB104, Waymouth's and DMEM (all from Gibco Europe), were tested on the cell lines for growth in different concentrations of FCS (10%, 5%, 2% and 0).

The serum-free conditions [15] were studied according to the method of Murakami and Masui [16]. Briefly, the media were supplemented with insulin (2 μg/ml), transferrin (2 μg/ml), hydrocortisone (10 ng/ml), epidermal growth factor (EGF:

Table 1. Characteristics of human colorectal tumour cell lines

Cell line (passages)	Origin (Dukes staging)	Chromosomes	CEA	Growth factor requirements (in DMEM medium)	Differentiation of tumour cells
SW480 (115–130)	Metastatic lymph node (C)	54	Low	None	Poor
SW480 (100–130)	Colon (B)	55	Low	None	Poor
SW837 (50–80)	Rectum (C)	42	High	None	Poor
SW48 (120–150)	Colon (C)	47	Low	None	Poor
SW1222 (60–90)	Colon (C)	44	High	2 μg/ml T 1 ng/ml EGF 10 μg/ml A 25 nmol/l N	Moderate
HT29 (150–180)	Colon (NA)	71	Low	2 μg/ml T 0.3 ng/ml T_3 25 nmol/l N (serum-coated dishes)	Moderate
LS174T (105–135)	Colon (B)	45	High	2 μg/ml T 2 μg/ml I	Moderate
PC/JW (35–50)	Colon – from FAP patient (NA)	46	High	2 μg/ml T 2 μg/ml I	Moderate

FAP = familial adenomatous polyposis, NA = not available, T = transferin, A = ascorbic acid, N = sodium selenite, I = insulin. All cell lines were tumorigenic (SW837 weakly so).

1 ng/ml), glucagon (0.2 μg/ml), tri-iodothyronine (T_3; 0.3 ng/ml), sodium selenite (25 nmol/l) and ascorbic acid (10 μg/ml) (all from Sigma).

Serum-coated dishes for the growth of HT29 cells were prepared by leaving FCS in tissue culture dishes overnight and then washing three times in DMEM, with no serum.

Growth experiments

Cells in log-phase of growth were harvested with 0.25% trypsin containing 0.01% EDTA and then plated at appropriate cell densities into tissue culture dishes (Nunc). The cells were incubated at 37°C in 10% CO_2 in humidified air. In the first series of experiments, an aliquot of trypsinised and well dispersed cells was taken and counted at specified times by using a Coulter Counter (model ZB1).

In later experiments, a rapid method to score cell numbers had been devised [17]. In brief, cells were plated onto 96-well trays (Falcon Microtest III Testplate, Becton-Dickinson Labware). At appropriate times the wells were washed in phosphate-buffered saline (Dulbecco's PBS'A'), drained and then 200 μl of freshly made assay solution at 37°C (0.2 mmol/l boric acid, 1 mmol/l $MgCl_2$, 1.2 mmol/l 4-methyl-umbelliferyl-phosphate (Sigma) was added to each well. After incubating the tray for 3 h at 37°C, the relative fluorescence was measured using a Dynatech Fluor Tm reader. Before this assay was used it was necessary to establish a linear correlation between increasing cell number and relative fluorescence for each cell line studied [17]. Growth was expressed as a ratio: cell numbers obtained with growth factors or serum divided by cell numbers with no serum.

RESULTS

Growth requirements of SW1222 cells

Initially we examined the growth requirements of human colorectal cell lines that retain a differentiated phenotype (Table 1). SW1222 is a cell line that can be induced to differentiate *in vitro* to form lumen-like structures [18]. Growing cultures of these cells were trypsinised and plated (10^4 cells/cm^2) in DMEM containing either 0.1 or 2% FCS. Figure 1 shows that cells inoculated in the presence of 2% FCS continued to proliferate even after 17 days in culture. A similar growth response was obtained when FCS was added at 5 or 10% instead of 2% (results not shown). In contrast, SW1222 cells failed to proliferate in DMEM supplemented with 0.1% FCS (Fig. 1). The range of initial cell densities at which SW1222 did not grow in DMEM alone or supplemented with 0.1% FCS was 1–3.5 × 10^4 cells/cm^2 in six independent experiments. It is noteworthy that SW1222 cells seeded at higher cell densities were able to proliferate in this medium.

SW1222 cells maintained in DMEM with 0.1% FCS retained viability, as judged by their ability to proliferate when serum or a mixture of growth factors were added to the medium after 4 days of incubation (Fig. 1). The mixture of growth factors contained insulin, transferrin, hydrocortisone, EGF, glucagon, T_3, sodium selenite and ascorbic acid [16]. Additional experiments showed that transferrin, EGF, ascorbic acid, sodium selenite and glucagon enhanced the growth of SW1222 cells (Fig. 2a), whereas hydrocortisone, T_3 and insulin had no effect. Transferrin added with EGF, sodium selenite and ascorbic acid stimulated growth to the level achieved in DMEM supplemented with 2% FCS (Fig. 2b). The optimal concentrations were 2 μ/ml transferrin, 1 ng/ml EGF, 25 nmol/l sodium selenite and 10 μg/ml ascorbic acid. SW1222 cells were grown in this medium

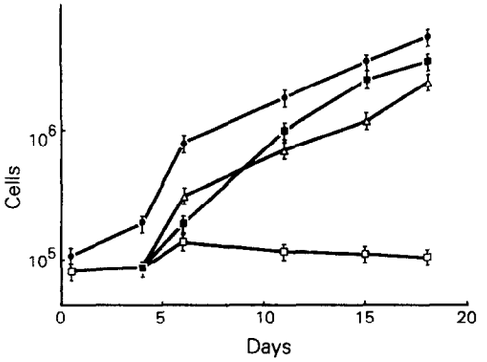

Fig. 1. Growth curves of SW1222 cells in DMEM supplemented with 0.1% FCS (□) 2% FCS (●); after 4 days growth in 0.1% FCS either 2% FCS (■) or a mixture of growth factors (△) were added. Means of three determinations. Growth in 2% FCS was similar to that in 5% or 10% FCS.

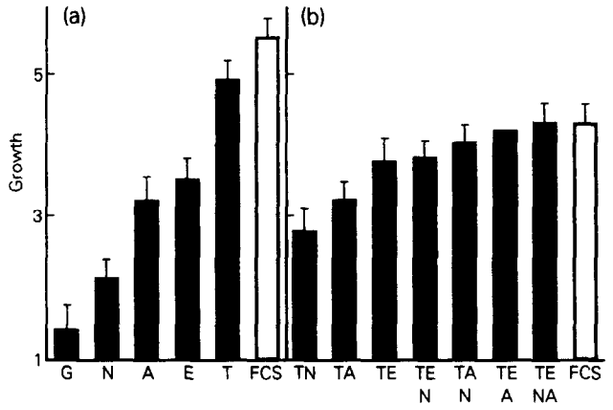

Fig. 2. Effect of various growth promoting factors on the growth of SW1222 cells. Serum control (2% FCS) and different combinations of growth factors (added at 2 days). Average cell number at 15 days' incubation. T = transferrin, E = EGF, A = ascorbic acid, N = sodium selenite, G = glucagon. Three dishes were scored for each combination. Hydrocortisone and T_8 had no effect. Insulin was inhibitory in some experiments. The combination of growth factors is indicated in each bar.

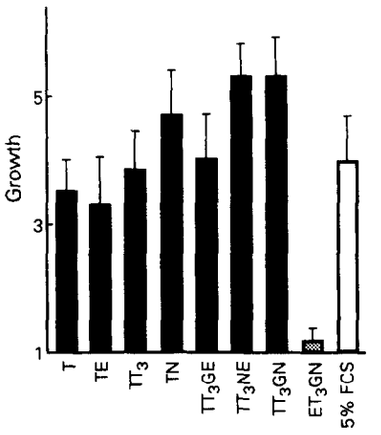

Fig. 3. Growth requirements of HT29 cells. The combination of growth factors is indicated in each column. T = transferrin, T_3 = triiodothyronine, N = sodium selenite, G = glucagon. A serum control and different combinations of growth factors were added at 2 days' growth and cell growth calculated 9 days later, the time required to just reach confluence. (□) Growth of cells incubated with growth-promoting factors in the absence of transferrin.

continuously for 6 weeks with a split ratio of 1:8 and 1:16, that is about 18–24 generations (data not shown).

Growth of HT29, PC/JW and LS174T cells in defined medium

Since SW1222 could be grown in serum-free medium supplemented with additional growth-promoting factors, we next examined the serum-free growth conditions for other colorectal cell lines that are moderately differentiated, namely HT29, LS174T and PC/JW (Table 1). HT29 has been shown to differentiate *in vitro* [19] and it has been studied in many laboratories since its isolation. These cells did not attach or grow when they were seeded onto tissue culture dishes, unless the plastic dishes were coated with FCS [20]. Other extracellular matrix components tested, such as fibronectin and collagen types I and IV, were not as efficient in promoting cell attachment and long-term growth as treating the plastic surface with serum (results not shown). Initial experiments compared the growth of HT29 cells in DMEM supplemented with 2, 5 or 10% FCS. Optimal growth was achieved in the presence of 5% FCS. In contrast, the growth of HT29 cells seeded at densities of 2–3 × 10^4 cells/cm^2 was markedly restricted in DMEM containing 0.1% FCS. As shown in Fig. 3, transferrin was obligatory for growth of HT29 cells with enhanced proliferation in the presence of sodium selenite and T_3. These cells were continuously passaged in DMEM-TT_3NE (see Fig. 3) for 4 weeks with a split ratio for 1:8 and they did not slow down in this time (data not shown).

The PC/JW and LS174T cell lines, which exhibit many characteristics of normal colonic epithelial cells, also failed to grow in DMEM when seeded at densities of 1.6–4 × 10^4 cells/cm^2. Addition of insulin at 2 μ/ml caused a marked simulation of the growth of LS174T cells. Both cell lines required insulin and transferrin for maximal growth and, in fact, the cultures grew better in this medium than in high serum (Fig. 4). These cell lines could be passaged continuously for 4 weeks in DMEM supplemented with transferrin and insulin with a split ratio of 1:8.

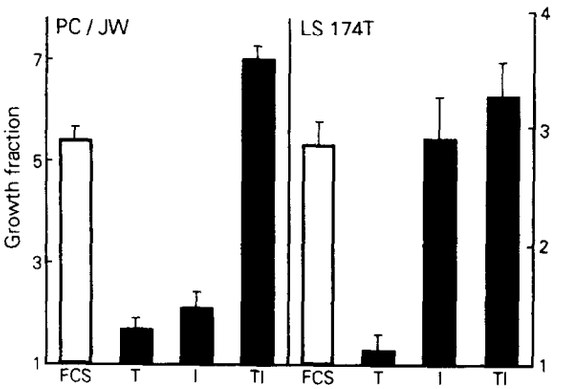

Fig. 4. Growth requirements of PC/JW and LS174T cells. The addition of growth factors is indicated in each column. T = transferrin, I = insulin. At 2 days' growth a serum control (5% FCS) and different combinations of growth factors were added for 11 days.

Serum-free growth of poorly differentiated cells

The poorly differentiated tumour colorectal cell lines, SW620, SW480, SW48 and SW837 (Table 1) were able to grow in a variety of media supplemented with only 0.1% FCS when seeded at cell densities between 2–4 × 10^4 cells/cm^2. These poorly differentiated cell lines were able to proliferate in media such as DMEM, Waymouths, RPMI-1640, MCDB104 and in the combinations DMEM:Ham's F12 (1:1), DMEM:Waymouths (1:1), RMPI-1640:Waymouths (1:1) and RMPI-1640: MCDB104 (1:1) containing 0.1% FCS (for examples see Fig. 5). In contrast, neither growth nor viability of SW1222 cells was maintained in media such as RMPI-1640, Ham's F12, McCoys 5A, MCDB104 or Waymouths at similar cell densities.

The growth of the poorly differentiated cell lines in synthetic growth medium was somewhat slower than in serum-supplemented medium, specially SW48 (Fig. 5). However, SW620 and SW837 could be passaged routinely for 4 weeks with a split ratio of 1:8 per week in only DMEM (results not shown). Thus, poorly differentiated human colorectal cell lines can be grown and propagated in a variety of synthetic media devoid of exogenously added growth factors.

DISCUSSION

Several laboratories have been successful in defining the growth conditions of human colorectal cell lines and in estab-

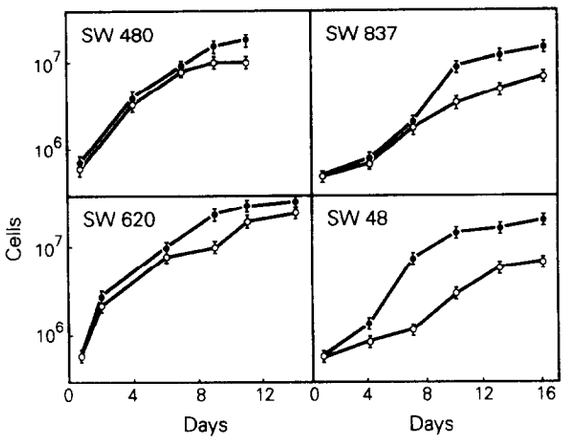

Fig. 5. Growth curves of poorly differentiated cell lines SW480 in RPMI-1640:Waymouths (1:1), SW620 in DMEM:Waymouths (1:1), SW837 in RPMI-1640:Waymouths (1:1), and SW48 in RPMI-1640 medium. 6×10^5 cells were seeded for all cell lines except SW480, which had 7×10^5, into 5 ml medium containing 0.1% (○) or 2% FCS (●). At each time point, three dishes were used to calculate the average cell number.

lishing lines from xenografted tumours in nude mice and primary explants from colon carcinomas [16, 21–24]. The purpose of the present study was to develop synthetic media for several human colorectal tumour cell lines with varying degrees of differentiation. The salient feature of our results is that these cell lines were shown to fall into three main categories. Firstly, the poorly differentiated colorectal tumour cell lines (SW620, SW480, SW48 and SW837) were able to grow in a variety of synthetic media devoid of exogenously added growth factors. Secondly, the moderately differentiated tumour cell lines SW1222 and HT29 required transferrin for growth and this was improved when other growth factors were added. Finally, the moderately differentiated tumour cell lines LS174T and PC/JW required both transferrin and insulin for maximal growth. All the cell lines were able to grow continously in growth factor-supplemented medium and the overall growth was better than in serum-supplemented medium for some differentiated cell lines.

In recent years it has become evident that neoplastic cells acquire complete or partial independence of mitogenic signals in the extracellular environment through different mechanisms [1, 2, 5–7]. These include production of growth factors that act in an autocrine or paracrine manner, alterations in the number or structure of cellular receptors and changes in the activity of postreceptor signalling pathways that either stimulate or suppress cell growth [1, 2, 6, 9, 25]. Although the results presented here do not provide direct evidence to distinguish between these possibilities, the influence of cell density on the ability of human colorectal cell lines to proliferate in serum-free medium strongly suggests that the release of factors that act in a paracrine manner may play a significant role: for example, SW1222 and HT29 may produce insulin-like growth factors, thus releasing them from insulin-dependent growth. In fact, Colouscou *et al.* [26, 27], have shown that the conditioned medium from HT29 cells can stimulate the proliferation of fibroblasts in the absence of serum. Two growth factors were elucidated, one exhibiting insulin-like growth factor I competing activity and the other one inhibiting EGF binding to its receptor. In addition, the colorectal lines SW620, SW480, SW48 and SW837, which grow in unsupplemented medium, could also produce their own factors. Coffey *et al.* [28] demonstrated that the serum-free conditioned medium of SW480 cells contained TGF-β activity although the cells had no TGF-β receptors, whilst SW620 produced TGF-α but had no EGF receptors. Further studies, however, are needed to define the role of these and other putative growth factors in autocrine and/or paracrine stimulation of growth of human colorectal cell lines.

Among the most striking genetic changes in colorectal cancer are those in the gene for p53 protein [29, 30], which has been identified as an antimitogenic signal [25]. Recently, Rodrigues *et al.* [30] have shown gross overexpression of mutated p53 in 50% of the malignant colorectal carcinomas whereas benign adenomas were all negative. The expression of p53 in colorectal cancer cell lines was also determined. Together with the results presented here, an intriguing correlation has been noted. Poorly differentiated cell lines which are able to grow in synthetic medium (e.g. SW480, SW620 and SW837) express very high levels of mutated p53 protein. In sharp contrast, moderately differentiated cell lines such as SW1222, LS174T and PC/JW, which required additional factors to grow (e.g. transferrin, EGF, insulin) did not express detectable levels of p53, as judged by immunoprecipitation and immunoblotting [30]. These results suggest that loss of growth factor requirement correlates with gross overexpression of p53. Whether these changes are causally linked or represent two independent changes in a multistep process leading to a poorly differentiated phenotype is an important question that warrants further experimental work.

In conclusion, we have shown that human colorectal tumour cell lines which are moderately differentiated will grow only in growth factor-supplemented medium, while those which are poorly differentiated will grow in unsupplemented medium. These serum-free growth conditions should prove useful for studies designed to elucidate the interplay between growth and differentiation in colorectal cells.

1. Heldin CH, Westermark B. Growth factors: mechanism of action and relation to oncogenes. *Cell* 1984, **37**, 9–20.
2. Sporn MB, Roberts AB. Autocrine growth factors and cancer. *Nature* 1985, **313**, 745–747.
3. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. *Cancer Res* 1986, **46**, 1015–1029.
4. Rozengurt E. Early signals in the mitogenic response. *Science* 1986, **234**, 161–166.
5. Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell* 1986, **46**, 155–169.
6. Pignatelli M, Bodmer WF. Integrin cell adhesion molecules and colorectal cancer. *J Pathol* 1990, **162**, 95–97.
7. Rozengurt E. Growth factors, cell proliferation and cancer: an overview. *Mol Biol Med* 1983, **1**, 169–181.
8. Silverberg E, Lubera J. Cancer statistics, 1987 *Cancer J Clin* 1987, **37**, 2–19.
9. Bodmer W. Somatic cell genetics and cancer. *Cancer Surv* 1988, **7**, 239–250.
10. Whitehead RH, Gardner J. *In vitro* growth of epithelial cells from mucosa derived from different regions of the gastrointestinal tract. *J Gastr Hepatol* 1987, **2**, 59–66.
11. Leibovitz A, Stinson JC, McCombs WB III, McCoy CE, Mazur KC, Maory ND. Classification of human colorectal adenocarcinoma cell lines. *Cancer Res* 1976, **36**, 4562–4569.
12. Fogh M, Trempe C. New tumour cell lines. In: Fogh J, ed. *Human Tumour Cell in vitro*. New York, Plenum Press, 115.
13. Tom BH, Rutzky LP, Jakstys MM, Oyasu R, Kaye CI, Kahan BD. Human colonic adenocarcinoma cells. I. Establishment and description of a new line. *In Vitro* 1976, **12**, 180–191.
14. Paraskeva C, Buckle BG, Sheer D, Wigley CB. The isolation and characterisation of colorectal epithelial cell lines at different stages of malignant transformation from familial polyposis coli. *Int J Cancer* 1984, **34**, 49–56.

15. Barnes D, Sato G. Methods for growth of cultured cells in serum-free medium. *Anal Biochem* 1980, **102**, 225–270.
16. Murakami H, Masui B. Hormone control of human colon carcinoma cell growth in serum-free medium. *Proc Natl Acad Sci USA* 1980, **77**, 3464–3468.
17. Huschtscha LI, Lucibello FM, Bodmer WF. A rapid micro method for counting cells *in situ* using a fluorogenic alkaline phosphatase enzyme assay. *In Vitro* 1989, **25**, 105–108.
18. Pignatelli M, Bodmer WF. Genetics and biochemistry of collagen binding triggered glandular differentiation in a human colon carcinoma cell line. *Proc Natl Acad Sci USA* 1988, **85**, 5561–5565.
19. Pinto M, Appay MD, Simon-Assman P *et al.* Enterocytic differentiation of cultured human colon cancer cells by replacement of glucose by galactose in the medium. *Biol Cell* 1982, **44**, 193–196.
20. Bellot F, Luis H, El Battari A, *et al.* Extracellular material secreted by human colonic adenocarcinoma cell lines promotes spreading in serum-free medium and induces neurite outgrowth of PC-12 cells. *Int J Cancer* 1985, **36**, 609–615.
21. Van der Bosch J, Masui H, Sato G. Growth characteristics of primary tissue cultures from heterotransplanted human colorectal carcinomas in serum-free medium. *Cancer Res* 1981, **41**, 611–618.
22. Zirvi KA, Chee DO, Hill GJ. Continuous growth of human tumour cell lines in serum-free media. *In Vitro* 1986, **22**, 369–374.
23. Park J-G, Oie HK, Sugarbaker PH, *et al.* Characteristics of cell lines established from human colorectal carcinoma. *Cancer Res* 1987, **47**, 6710–6718.
24. Boyd DD, Levine AE, Brattain DE, McKnight MK, Brattain MG. A comparison of growth requirements of two human intratumoral colon carcinoma cell lines in monolayer and soft agarose. *Cancer* 1988, **48**, 2469–2474.
25. Sager, R. Tumor suppressor genes: The puzzle and the promise. *Science* 1989, **246**, 406–412.
26. Culouscou J-M, Remaclc-Bonnet M, Garrouste F, Marvaldi J, Pommier G. Simultaneous production of IGF-I and EGF competing growth factors by HT29 human colon cancer line. *Int J Cancer* 1987, **40**, 646–652.
27. Culouscou J-M, Garrouste F, Remacle-Bonnet M, Bettetini D, Marvaldi J, Pommier G. Autocrine secretion of a colorectum-derived growth factor by HT29 colon carcinoma cell line. *Int J Cancer* 1988, **42**, 895–901.
28. Coffey RJ, Goustin AS, Soderquist AM, *et al.* Transforming growth factor α and β expression in human colon cancer lines: implications for an autocrine model. *Cancer Res* 1987, **47**, 4590–4594.
29. Nigro JM, Baker SJ, Preisinger AC *et al.* Mutations in the p53 gene occur in diverse human tumour types. *Nature* 1989, **342**, 705–708.
30. Rodrigues NR, Rowan A, Smith MEF *et al.* p53 mutations in colorectal cancer. *Proc Natl Acad Sci USA* 1990, **87**, 7555–7559.

Acknowledgements—We thank Dr Christos Paraskeva for supplying us with the PC/JW line.

Eur J Cancer, Vol. 27, No. 12, pp. 1684–1689, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

In vitro Methods for Screening Agents with an Indirect Mechanism of Antitumour Activity: Xanthenone Analogues of Flavone Acetic Acid

Lai-Ming Ching, Graeme J. Finlay, Wayne R. Joseph and Bruce C. Baguley

Xanthenone-4-acetic acid (XAA) resembles flavone acetic acid (FAA) in its effects on solid tumours in mice. The activity of methyl-substituted XAA derivatives *in vitro* was determined using 18 h ^{51}Cr-release assays, continuous exposure growth inhibition assays and stimulation of tumouricidal activity of cultured murine resident peritoneal macrophages. The macrophage assay identified the high biological activity and dose potency of 5-MeXAA *in vivo*, and was the most accurate *in vitro* predictor of the ability of congeners to induce either haemorrhagic necrosis of subcutaneous Lewis lung and colon 38 tumours or splenic natural killer activity. *In vitro* immune stimulation may be more appropriate than direct cytotoxicity for screening compounds with indirect mechanisms of antitumour activity.
Eur J Cancer, Vol. 27, No. 12, pp. 1684–1689, 1991.

INTRODUCTION

FLAVONE-8-ACETIC ACID (FAA), a synthetic flavonoid [1] has shown impressive preclinical activity against a broad spectrum of murine transplantable solid tumours [2–5], but clinical trials of FAA have been disappointing [6, 7]. Non-linear pharmacokinetics [8], low dose potency and problems of drug precipitation [9] have pointed to the need for better analogues of FAA. Towards this end, work from this laboratory has identified a series of xanthenone-4-acetic acid (XAA) derivatives with a similar antitumour action to that of FAA [10–12] and, in some cases, much greater dose potency.

FAA has been tested *in vitro* and found to have greater toxicity against leukaemic lines than solid tumour lines [2, 13]. On the other hand, solid tumours are more susceptible than leukaemic lines *in vivo* [4]. These discrepancies, as well as other observations, have led to the suggestion that FAA has a mechanism of action different from that of conventional direct cytotoxic agents [2, 13, 14], a suggestion strengthened by the observations

Correspondence to L.-M. Ching.
The authors are at the Cancer Research Laboratory, Auckland University School of Medicine, Auckland, New Zealand.
Revised 24 June 1991; accepted 8 Aug. 1991.