Culture of low passage colorectal cancer cells and demonstration of variation in selected tumour marker expression

Melanie Arul · April Camilla Roslani ·
Colin Leong Liong Ng · Swee Hung Cheah

Received: 10 January 2013 / Accepted: 3 June 2013 / Published online: 4 July 2013
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Abstract There is increasing evidence that a tumour comprises of heterogeneous population of cells. Thus, studying homogenous cell lines in vitro may yield results that are not reflective of the true situation in a tumour and studying low passage cell lines maintained in a heterogeneous population before they transform away from the original state may provide a more complete picture of colorectal cancer. A method was developed to isolate and establish low passage colorectal cancer cell lines from tumour biopsies. The media contents, combination of antimicrobials and specimen collection and transport conditions employed, successfully eliminated microbial contamination which is frequently present in samples obtained from the gastrointestinal tract. A variety of growth forms indicating a heterogeneous mixture of cells was seen in the initial cultures. Using fluorescence immunocytochemistry,

primary tumour cultures were shown to variably express selected tumour markers, carcinoembryonic antigen and C2 antigen. These low passage cell lines growing in a heterogeneous environment would more closely reflect the characteristics of the cells of the original tumour.

Keywords Colorectal · Cancer · Cell · Culture · Immunocytochemistry

Background

Colorectal cancer is one of the major malignancies in the world (WHO 2011) and a significant amount of research has been conducted to study the pathogenesis, progression and treatment of the disease. Pathologically, tumours originate from normal cells that accumulate genetic and epigenetic changes. The types and number of changes necessary for malignant transformation differ among tumour types, but a common feature among most types is variability in both genotype and phenotype among the cancer cells within a single tumour (Campbell and Polyak 2007; Heppner 1984; Marusyk et al. 2012; Nowell 1986; Shackleton et al. 2009; Fidler 1978). The progression and growth of a tumour is a complex process, and as cells develop and proliferate, further mutations occur. This could be due to interactions with other cells and changes in the microenvironment that allow certain cells to gain differential abilities to proliferate, maintain their “stemness” in particular niches, gain resistance to

M. Arul · S. H. Cheah (✉)
Department of Physiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia
e-mail: cheahsh@ummc.edu.my

M. Arul
e-mail: melanie_arul@yahoo.com.sg

A. C. Roslani
Department of Surgery, Faculty of Medicine, University
of Malaya, Kuala Lumpur, Malaysia

C. L. L. Ng
Tropicana Medical Centre, 11 Jalan Teknologi, Taman
Sains Selangor 1, PJU 5, Kota Damansara, 47810 Petaling
Jaya, Selangor Darul Ehsan, Malaysia

various drugs and treatment modalities and metastasize even though they may originate from a single cell or a homogenous group of cells (Li et al. 2008; Lodish et al. 2000; Marusyk and Polyak 2010). This tumour diversity leads to various responses to treatment, recurrence and relapses after initial treatment and is therefore recognized as the major hurdle to cure cancer (Li et al. 2008; Quintana et al. 2010; Sharma et al. 2010).

Therefore, the study of cancer cell population diversity is of great importance due to its profound implications for disease progression, diagnosis and treatment (Botchkina et al. 2009; Heppner and Miller 1983; Perkins and Swain 2009). A great deal of information concerning the cell biology of colorectal cancer has come from studies on homogenous cell lines derived from various laboratories and sources. However, the use of established homogenous cell lines from different sources may not reflect the possible spectrum of tumour cells that exists in a single population or even a single person. Moreover, within a supposedly “homogenous cell” population, genetic differences among cells have been found presumably because cells will mutate and transform away from their original primary characteristics with each increasing passage (Marusyk and Polyak 2010; Rubin 2001, 2005; Stockholm et al. 2007).

Thus, questions have been frequently raised concerning the validity of some of the findings in studies using established cell lines from various sources (Burdall et al. 2003; Failli et al. 2009; Hu et al. 2007; Lacroix and Leclercq 2004; MacLeod et al. 1999). Sometimes, the sources of the cells are ambiguous and the number of passages they have undergone are uncertain. Furthermore, studies have shown that repeated subculturing of cancer cells tend to transform their characteristics away from those of the primary tumour from which they originate (ATCC 2007).

Therefore, instead of focusing on homogenous cultures as representing an individual cell type or a bulk population of cells, it would be useful to establish low-passage cell lines from individuals in whom disease progression is known and in which the tumour heterogeneity is maintained. Cells sustained in low passages will more closely reflect the characteristics of the primary tumour, before the cells have an opportunity to transform from their primary characteristics and thus provide more meaningful information on the disease. The establishment of low-passage cell lines also allows for further study of the efficacy or

resistance to treatment modalities of fresh tumour cells obtained from patients and the design of individualized therapies. Therefore, a procedure to routinely and successfully obtain cell lines from patients would be expedient.

Culture of tumours from the colon is a challenge because of the high microbial content. Our initial attempts to establish cell lines from colorectal tumours of patients using published methods (Baten et al. 1992; Gibson et al. 1989) met with little success because we could not totally eliminate the unique population of microbes found within colon micro fauna resulting in contamination of the cells in culture. We therefore decided to review and redesign the procedures in order to overcome the contamination problems stated. Here, we describe the procedures and media contents that we have developed for the collection of tumour biopsies from colorectal cancer patients, transport of samples and isolation and establishment of cell lines *in vitro*. The cells were characterized by morphology and immunocytochemistry to probe for selected cell surface cancer markers.

Materials and methods

Tissue

Tumour samples were obtained from patients after approval of the study by the University of Malaya Medical Center ethics committee and with written informed consent from the patients. Immediately after surgery, specimens were handled under aseptic conditions by the pathologist and the tumour specimens were transferred into 30 ml of serum-free culture medium I (CM-I) (Roswell Park Memorial Institute (RPMI)-1640 (Hyclone, Thermo Scientific, Logan, UT, USA), 4 % of 100 unit/100 µg penicillin/streptomycin (PAA, Linz, Austria), 100 µg/ml gentamicin (ATC, Thailand), 100 µg/ml Primocin (InvivoGen, San Diego, CA, USA) and 100 µg/ml Normocin (InvivoGen)). Specimens were transported to the research lab on ice within 24 h. Generally, specimens of between 0.5 and 1.5 cm³ cubes were preferred. Upon arrival at the research laboratory, the tumour specimens were vibrated for about 10 min to remove fecal matter and then the tissue was washed with CM-I until the medium was clear of any dirt or particles. Connective and fatty tissues were dissected from the

tumour specimens and samples were incubated in 20 ml of CM-I for 20 min at 37 °C in a 95 % air/5 % CO₂ atmosphere. Specimens were meticulously dissected with sharp instruments into approximately 1 mm³ pieces while immersed in 10 ml of CM-I. The tissues were rewashed with CM-I and the procedure was repeated 2 more times. After the final wash, the cells were disaggregated and isolated from tumour biopsy by enzymatic disaggregation. First, the minced tissue was immersed in digestion solution I (DS-I) (0.25 % wt/vol trypsin (Gibco, Invitrogen, Carlsbad, CA, USA) solution, 2 % of 100 unit/100 µg penicillin/streptomycin (PAA), 50 µg/ml gentamicin (ATC), 100 µg Primocin (InvivoGen) and 100 µg Normocin (InvivoGen)). For every 1 cm³ of original tissue 20 ml of DS-I were used. The specimen was incubated overnight at 4 °C and then at 37 °C in a 95 % air/5 % CO₂ atmosphere for 30 min. The trypsin activity was stopped by the addition of serum-containing culture medium II (CM-II) (RPMI-1640 (Hyclone, Thermo Scientific), 10 % fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA), 4 % of 100 unit/100 µg penicillin/streptomycin (PAA), 100 µg/ml gentamicin (ATC), 100 µg/ml Primocin (InvivoGen) and 100 µg/ml Normocin (InvivoGen), 4 mM L-glutamine (Applichem, Darmstadt, Germany), 1 % Non-essential amino acids (NEAA) (Hyclone, Thermo Scientific) and 1 mM Na-pyruvate (Hyclone, Thermo Scientific)). The remaining undigested tissue was immersed in digestion solution II (DS-II containing collagenase (Gibco, Invitrogen) at 500 units/ml), with approximately 10 ml of DS-II for every 1 cm³ of the original tissue, and incubated at 37 °C for 1 h. Following dissociation of the tumour cells, the cells were suspended in CM-II (2 ml for every 1 cm³ of the original tissue) and seeded into T-25 culture flasks. Cell viability was not ascertained at this initial step and seeding density was not tightly controlled. Typically, 500 µl of suspended cells were seeded in each T-25 tissue culture flask. Once the cells had attached as observed under the phase contrast microscope, an additional 5–10 ml of CM-II were added to the culture flasks.

Maintenance of cell culture

Following adhesion of the cells, medium was replaced with culture medium III (CM-III) (RPMI-1640 (Hyclone, Thermo Scientific), 10 % FBS (Sigma), 1 % of 100 unit/100 µg penicillin/streptomycin (PAA),

50 µg/ml gentamicin (ATC), 100 µg/ml Primocin (InvivoGen), 100 µg/ml Normocin (InvivoGen), 4 mM L-glutamine (Applichem), 1 % NEAA (Hyclone, Thermo Scientific) and 1 mM Na-pyruvate (Hyclone, Thermo Scientific)) approximately every 3–4 days, depending on the cells' growth rate. During the first week of primary culture, when medium was changed, any unattached cells in the discarded medium were routinely recovered by bench centrifugation (110×g for 5 min) and replated with CM-III into the original flasks. Subsequently, cells were provided with conditioned medium by replacing only 50–80 % of old culture medium with a fresh CM-III. Cultures were subcultured after cells became confluent. At the first subculture, cells were detached with Dispase (BD Biosciences, Franklin Lakes, NJ, USA) (2 units/ml per 25 cm² culture flask) when the cells were still in log phase and at their healthiest state. Subsequently, cells were passaged with routine 0.25 % trypsin (Gibco)-0.02 % EDTA (Sigma) (Ethylenediaminetetraacetic acid) procedures [adapted with modifications from protocols described by (Paraskeva et al. 1984, 1989)]. Cell viability was assessed by the trypan blue dye exclusion method and the number of cells was determined using a hemocytometer (Macleod and Langdon 2004). In general, passage ratio and frequency was dependent on growth rate and initial cell passage was delayed until heavy tumour cell growth was observed. Subculturing from primary cultures was usually carried out in relatively small split ratios, e.g. 1/2 or 1/3. The time from initial plating to first passage usually ranged from 1 to 4 weeks but it may, in some cases, go up to 2–3 months. Absence of bioburden (bacteria, mycoplasma and etc.) was confirmed by standard testing procedures routinely employed in the laboratory; i.e. using Roche's mycoplasma detection kit (Cat. No. 11296744001, Roche, Mannheim, Germany) and Molecular Probe's cell culture contamination detection kit (Cat. No. C-7028, Molecular Probe, Eugene, OR, USA). Whenever possible, aliquots of cells were cryopreserved after each passage in FBS/10 % dimethyl sulfoxide (Applichem) and stored in liquid nitrogen.

Selective removal of fibroblasts

Fibroblasts were, if necessary, removed selectively by differential trypsinization between passages 1 and 3 (Kirkland and Bailey 1986). At confluence, the

medium was aspirated and the cells were incubated with 1 ml of 0.02 % EDTA for 5 min or longer at 37 °C to remove fibroblast from the heterogeneous culture of primary cells. To remove confluent fibroblastic sheets, 1 ml of 0.05 % trypsin in PBS (phosphate buffered saline) (Oxoid, Basingstoke, England) was added and incubation was done at 37 °C for not more than 5 min. 1 ml of fresh 0.1 % trypsin was added for an additional 5 min at 37 °C to harvest epithelial cells after fibroblasts had been removed. Trypsin activity was neutralized by adding 5 ml of CM-III. The cell suspension was centrifuged and the pellet was resuspended in fresh growth medium and seeded into a new culture flask.

Confirmation of tumoral nature of isolated cells by immunocytochemistry

Direct immunocytochemistry was used to detect for the expression of the tumour markers carcinoembryonic antigen (CEA) and C2 antigen. CEA is a tumour marker widely used in colorectal cancer (Duffy et al. 2007; Hammarström 1999) while C2 antigens are specific antigens secreted by colorectal and breast carcinoma cells (Iznaga-Escobar et al. 2004).

Primary cells were grown subconfluently on Lab-Tek chamber slides (Nunc, Penfield, NY, USA) according to the manufacturer's protocol. Each slide was overlaid with sufficient medium or buffer depending on the chamber slides surface area. For a 4-well chamber slide, 500–1000 µl were sufficient for each chamber. Each slide was fixed in 4 % paraformaldehyde (Sigma) and cells were permeabilized with 0.1–0.5 % Triton X-100 (Sigma) in PBS. After washing with PBS, non-specific antibody binding was blocked with 5 % BSA (bovine serum albumin) (Sigma) in PBS for 30 min at room temperature.

In indirect immunocytochemistry studies to probe for CEA and C2 antigen, cells were first incubated with unlabelled primary antibody. Primary antibodies used were anti-CEA monoclonal antibody (Dako, Glostrup, Denmark; Clone 11-7 antibody; Code No. M 7072) and anti-C2 antibody (gift from Innobiologics, Nilai, Malaysia). These antibodies were appropriately diluted optimally as determined from preliminary experiments (CEA was diluted in Dako antibody diluents whereas C2 was diluted in Dulbecco's Modified Eagle Medium or DMEM (Cellgro, Mediatech Inc, Manassas, VA, USA)) and slides were incubated for 60 min at room

temperature. The cells were rinsed three times with PBS (5 min each wash). The bound antibodies were detected by incubation with FITC conjugated CF 488A Goat Anti-mouse IgG (Biotium, Hayward, CA, USA, Cat. No. 20010) (1–10 µg/ml) for 60 min at room temperature. After washing with PBS, cells were further counterstained with 4', 6-diamidino-2-phenylindole (DAPI) (Invitrogen) in order to visualize the nuclei (which stained blue) and the slides were observed using a Nikon fluorescence Eclipse 80i microscope (Tokyo, Japan).

Direct immunocytochemistry using primary antibodies conjugated with different fluorescent dyes were used in order to simultaneously detect for the presence of CEA and C2 antigen in the same preparation of cells. First the primary antibodies were labelled with fluorescent dyes using the Mix-n-Stain antibody labelling kit (Biotium). CEA was covalently linked to CF488 dye (Biotium, Cat. No. 92233), a green fluorescent dye which is similar to Alexa Fluor 488 and FITC dyes. Meanwhile, C2 antibody was conjugated with a deep red fluorescent CF594 dye (Biotium, Cat. No. 92236), which is similar to Alexa Fluor 594 or Texas Red dyes. Both antibodies were labelled at an optimum concentration of 0.5–1 mg/ml according to the manufacturer's protocol and slides were incubated for 60 min at room temperature or overnight at 4 °C. Concentrations of the fluorescence labelled primary antibodies in the range of 30–50 µg/ml were preferred. The cells were rinsed three times with PBS (5 min each wash). After washing with PBS, cells were further counterstained with 4', 6-diamidino-2-phenylindole (DAPI) in order to visualize the nuclei (which stained blue). Staining was verified using appropriate positive (SW620 cell line; ATCC CCL-27) and negative controls (HeLa cell line; ATCC CCL-2) to ensure specificity of staining. Stained slides were viewed with a Nikon fluorescence Eclipse 80i microscope (Nikon Instruments Inc.).

Results

Establishment of primary colorectal cancer cell lines in vitro

Using this protocol, we have been able to culture and generate primary cell lines from colorectal cancer specimens without contamination. The cells were observed under the phase contrast microscope as they proliferated and attached.

Fig. 1 Progression of cell culture from initial culture (A), (B) and (C), and at third passage (D) from a tumour specimen of a patient with colorectal cancer. **A** Attached cells started to form polarized islets and pile up on the top of the first support—attached cell layer within few days after seeding the cells in culture flask (3–5 days in culture). **B** Cells started to grow in multilayers and form almost round, ball-like clumps on top of the first cell layer (5–8 days in culture). **C** Cells started to flatten out around a central, multilayered aggregate and started proliferating (usually within 4–5 days after spreading). **D** At third passage, the cells were polymorphic which indicated a heterogeneous population of cells in the culture flask (the

morphology of cultured cells under microscope at passage 3). Some were polygonal or cubical with distinct cell borders (i), others had irregular or poorly defined borders (ii), and others consisted of a more spindle-like (iii) or elongated (iv) morphologies. Most cells grew with large prominent nuclei, while some contained cytoplasmic vacuoles or granules. All images were captured from the initial culture after cell seeding till the subsequent subcultures (up to passage 3). The images were captured under the phase contrast microscope at $\times 10$ magnification (A), and at $\times 20$ magnification (B, C, and D). Similar growth characteristics were seen in other specimens obtained from different patients

Figure 1 shows the typical changes in tissue and cell morphology after plating and in the first few weeks of cell proliferation. First, the disaggregated cells started to attach to the substrate and formed polarized islets (Fig. 1A). The cells formed almost round, ball-like clumps on top of the first, support-attached cell layer. Cells started to pile up over the first cell layer and could grow preferentially in multilayers (Fig. 1B). The cells grew out from the pieces of tissue and gradually flattened out into thin monolayers around a central multilayered aggregate of cells (Fig. 1C). Proliferation was evident within 4–5 days once cells started spreading in the substrate. The photomicrographic results show that cells could be cultured from primary tumours. The cells cultured were heterogeneous as shown by the different morphologies, even at the third passage (Fig. 1D).

We have been able to isolate low passage cells from tumours about 20 times consecutively without contamination by this method. Selected established cell lines tested negative for the presence of bacteria/yeast and mycoplasma.

Immunocytochemistry

Immunocytochemistry studies show that the low passage cell cultures contain cancer cells that secrete tumour markers CEA and C2 antigen.

Figures 2 and 3 show the results of verifications of the immunocytochemistry protocols using the positive control SW620 colorectal cancer cell line and the negative control HeLa cell line. Results show that the antibodies stained positively for SW620 cell line while HeLa cells failed to stain.

Figure 4A shows the visualization of CEA expression (green fluorescence) in low passage cells cultured from a colorectal cancer specimen after the exposure of the cells to anti-CEA antibody followed by FITC-conjugated anti-IgG antibody. Most of the cells in this preparation, the presence and positions of which are indicated by the nuclei which are stained blue, appeared to express the CEA tumour marker. CEA is a glycoprotein and its expressions are mainly localized in the surface and cytoplasm of colorectal cancer (according to the antibody manufacturer's guideline).

Figure 4b similarly shows the presence of the tumour marker C2 antigen when cells were exposed to anti-C2 antibody followed by FITC conjugated secondary antibody. In this slide, it can be clearly shown that not all cells expressed C2 antigen. (e.g. cell indicated by the arrow). C-2 antibodies are specific for a colorectal tumour associated antigen C2; which is preferentially expressed on the surface of malignant colorectal cells (Iznaga-Escobar et al. 2004).

Fig. 2 Indirect immunofluorescence for the detection of CEA and C2 in SW620 and HeLa cell lines (control slides). The presence of CEA and C2 was detected by exposure of cells to primary anti-CEA antibodies and anti-C2 antibodies followed by secondary anti-IgG antibodies conjugated to FITC. The cells

were viewed under a fluorescence microscope. The presence and positions of the cells were detected by the nuclei which were stained blue with DAPI while the regions positive for CEA (A) and C2 antigen (B) fluoresced green. Magnification = $\times 20$

Fig. 3 Double direct immunofluorescence analysis of SW620 and HeLa cell lines for the expression of CEA and C2. Cells were exposed to primary antibodies against CEA and C2 antigen that had been directly conjugated to different fluoresceins that fluoresce at different wavelengths: Anti-CEA was conjugated to

CF488 dye which fluoresced green while anti-C2 antibody was conjugated to CF594 dye which fluoresced red. The nuclei of cells were stained blue and indicate the presence and positions of the cells. Areas where both CEA and C2 antigen were present appear beige. Magnification = $\times 20$

When a single preparation of cells was probed simultaneously with primary antibodies against CEA and C2 antigen to which fluoresceins emitting at different wavelengths were directly conjugated (anti-CEA antibody with CF488 dye, a green fluorescent

dye; and anti-C2 antibody with CF594 dye, a deep red fluorescent dye) the differential expression of CEA and C2 antigen by cells in a single population could be detected (Figs. 5, 6). The immunofluorescence varied considerably from cell to cell. Denser cell populations

Fig. 4 Indirect immunofluorescence for the detection of CEA (**A** series) and C2 (**B** series) in cells derived from low passage colorectal cancer specimen of a patient at third passage. The protocol and staining are the same as described in Fig. 2. **A4**, **A5**

are higher magnifications (at $\times 40$) of the areas shown in **A3**. **B4** is the magnification of the area in **B3**. Magnification (**A1–A3** and **B1–B3**) = $\times 20$; Magnification (**A4–A5** and **B4**) = $\times 40$

appeared brighter with increased fluorescence. Figure 5A1 is the bright field image of the cells under study. Figure 5A2 shows the presence and positions of cells as indicated by the DAPI stained nuclei (blue). Figures 5A3 shows the staining for CEA (green), Fig. 5A4 for C2 antigen (red), Fig. 5A5 is a composite of Figs. 5A2–A4, while Fig. 5A6 is a higher magnification ($40\times$) of the area indicated in Fig. 5A4. In

these latter pictures, the presence of CEA and C2 at the same area is indicated by a beige colour. It can be observed that both CEA and C2 antigens are expressed by the cells present, however the expression was variable (Fig. 5A5). In Fig. 5A6, at $40\times$ magnification it can be seen that there is a cell secreting both CEA and C2 antigen (i) and one secreting C2 antigen alone (ii). The C2 antigen appears to be associated

Fig. 5 Double direct immunofluorescence analysis of cultured cells for the expression of CEA and C2. The protocol and staining are the same as described in Fig. 4. **A1** shows the bright field image of the area of the slide tested, **A2** Dapi staining for nuclei (blue), **A3** staining for CEA (green) and **A4** staining for C2 antigen (red). **A5** is the merged image from **A2–A4**. **A6** is the

higher magnification ($\times 40$) of the area shown in **A5**. Areas with both CEA and C2 antigen appear beige. Some cells express both C2 antigen and CEA, e.g. (i) in **A6** whereas others primarily C2 antigen, (ii) in **A6**. Magnification (**A1–A5**) = $\times 20$; Magnification (**A6**) = $\times 40$

with the cell membrane, while CEA was somewhat patchy and much of it appears to be found in the cytoplasm. Similar patterns are seen in other areas of the preparation (Fig. 6). In Fig. 6A1, there is a cell that does not secrete either CEA or C2 (iii). In summary it can be observed that the cells in the preparation expressed CEA and C2 to varying degrees; some cells that secreted C2 antigen with little or no CEA and some cells that did not secrete either C2 antigen or CEA. Similar patterns were observed in preparations from 2 different patients.

Discussion

Tumours are complex structures which contain many different kinds of cells (Fidler 1978). While the study of homogenous cell lines has yielded much useful information, however, it must be realized that interaction between cells in a heterogeneous structure may be important in the progression and development of a tumour (Ogino et al. 2012; Stuelten et al. 2010). A homogeneous cell line involves a bulk population of cells that represents an average measurement (NCI 2012). During repeated culture of cell lines, the cells may undergo transformation which then may alter their basic biological characteristics away from the primary cells from which they are derived (Briske-Anderson et al. 1997; Chang-Liu and Woloschak 1997; Esquenet et al. 1997; Sambuy et al. 2005; Wenger et al. 2004; Yu et al. 1997). Importantly, primary cells derived from tumour biopsies and immortalized to homogeneous cells might represent only a small region of a tumour and might not be representative of the totality of a diverse cancer cell

population. Hence, treatment choices based upon homogenous cells might not inhibit all tumour cells and thereby lead to relapse in many patients (Iwasa and Michor 2011).

Since the tumour microenvironment may be unique in each individual, it is useful to study the cells at the early stages of culture and possibly in a heterogeneous state instead of using homogenous cell lines. The isolation and study of only a small number of cells derived from a heterogeneous population is now possible with the advent of single cell genetics and proteomic techniques which could provide good insight into the biology of cancers in their more natural state. The study of cancers from individual patients, the types of cell they possess and the biology and response to drugs will provide great insight into the biology and individualized treatment of the disease. To do this, it is desirable to be able to routinely culture cells from patients.

In this communication, we have focused on the culture of cells from colorectal cancer as this is one of the major cancers afflicting mankind. However, this poses a particular challenge because of the high incidence of contamination when specimens (in this case from the Malaysian population) were cultured. It could be due to the particular diet or exposure to the different flora present in the intestines of the Malaysian population which are resistant to the standard concentration and types of antimicrobials used.

We have therefore reviewed and readjusted the procedures with judicious use of different levels and combinations of antimicrobials. The procedure that we have developed has been successful in eliminating the contamination problems and we have been able to obtain cells from patient specimens without contamination. The cells obtained in culture displayed a

Fig. 6 Double direct immunofluorescence analysis of cultured cells for the expression of CEA and C2. **A** and **B** series show merged images from other areas of the preparation shown in Fig. 5 indicating varying expression of CEA and C2 antigen in

different cells. Notice that some cells do not stain for either CEA or C2, e.g. (iii) in **A1**. Magnification (**A1** and **B1**) = $\times 20$; Magnification (**A2** and **B2**) = $\times 40$

multiplicity of morphologies, indicating the heterogeneity of the cells present.

The cultures derived from the tumour specimens contain cancer cells as evidenced by the presence of the cancer markers CEA and C2 antigen; expressed at the cytoplasm and cell membrane. However, interestingly not all cells expressed the markers to the same degree, and indeed some cells seem to secrete only one marker. Some cells did not seem to express either of the markers tested, and so either they were not malignant cells, or they may be expressing cancer markers other than CEA and C2 that were not tested. This suggests that not all cells in population are homogenous. These low passage cells may not have transformed very far, if at all, from the primary cells and may still possess the biological properties of the original tumour. Moreover they are still in contact with other cells of the original tumour and might still preserve much of their cell-to-cell interactions. Thus it would be very educational and useful to study these cells at this point. The ability to derive these cells routinely also allows the examination of these cells from different individuals and perhaps drive individualized targeted treatment for colorectal cancer.

Conclusions

In conclusion, in this study, we have developed a method to culture and obtain low passage cell lines

from colorectal cancer specimens without contamination. These cultures contain cells with a multiplicity of morphologies. Fluorescence immunocytochemistry show that many cells variably secrete the cancer markers CEA and C2 antigen. These results suggest that the cells derived are heterogeneous in nature. Work is under way or planned to look for further markers and examine the genetic expression of individual cells in a population of cells obtained from tumours derived from different individuals. A fundamental understanding of the biology of the heterogeneity of cells within a tumour can give rise to targeted therapies that optimises positive treatment outcome.

Acknowledgments We acknowledge the financial support received from the MAKNA Cancer Research Award 2007, University of Malaya Research University Grant RG014/09HTM and University of Malaya Postgraduate Research Fund PS288/2007B. We thank the University Malaya Medical Centre, Kuala Lumpur surgeons who were involved in providing the tumour specimens for this study.

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