

## MINI REVIEW

# Cell exfoliation in the human colon: Myth, reality and implications for colorectal cancer screening

Alexandre Loktionov\*

Colonix Medical, Babraham Research Campus, Cambridge, United Kingdom

Colonocyte exfoliation in the human colon constitutes a unique mechanism of cell population control that can undergo significant changes under different physiological and pathological conditions. Being closely related to the apoptosis and anoikis, cell exfoliation from colonic epithelium appears to be a relatively rare event in normal conditions, but its rate dramatically increases in neoplasia, when cell removal by apoptosis *in situ* does not function properly. Several studies show that significant numbers of exfoliated colonocytes are not lost in the faecal contents of the gut, but retained in the mucocellular layer overlying colonic mucosa. Recent observations allow hypothesizing that the mucocellular layer containing exfoliated colonocytes may gradually migrate distally, eventually leading to the accumulation of the cells exfoliated from malignant colorectal tumours on the surface of the rectal mucosa. Implications of exfoliated colonocyte analysis to colorectal cancer screening and early diagnosis are discussed.

© 2007 Wiley-Liss, Inc.

**Key words:** colonic epithelium; cell exfoliation; mucocellular layer; neoplastic growth; colorectal cancer screening

Intense investigation of epithelial cell proliferation in the mammalian intestinal mucosa has resulted in a significant progress in understanding regulatory mechanisms governing cell dynamics in this rapidly self-renewing cell population. This understanding greatly assisted in developing theoretical models of human colorectal carcinogenesis addressing both its molecular mechanisms<sup>1–4</sup> and sequences of events in tumour morphogenesis.<sup>5–10</sup> As numerous studies in this area were devoted to the investigation of colonocyte proliferation and differentiation, relatively little remained known about the final natural destiny of these cells. Obligatory exfoliation of terminally differentiated colonocytes was traditionally believed to be the predominant way of cell loss in colonic epithelium,<sup>11</sup> but this popular notion remained essentially hypothetical in the absence of convincing firm evidence. Given that it has become generally admitted that exfoliated colonocyte analysis may open new approaches to colorectal cancer screening and early diagnosis, detailed knowledge and better understanding of the exfoliation of colonic epithelium and its changes in neoplasia is of high practical relevance. The present brief review addresses this surprisingly obscure area with the purpose of clarifying a few points still provoking controversy and confusion. Perspectives of using the phenomenon of colonic cell exfoliation for screening and diagnosis of colorectal cancer and related diseases are then re-evaluated in view of recent advances in the field.

### Cell exfoliation in the colonic epithelium: Its role in normal physiological conditions and neoplasia

Colonic epithelium is known to be one of the most dynamic cell populations of the human organism.<sup>4,8,11,12</sup> Meticulous investigation of the well-structured organisation of the colonic mucosa allowed constructing a detailed and credible model of colonocyte proliferation, differentiation and migration especially important in relation to colorectal carcinogenesis. It is generally accepted that in normal physiological conditions stem cells of colonic epithelium are located at the base of the crypt.<sup>4,7,8,12</sup> Their progeny migrates upward, gradually undergoing differentiation and losing

proliferative capacity until terminally differentiated nondividing colonocytes reach surface (luminal) epithelium.<sup>4,11,12</sup> There these cells end their short lives, being replaced by next generations of differentiating counterparts. Indeed, extremely high colonocyte proliferation rate and constant flow of new cells migrating from crypts toward the lumen demand an adequate efficiency of cell elimination. For decades it was presumed that all highly differentiated colonocytes of the luminal compartment of colonic epithelium are eventually exfoliated into the lumen.<sup>7,11</sup> This theory may be generally correct for the colonic epithelium of rodents,<sup>13</sup> but there are good reasons for doubts about its applicability to humans. Although some authors still believe that massive exfoliation is constantly going on with millions of colonocytes shed into the faecal stream daily,<sup>14,15</sup> there is accumulating evidence that the rate of cell exfoliation from normal human colonic mucosa is much lower than it was previously believed.<sup>16–19</sup> Apoptosis *in situ* followed by the engulfment of apoptotic cells by adjacent colonocytes or subepithelial macrophages appears to be the main pathway of cell death in healthy colonic epithelium,<sup>16,17,20,21</sup> colonocyte exfoliation being an important, but auxiliary mechanism of cell loss. This situation can, however, be completely reversed in neoplastic growth. Despite the absence of a complete consensus on the precise order of the emergence of initial neoplastic lesions with both “bottom-up”<sup>6–10</sup> and “top-down”<sup>5,7</sup> theories in use, it is apparent that intercryptal (luminal) epithelium becomes involved in the process very early. Although even small neoplastic loci can bear severe alterations in the homeostatic control of cell reproduction and loss, little is known about colonocyte exfoliation in early tumours. Later exfoliation changes are much better documented. It is well established that profound deregulation of apoptosis is a characteristic feature of cancer.<sup>2,22,23</sup> Moreover, the loss of cell adhesion associated with malignant progression and normally regarded in relation to the metastatic process<sup>24–26</sup> can further change the pattern of cell loss in a growing tumour in favour of exfoliation. Indeed, a dramatic increase of colonocyte exfoliation in colorectal cancer patients has been reported by several groups.<sup>18,27–31</sup> Ahlquist *et al.* observed accumulation of exfoliated malignant cells in the mucocellular layer overlying tumour surface, but not over unchanged colonic mucosa.<sup>18</sup> In a recent study<sup>32</sup> we collected exfoliated cells from mucosal surface of resected colon specimens immediately after operations and were able to show that thousands of cells are exfoliated from the surface of malignant colorectal tumours. These cells as well as multicellular fragments of well-preserved neoplastic tissue (Fig. 1) were found within the mucocellular layer not only over tumours, but even at significant distances distally from tumours indicating that exfoliated material

There may be a potential financial conflict of interest resulting from the professional status of the author as a director of a private company (Colonix Medical Ltd).

Grant sponsor: EEDA (East of England Development Agency).

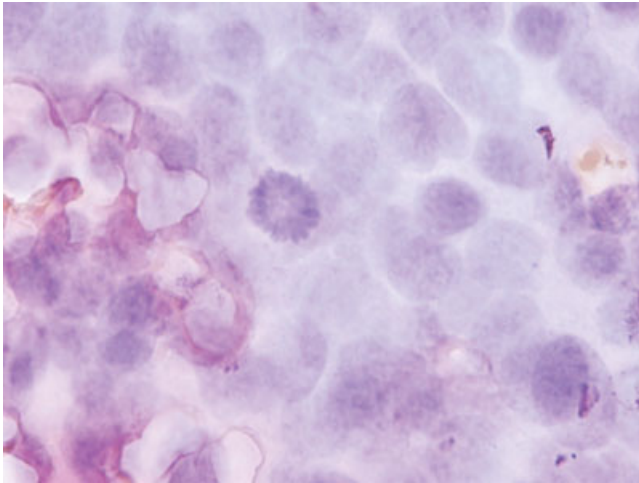
\*Correspondence to: Colonix Medical Limited, Babraham Research Campus, Cambridge, CB22 3AT, United Kingdom.

E-mail: alex.loktionov@colonixltd.co.uk

Received 14 June 2006; Accepted after revision 11 January 2007

DOI 10.1002/ijc.22647

Published online 9 March 2007 in Wiley InterScience (www.interscience.wiley.com).



**FIGURE 1** – Mitotic cell (metaphase) in a tissue fragment exfoliated from a rectal adenocarcinoma. The fragment collected directly from the surface of unchanged rectal mucosa 7 cm distally from the tumour margin (material collected from a resected colon segment within 30 minutes after operation).

tends to migrate alongside the faecal flow, but mostly without being incorporated into the faeces. Even mitotic cells (Fig. 1) could be occasionally detected in the exfoliated clusters of malignant cells, clearly indicating that these cells preserved viability and in some cases proliferative potential. The findings were perfectly in line with clinical reports describing secondary distal, especially anal, tumours in colorectal cancer patients.<sup>33–35</sup> Such tumours, albeit rare, appear to result from an unusual metastatic process involving the reimplantation of viable exfoliated malignant cells migrating within the mucocellular layer. Likewise, occasional intraperitoneal seeding of exfoliated cancer cells during surgery for colorectal cancer can lead to peritoneal carcinomatosis.<sup>36</sup>

Analysis of the available literature combined with recent observations of our group allows proposing a partially hypothetical scheme of changes in colonocyte exfoliation occurring alongside the growth of malignant tumours (Fig. 2). The scheme highlights the following 3 hallmarks characterizing cell exfoliation in colorectal cancer:

- a. extremely high level of cell exfoliation from the surface of malignant tumours;
- b. preferential location of exfoliated cells and their remnants in the mucocellular layer between colonic mucosa and faecal contents of the gut; and
- c. gradual distal movement of the cell-containing mucocellular layer.

The first two of these points have already been discussed in a recent review.<sup>21</sup> Observations of our group supporting the last point are still awaiting peer-reviewed publications; thus, the author has to consider it as a new hypothesis.

It should be admitted that little is known about cell exfoliation at early stages of carcinogenesis in the human colon. Further studies are needed to reveal molecular mechanisms triggering the dramatic increase of colonocyte exfoliation as well as the precise moment when this “switch” occurs. It seems to be logical that the exfoliation increase coincides with apoptosis deregulation and/or cell adhesion loss, but firm evidence is still absent. It also remains unclear whether cell exfoliation from the surface of colorectal carcinomas should be regarded as a protective mechanism or a manifestation of increasing tumour invasiveness.

One can suggest that the enhancement of exfoliation is initially a protective response directed to activating anoikis<sup>37,38</sup> by cell detachment from the basal membrane when apoptosis *in situ* fails to occur. Alternatively, it may be just another manifestation of neoplastic progression when tumour starts producing autonomous potentially metastatic cells ready for secondary implantation. The latter assumption is corroborated by the cited reports of secondary anal metastases after removal of primary colorectal cancers<sup>33–35</sup> or peritoneal cancer dissemination during operations.<sup>36</sup> However, the 2 mechanisms are likely to be inseparably entangled since well-preserved colonocytes, apoptotic cells and cell debris are all abundant in the mucocellular layer of cancer patients.

It should be accepted that even phenomenology of cell exfoliation in colorectal cancer is still far from being completely clear. The scheme in the Figure 2 may be correct till some point in the progression of cancer (*e.g.*, Fig. 2d), but it is impossible to exclude a secondary decrease in cell exfoliation from the primary tumour associated with the development and growth of its local and remote metastases. This is another obscure and theoretically challenging area remaining to be studied.

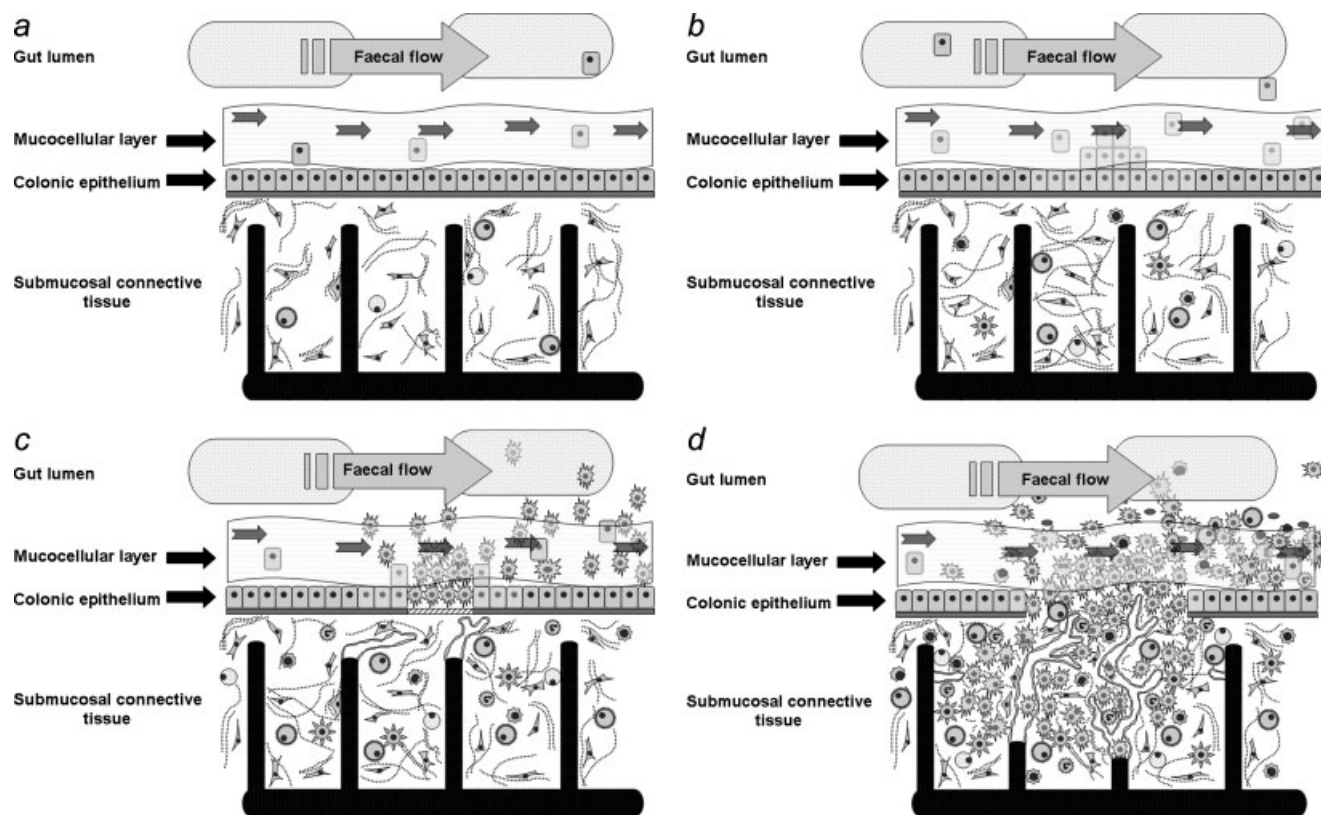
#### Methodological approaches to the isolation and analysis of human exfoliated colonocytes

The possibility of obtaining well-preserved epithelial cells from colorectal washings and their use for cytological diagnosis of neoplastic conditions had been described for the first time over 50 years ago.<sup>27</sup> However, the proposed procedures of colorectal irrigation and material collection<sup>27,28</sup> looked complicated and were difficult to standardize. This, together with the exclusively cytological approach to colonocyte analysis, resulted in a slow progress in the development of exfoliated cell collection techniques.

The interest to cell exfoliation in the human colon had re-emerged with the introduction of the Vogelstein’s genetic model for colorectal carcinogenesis.<sup>1</sup> As analytical methods for the detection of cancer-associated molecular markers became available, it was logical to suggest that cells exfoliated from colorectal tumours could provide sufficient amounts of DNA for such analyses. The idea of obligatory colonocyte shedding into the faecal stream discussed in the first section of this review was generally accepted at the time, so it looked transparent that human stool samples could provide exfoliated colonocytes for either cytological characterization or nucleic acid and protein isolation for molecular biomarker analysis. This noninvasive approach to cellular material collection promised to open a convenient way to colorectal cancer early diagnosis and screening.

In the beginning of the nineties, Nair *et al.* published 2 papers describing exfoliated colonocyte isolation from dispersed samples of human stool by Percoll density gradient centrifugation. The group claimed that millions of viable exfoliated colonocytes could be obtained from a few grams of stool.<sup>39,40</sup> Although those publications certainly had a positive effect attracting attention to an important physiological phenomenon, the main claim of the authors on the isolation of millions of viable colonocytes from small amounts of faecal material was not convincingly substantiated morphologically and remained debatable.

Several groups of scientists attempted exfoliated colonocyte collection from either stool samples or colorectal mucosa (see Table I). It is apparent that attempts of using human faeces as the source of exfoliated colonocytes were only partially successful. Indeed, it is difficult to expect finding viable colonocytes in stool homogenates even if they were entering the faecal milieu in millions. The likelihood of the presence of well-preserved colonocytes in the anaerobic faecal environment rich in bile acids and other cytolytic agents appears to be very low. One possible minor



**FIGURE 2** – Scheme of cell exfoliation changes during colorectal carcinogenesis. *a*: Physiological conditions—low exfoliation. *b*: Benign tumour (small polyp)—low exfoliation (possibly slightly higher than normal). *c*: Malignisation/early cancer—increased exfoliation (change of apoptosis/exfoliation ratio). *d*: Invasive cancer—dramatically increased exfoliation (free cells and blood also present). It should be understood that the scheme does not reflect the complexity of the structure of colorectal mucosa (epithelium is shown as a simple monolayer without crypts, blood vessels as straight tubes etc). Likewise, the presence of apoptotic cells and cell debris that should be abundant in the mucocellular layer is not shown.

source of cells inside faecal samples can be provided by strands of colonocyte-impregnated mucus from the mucocellular layer that are folded into the stool.<sup>21</sup> The latter assumption remains to be proven, but fragments of the mucocellular layer are certainly excreted on the stool surface. In our earlier studies we explored this phenomenon initially in a rat model of colon carcinogenesis,<sup>48</sup> and then developed a method for human exfoliated colonocyte isolation based on the use of surface washes from whole stool samples in combination with immunomagnetic bead-based cell separation.<sup>19,30</sup> The method allowed isolation of well-preserved colonocytes and was later employed by other groups for the identification of cancer-associated cellular markers<sup>49,50</sup>; however, few colonocytes were usually obtained, whereas squamous cells of the anal canal epithelium often dominated the pool of isolated cells. In addition, the standardization of the technique was extremely difficult because of the necessity to use intrinsically variable whole stool samples. Two other groups also tried immunomagnetic cell capture, using stool homogenates.<sup>42,43</sup> Although application of diagnostic cytology was attempted by one of these groups upon collection of material from cancer patients, no information on cell yield was provided, and cytology results were inconclusive in most cases.<sup>43</sup> It is apparent that obtaining material for DNA or RNA examination was the main goal of exfoliated cell isolation in most studies of this type.<sup>42-43</sup>

The analysis of the reviewed reports on colonocyte isolation from human stool samples shows that none of the employed techniques could provide significant amounts of exfoliated cells

suitable for reliable cytological assessment or intracellular biomarker identification. Given that recent efforts focused on the direct extraction of human DNA and RNA from stool samples have produced encouraging results (see later), the idea of preliminary isolation of exfoliated colonocytes from stool with the same only purpose of extracting nucleic acids now starts to look less attractive.

It was known for decades that exfoliated colonocytes could be obtained by colorectal lavage (see Table I); however, this procedure, being inconvenient and difficult to standardize, is now rarely employed. Nevertheless, it appeared that direct collection of exfoliated colonocytes from the surface of human rectal mucosa could provide substantial amounts of well-preserved cells. Following the hypothesis on colonocyte migration within the mucocellular layer discussed earlier in this review, we have developed a simple sampling device for the direct collection of exfoliated cells from the surface of human rectal mucosa with minimal invasiveness.<sup>46</sup> The author believes that discussing promising preliminary results of our ongoing trials here is not appropriate since they have only been reported in a meeting,<sup>47</sup> but not published so far. At the same time presenting a few examples of cellular material collected from the surface of rectal mucosa (see Fig. 3) is essential for this review. One remarkable observation (Fig. 3f) clearly indicates that cells exfoliated from the surface of proximal colorectal cancers can reach rectum in the mucocellular layer without being lost. Although various aspects of the new technique require further investigation, there are good reasons to hope that direct collection of exfoliated cells from the surface of rectal mucosa can be prefer-

TABLE 1 - HUMAN EXFOLIATED COLONOCYTE ISOLATION METHODS (CRITICAL ASSESSMENT AND COMPARISON)

Cell collection sources	Method of cell isolation	Exfoliated cell yield	Exfoliated cell morphology and squamous (anal) epithelium presence	Faecal contamination of collected material	Technical (reproducibility, standardisation) problems	Applicability of analytical approaches (quantitative/qualitative)	References
<i>Stool-based techniques</i> Small amounts of faecal material (1 g).	Stool dispersion followed by density gradient centrifugation.	Claimed to be millions of viable cells per gram of stool; however, this claim seems to be doubtful.	No convincing evidence of well-preserved cells. The presence of cell components including those derived from squamous epithelium was likely.	Always strong.	The technique appeared to be standardized, but claimed results look exaggerated and not substantiated by cytological analysis, making reproducibility highly questionable.	Isolated material may contain some cells and cell components; thus, biochemical and molecular methods can be applied. Quantitative approaches highly questionable.	39, 40
Small amounts of faecal material (5 g).	Stool dispersion followed by a Percoll centrifugation-based technique (a modification of the technique of Nair <i>et al.</i> <sup>39-40</sup> ).	Appears to be low, however, no estimate given.	Cell debris rather than cells mostly obtained. The presence of squamous cell components was likely.	Always strong.	The technique was found to be difficult to standardize and reproduce. Later abandoned.	RNA isolation and gene expression analysis shown to be possible.	41
Small amounts of faecal material (1 g).	Stool dispersion followed by either a Percoll centrifugation-based technique (a modification of the technique of Nair <i>et al.</i> <sup>39-40</sup> ) or immunomagnetic bead-based separation.	Not assessed.	Isolated cells were not cytologically characterised. The presence of squamous cell components was likely.	Always strong.	Immunomagnetic separation was found to be superior in terms of reproducibility compared to Percoll-based isolation.	Cell isolation did not improve qualities of isolated DNA and RNA compared to direct isolation from faeces.	42
Small amounts of faecal material (5-10 g).	Stool homogenisation followed by immunomagnetic bead-based separation.	Not assessed.	Isolation of colonocytes reported to be cytologically confirmed. The presence of squamous cells not evaluated.	Always strong.	Immunomagnetic separation was found to be far superior in terms of reproducibility compared with Percoll-based isolation.	DNA extracted from isolated cells was suitable for amplification and molecular analysis.	43
Stool surface (whole stool samples).	Stool surface (part of the mucocellular layer excreted with faeces) was washed with a cell-dispersing solution. Cells isolated using immunomagnetic bead-based separation.	Very low in normal conditions, higher (hundreds) in samples from colorectal cancer patients.	Few well-preserved colonocytes could be isolated. Squamous epithelial cells were commonly present.	Usually strong.	The technique was reproducible; however, its standardization was hardly possible.	Isolated cells could be used for cytological and immunohistochemical assessment. DNA isolated from cells could be quantified and successfully amplified.	19, 30

TABLE I - HUMAN EXFOLIATED COLONOCYTE ISOLATION METHODS (CRITICAL ASSESSMENT AND COMPARISON) (CONTINUED)

Cell collection sources	Method of cell isolation	Exfoliated cell yield	Exfoliated cell morphology and squamous (anal) epithelium presence	Faecal contamination of collected material	Technical (reproducibility, standardisation) problems	Applicability of analytical approaches (quantitative/qualitative)	References
<p><i>Techniques based upon cell collection from colorectal mucosa</i></p> <p>Surface of the colorectal mucosa (distal segment).</p>	<p>Colorectal lavage followed by simple centrifugation (or gradient centrifugation<sup>44,45</sup>).</p>	<p>High (hundreds to thousands easily identifiable cells).</p>	<p>Well-preserved colonocytes can be isolated. Squamous cells always present.</p>	<p>Variable.</p>	<p>Standardization difficult (variability of the recovered lavage liquid volume).</p>	<p>Standardization problems make quantitation difficult. Cell-based and molecular qualitative analytical methods applicable.</p>	<p>27, 28, 44, 45</p>
<p>Surface of the rectal mucosa</p>	<p>Direct collection of exfoliated cells from the mucocellular layer overlaying the surface of rectal mucosa using a thin inflatable membrane.</p>	<p>High (hundreds in normal conditions; thousands in cancer cases or severe inflammatory conditions).</p>	<p>Very well-preserved colonocytes. Occasional squamous epithelium presence.</p>	<p>Mostly low (strong contamination in less than 10% cases).</p>	<p>Well standardized and reproducible.</p>	<p>Collected cells can be used for a wide range of quantitative and qualitative analytical techniques.</p>	<p>32, 46, 47, A. Loktionov <i>et al.</i>, unpublished observations</p>

able compared with stool-based techniques in terms of providing high quality material for a wide range of diagnostic and research applications.

**Exfoliated colonocytes, DNA extracted from stool and detection of molecular biomarkers of colorectal cancer**

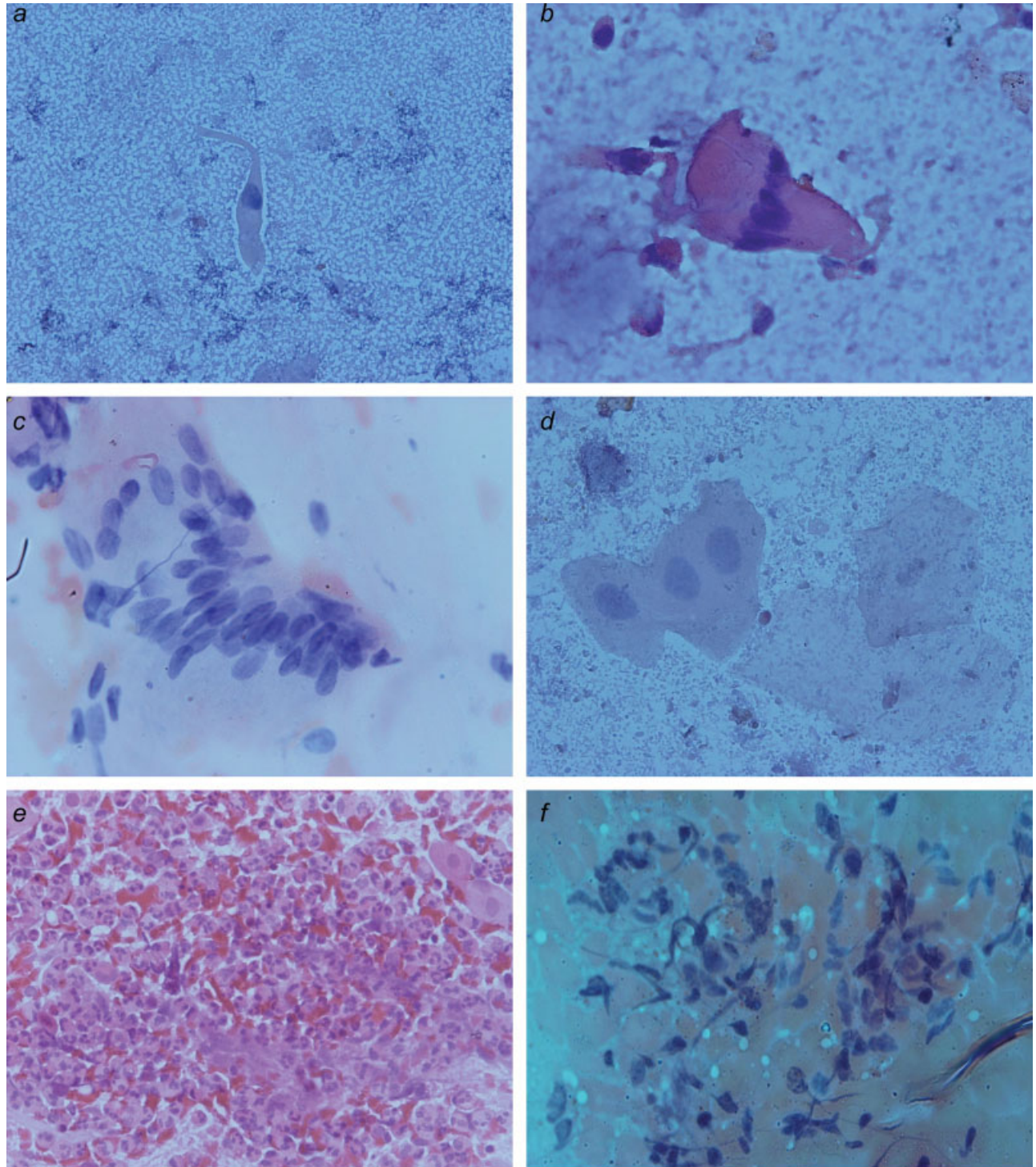
It has already been mentioned that with the development of the Vogelstein's genetic model for colon carcinogenesis in the early nineties,<sup>1</sup> the possibility of using tumour-derived DNA for colorectal cancer screening and early diagnosis attracted considerable attention. Initial report on the demonstration of the presence of *K-ras* oncogene mutations in DNA isolated from stool samples of patients with colorectal malignancies<sup>51</sup> provoked a surge of interest to the detection of cancer-associated molecular markers in the human DNA present in faeces. Although it is usually presumed that this DNA originates exclusively from exfoliated colonocytes excreted with stool,<sup>21,52-58</sup> one can argue that well-preserved squamous epithelial cells (see Fig. 3d) mechanically sloughed in abundance from the surface of the anal canal epithelium during every stool passage become attached to the stool surface and can considerably contribute to the final DNA yield. The impact of this DNA, which certainly "dilutes" colonocyte-derived material, should not be disregarded as an interfering influence in detecting the molecular changes relevant for colorectal cancer.

The most important problem with the diagnostic value of stool DNA analysis is related to the apparent complexity of colorectal carcinogenesis involving multiple metabolic and regulatory pathways governed by interacting networks of genetic control.<sup>3</sup> At the present level of our knowledge there is no identified reliable single cancer-specific molecular biomarker (*i.e.*, some "unifying" marker always present in tumour rather than normal cells making neoplastic cells unambiguously detectable). The main consequence of this unfortunate problem is the necessity of using overlapping sets of multiple complementary molecular markers (multitarget DNA assay panels or MTAPs) present in malignant cells at relatively high frequencies.<sup>21,52-58</sup>

Moreover, from the methodological point of view, human stool is a difficult material for DNA extraction: the abundance of bacteria and cytolytic substances in faeces can interfere with both colonocyte DNA stability and isolation procedures, often resulting in the coisolation of undesirable bacterial DNA and substances interfering with PCR. For these reasons the task of obtaining representative amounts of high quality human DNA from faecal material is always challenging.<sup>55,56,59,60</sup>

Although the stool DNA-based approach has already brought some promising results,<sup>52-58</sup> it is evident that its introduction as a common tool for colorectal cancer screening and early diagnosis is still impossible. Given that on one hand the only large prospective screening trial testing this approach resulted in a sensitivity of 51.6% (specificity 94.4%),<sup>57</sup> and on the other hand the cost of the test comprising a multitarget panel of 21 molecular markers (detection of multiple point mutations in the sequences of the *K-ras*, *APC* and *p53* genes, microsatellite instability of the *Bat-26* gene and long fragment DNA analysis) is over \$700 per assay (Pre Gen Plus test marketed by EXACT Sciences), we are still a long distance away from the ideal of cost-effective and simple colorectal cancer screening based on the molecular analysis of stool-derived DNA. Using a Markov model for cost-effectiveness assessment for mass colorectal cancer screening, Song *et al.*<sup>61</sup> concluded that the approach can become marginally cost-effective only at \$195 per assay presuming that its sensitivity reaches 65% for cancers and 40% for large polyps. Although rapid methodological progress allows room for future optimism, it is obvious that the assessment of stool samples for the presence of molecular cancer markers is excessively expensive in its present form.<sup>61-63</sup>

The use of relatively uncontaminated exfoliated colonocytes collected from rectum<sup>47</sup> might help in overcoming problems asso-



**FIGURE 3** – Examples of exfoliated material collected directly from human rectal mucosa of unprepared individuals. *a*: Single exfoliated colonocyte displaying typical morphological features of this cell lineage. Note that the cell does not display any apoptotic features. *b*: Simultaneously exfoliated small group of well-preserved colonocytes. *c*: Larger group of exfoliated normal-looking colonocytes. *d*: Occasionally collected typical cells of squamous (anal) epithelium. *e*: Active ulcerative colitis. Abundance of neutrophilic leucocytes. Macrophages, epithelial cells with changed morphology, apoptotic cells and blood also present. *f*: Ascending colon cancer. Malignant colonocytes with a pronounced nuclear polymorphism. Abundant blood presence.

ciated with the analysis of stool samples, but additional research is required to prove this point.

It should also be taken into account that recent advances in the understanding of the structure and function of the human genome

have resulted in a breathtakingly rapid development of such complex disciplines as genomics and proteomics making possible targeted detailed investigation of disease-related gene expression patterns. Presently there are only a few reports describing assess-

ment of specific RNA expression in human stool samples<sup>42,64,65</sup>; however, the opportunity of using directly collected colonocytes for RNA isolation should also be considered as a possible alternative to stool-based techniques.

### The place of exfoliated colonocyte analysis among other methods applied for colorectal cancer screening and early diagnosis

The problem of colorectal cancer screening has already been thoroughly discussed in a number of recent publications<sup>52,66–68</sup>; therefore, it is considered here very briefly, with the emphasis on the possible use of exfoliated colonocytes. Although benefits of the active screening are now generally accepted,<sup>52,66–68</sup> the disease remains among the leading causes of oncological mortality. The search for suitable approaches to colorectal cancer screening and early diagnosis is going on for decades, but the choice of available techniques is still strictly limited, faecal occult blood testing (FOBT) being the most common method.<sup>52,69,70</sup> Advantages and drawbacks of possible screening applications of full colonoscopy and flexible sigmoidoscopy as well as new approaches such as virtual colonoscopy and detection of molecular biomarkers in faeces (see previous section) have been discussed in several recent reviews.<sup>21,52,71,72</sup> Unfortunately, neither of these methods can offer the desirable combination of low invasiveness, simplicity and affordable cost with high sensitivity and specificity. FOBT is noninvasive, cheap and simple, but all versions of this test produce high rates of both false-negative and false-positive results.<sup>21,52,70,73,74</sup> Even its reported reducing effect on the incidence of colorectal cancer<sup>69</sup> is now questioned.<sup>75</sup> Colonoscopy, when performed by an experienced specialist, can be regarded as a precise and reliable diagnostic procedure; however, it is invasive and expensive,<sup>21,52,71</sup> thus its use for routine population mass screening appears to be problematic. Moreover, the cumulative risk of complications becomes a sizable negative factor in repeated screening colonoscopies of large groups of predominantly disease-free people.<sup>21</sup> Limitations of the flexible sigmoidoscopy are obvious since it allows only distal colon examination. The recently introduced computed tomographic colonography (CT colonography or virtual colonoscopy) is comparable with colonoscopy in terms of sensitivity and specificity<sup>72</sup>; however, high cost seriously hampers expectations of making this approach a mass screening method. In other words it appears that colonoscopy or its virtual analogue can be especially useful and cost-effective when applied as a “second line” or confirmatory screening in subjects preselected by a much wider “first line” screening procedure. The position of the latter is presently occupied by the inefficient FOBT.

Although rapid methodological progress in the development of molecular diagnostic procedures provokes optimistic predictions,<sup>21,53,71</sup> the reality shows that most advanced molecular approaches based on the stool analysis are still too complicated and expensive to be seriously considered for mass screening (see previous section of this review). In this situation the possibil-

ity of getting well-preserved exfoliated colonocytes (see Fig. 3) directly from the surface of human colorectal mucosa may present an interesting option. The availability of this material can facilitate the application of both cell-based and molecular approaches to cancer biomarker detection. In addition, there may be a good opportunity of using cheap and straightforward quantitative methods such as the detection of the increased cell exfoliation from malignant tumours by simple DNA quantitation. We previously tried the latter approach using exfoliated colonocytes isolated from stool,<sup>25</sup> but standardization difficulties made its validity questionable. The procedure of direct collection of exfoliated cells from rectum appears to be much easier to standardize.<sup>46</sup> Our preliminary results on the use of this material for DNA quantitation look encouraging<sup>47</sup>; however, these findings need to be meticulously scrutinised and confirmed in clinical trials.

### Concluding remarks

This review has briefly addressed problems surrounding the investigation of colonocyte exfoliation in the human colon. Although there was enough controversy and misunderstanding about the destiny of exfoliated colonocytes, it now appears that some basic principles of cell exfoliation in normal conditions and neoplasia finally emerge from obscurity. Malignant colorectal tumours definitely exfoliate huge amounts of cells, which mostly remain within the mucocellular layer overlying colorectal mucosa. Cell-containing fragments of the mucocellular layer excreted with stool are likely to constitute the main source of exfoliated colonocytes found in human faeces. Recent findings indicate that there may be a possibility of migration of the cell-containing mucocellular layer toward rectum creating conditions for the accumulation of exfoliated colonocytes on the surface of the rectal mucosa. It appears that direct collection of these cells can provide valuable material for various types of analysis. Methodological approaches valid for the development of new diagnostic and screening strategies for colorectal cancer based on the use of exfoliated colonocytes may include both advanced cytological techniques and the employment of cell-derived DNA, RNA and proteins for the identification of molecular biomarkers of neoplasia. Nevertheless, further profound research is required to address numerous unanswered questions still existing in the area of colonocyte population dynamics in normal conditions and cancer.

### Acknowledgements

The author thanks the members of the Colonix Medical team (Dr Tatiana Bandaletova, Dr Andrew Llewelyn, Rupert Lywood, Per Aniansson, Hugo Lywood) for their kind support and to our clinical collaborators (Prof. Christopher Marks, Dr Jeremy Gibson, Dr Colin Ferrett) for their efforts in providing us with excellent clinical materials relevant for this work. The author thanks Dr Tatiana Bandaletova for her help in preparing and selecting microphotographs.

### References

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–67.
2. Jass JR, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. *Gastroenterology* 2002;123:862–76.
3. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004;10:789–99.
4. Radtke F, Clevers H. Self-renewal and cancer of the gut: two sides of a coin. *Science* 2005;307:1904–9.
5. Shih IM, Wang TL, Traverso G, Romans K, Hamilton SR, Ben-Sasson S, Kinzler KW, Vogelstein B. Top-down morphogenesis of colorectal tumors. *Proc Natl Acad Sci USA* 2001;98:2640–4.
6. Wong WM, Mandir N, Goodlad RA, Wong BC, Garcia SB, Lam SK, Wright NA. Histogenesis of human colorectal adenomas and hyperplastic polyps: the role of cell proliferation and crypt fission. *Gut* 2002;50:212–7.
7. Lamprecht SA, Lipkin M. Migrating colonic crypt epithelial cells: primary targets for transformation. *Carcinogenesis* 2002;23:1777–80.
8. Brittan M, Wright NA. Stem cell in gastrointestinal structure and neoplastic development. *Gut* 2004;53:899–910.
9. Preston SL, Wong WM, Chan AO, Poulsom R, Jeffery R, Goodlad RA, Mandir N, Elia G, Novelli M, Bodmer WF, Tomlinson IP, Wright NA. Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. *Cancer Res* 2003;63:3819–25.
10. Greaves LC, Preston SL, Tadrous PJ, Taylor RW, Barron MJ, Oukrif D, Leedham SJ, Deheragoda M, Sasieni P, Novelli MR, Jankowski JA, Turnbull DM, et al. Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. *Proc Natl Acad Sci USA* 2006;103:714–9.

11. Eastwood GL. Gastrointestinal epithelial renewal. *Gastroenterology* 1977;72:962-75.
12. Bach SP, Renehan AG, Potten CS. Stem cells: the intestinal stem cell as a paradigm. *Carcinogenesis* 2000;21:469-76.
13. Van Lieshout EM, Van Doesburg W, Van der Meer R. Real-time PCR of host DNA in feces to study differential exfoliation of colonocytes between rats and humans. *Scand J Gastroenterol* 2004;39:852-7.
14. Nair P, Lagerholm S, Dutta S, Shami S, Davis K, Ma S, Malayeri M. Coprocitobiology: on the nature of cellular elements from stools in the pathophysiology of colonic disease. *J Clin Gastroenterol* 2003;36 (Suppl. 5):S84-93.
15. Kamra A, Kessie G, Chen JH, Kalavapudi S, Shores R, McElroy I, Gireesh T, Sudhakaran PR, Dutta SK, Nair PP. Exfoliated colonic epithelial cells: surrogate targets for evaluation of bioactive food components in cancer prevention. *J Nutr* 2005;135:2719-22.
16. Hall PA, Coates PJ, Ansari B, Hopwood D. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. *J Cell Sci* 1994;107:3569-77.
17. Barkla DH, Gibson PR. The fate of epithelial cells in the human large intestine. *Pathology* 1999;31:230-8.
18. Ahlquist DA, Harrington JJ, Burgart LJ, Roche PC. Morphometric analysis of the "mucocellular layer" overlying colorectal cancer and normal mucosa: relevance to exfoliation and stool screening. *Hum Pathol* 2000;31:51-7.
19. Bandaletova T, Bailey N, Bingham SA, Loktionov A. Isolation of exfoliated colonocytes from human stool as a new technique for colonic cytology. *APMIS* 2002;110:239-46.
20. Renehan AG, Bach SP, Potten CS. The relevance of apoptosis for cellular homeostasis and tumorigenesis in the intestine. *Can J Gastroenterol* 2001;15:166-76.
21. Osborn NK, Ahlquist DA. Stool screening for colorectal cancer: molecular approaches. *Gastroenterology* 2005;128:192-206.
22. Lowe SW, Lin AW. Apoptosis in cancer. *Carcinogenesis* 2000;21:485-95.
23. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. *Nat Rev Cancer* 2005;5:231-7.
24. Haier J, Nicolson GL. The role of tumor cell adhesion as an important factor in formation of distant colorectal metastasis. *Dis Colon Rectum* 2001;44:876-84.
25. Christofori G. Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression. *EMBO J* 2003;22:2318-23.
26. Bogenrieder T, Herlyn M. Axis of evil: molecular mechanisms of cancer metastasis. *Oncogene* 2003;22:6524-36.
27. Bader GM, Papanicolaou GN. The application of cytology in the diagnosis of cancer of the rectum, sigmoid, and descending colon. *Cancer* 1952;5:307-14.
28. Miller DF, Sikorski JJ, Moritz MM, De Luca VA. An evaluation of a simplified technique for colonic exfoliative cytology. *Acta Cytol* 1969;13:53-6.
29. Villa E, Dugani A, Rebecchi AM, Vignoli A, Grottola A, Buttafoco P, Losi L, Perini M, Trande P, Merighi A, Lerose R, Manenti F. Identification of subjects at risk for colorectal carcinoma through a test based on K-ras determination in the stool. *Gastroenterology* 1996;110:1346-53.
30. Loktionov A, O'Neill IK, Silvester KR, Cummings JH, Middleton SJ, Miller R. Quantitation of DNA from exfoliated colonocytes isolated from human stool surface as a novel noninvasive screening test for colorectal cancer. *Clin Cancer Res* 1998;4:337-42.
31. Klaassen CH, Jeunink MA, Prinsen CF, Ruers TJ, Tan AC, Strobbe LJ, Thunnissen FB. Quantitation of human DNA in feces as a diagnostic test for the presence of colorectal cancer. *Clin Chem* 2003;49:2112-3.
32. Loktionov A, Bandaletova T, Llewellyn A, Lywood R, Marks C. Quantitation of colonic cell exfoliation as a new approach to colorectal cancer screening. In 7th World Congress on Gastrointestinal Cancer, Barcelona, 15-18 June 2005, p 107.
33. Wind P, Douard R, Poupardin E, Cugnenc PH. Anal implantation of exfoliated tumor cells from a rectal adenocarcinoma after colorectal stapled anastomosis. *Eur J Surg* 1999;165:905-6.
34. Baig MK, Stebbing JF, Marks CG. Anal canal metastases from left sided colorectal cancer. *Colorectal Dis* 2002;4:371-2.
35. Maeda K, Maruta M, Hanai T, Sato H, Horibe Y. Irrigation volume determines the efficacy of "rectal washout". *Dis Colon Rectum* 2004;47:1706-10.
36. Koppe MJ, Boerman OC, Oyen WJ, Bleichordt RP. Peritoneal carcinomatosis of colorectal origin: incidence and current treatment strategies. *Ann Surg* 2006;243:212-22.
37. Shanmugathan M, Jothy S. Apoptosis, anoikis and their relevance to the pathobiology of colon cancer. *Pathol Int* 2000;50:273-9.
38. Valentijn AJ, Zouq N, Gilmore AP. Anoikis. *Biochem Soc Trans* 2004;32:421-4.
39. Iyengar V, Albaugh GP, Lohani A, Nair PP. Human stools as a source of viable colonic epithelial cells. *FASEB J* 1991;5:2856-9.
40. Albaugh GP, Iyengar V, Lohani A, Malayeri M, Bala S, Nair PP. Isolation of exfoliated colonic epithelial cells, a novel non-invasive approach to the study of cellular markers. *Int J Cancer* 1992;52:347-50.
41. Yamao T, Matsumura Y, Shimada Y, Moriya Y, Sugihara K, Akasu T, Fujita S, Kakizoe T. Abnormal expression of CD44 variants in the exfoliated cells in the feces of patients with colorectal cancer. *Gastroenterology* 1998;114:1196-205.
42. Spethmann S, Fisher C, Wagener C, Streichert T, Tschentscher P. Nucleic acids from intact epithelial cells as a target for stool-based molecular diagnosis of colorectal cancer. *Int J Mol Med* 2004;13:451-4.
43. Matsushita H, Matsumura Y, Moriya Y, Akasu T, Fujita S, Yamamoto S, Onouchi S, Saito N, Sugito M, Ito M, Kozu T, Minowa T, et al. A new method for isolating colonocytes from naturally evacuated feces and its clinical application to colorectal cancer diagnosis. *Gastroenterology* 2005;129:1918-27.
44. Umpleby HC, Fermor B, Symes MO, Williamson RC. Viability of exfoliated colorectal carcinoma cells. *Br J Surg* 1984;71:659-63.
45. Keller R, Brandt B, Terpe HJ, Winde G, Foerster EC, Domschke W. Density gradient centrifugation of colonic fluid after segmental lavage: a method of purification of exfoliative epithelial colonic cells for cytological interpretation and image cytometry in patients with long-standing ulcerative colitis. *Am J Gastroenterol* 1999;94:404-9.
46. Loktionov A, Bandaletova T, Llewellyn AH, Ferrett CG, Lywood RCG, Lywood HGG. Colorectal cell sampling device. World Intellectual Property Organisation. 2006; International Publication Number WO 2006/003447 A1.
47. Loktionov A, Lywood H, Bandaletova T, Llewellyn A, Ferrett C, Gibson J, Lywood R, Broughton M, Caffarey S, Marks C. Analysis of exfoliated colonic cells and colorectal cancer detection. *Ann Oncol* 2006;17 (Suppl. 6):vi42.
48. Loktionov A, O'Neill IK. Early detection of cancer-associated gene alterations in DNA isolated from rat feces during intestinal tumor induction with 1,2-dimethylhydrazine. *Int J Oncol* 1995;6:437-45.
49. Davies RJ, Freeman A, Morris LS, Bingham S, Dilworth S, Scott I, Laskey RA, Miller R, Coleman N. Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. *Lancet* 2002;359:1917-9.
50. Lewin MH, Bailey N, Bandaletova T, Bowman R, Cross AJ, Pollock J, Shuker DE, Bingham SA. Red meat enhances the colonic formation of the DNA adduct O<sup>6</sup>-carboxymethyl guanine: implications for colorectal cancer risk. *Cancer Res* 2006;66:1859-65.
51. Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992;256:102-5.
52. Ransohoff D. Colon cancer screening in 2005: status and challenges. *Gastroenterology* 2005;128:1685-95.
53. Haug U, Brenner H. New stool tests for colorectal cancer screening: a systematic review focusing on performance characteristics and practicality. *Int J Cancer* 2005;117:169-76.
54. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Piercalle WE, Thibodeau SN, Shuber AP. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000;119:1219-27.
55. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, Hibi K, Goodman SN, D'Allesio M, Paty P, Hamilton SR, Sidransky D, et al. Detecting colorectal cancer with the use of multiple genetic targets. *J Natl Cancer Inst* 2001;93:858-65.
56. Traverso G, Shuber A, Levin B, Johnson C, Olsson L, Schoetz DJ, Jr, Hamilton SR, Boynton K, Kinzler KW, Vogelstein B. Detection of APC mutations in fecal DNA from patients with colorectal tumors. *N Engl J Med* 2002;346:311-20.
57. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704-14.
58. Syngal S, Stoffel E, Chung D, Willett C, Schoetz D, Schroy P, Jagadeesh D, Morel K, Ross M. Detection of stool DNA mutations before and after treatment of colorectal neoplasia. *Cancer* 2006;106:277-83.
59. Whitney D, Skoletsky J, Moore K, Boynton K, Kann L, Brand R, Syngal S, Lawson M, Shuber A. Enhanced retrieval of DNA from human fecal samples results in improved performance of colorectal cancer screening test. *J Mol Diagn* 2004;6:386-95.
60. Olson J, Whitney DH, Durkee K, Shuber AP. DNA stabilization is critical for maximum performance of fecal DNA-based colorectal cancer tests. *Diagn Mol Pathol* 2005;14:183-91.
61. Song K, Fendrick AM, Ladabaum U. Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis. *Gastroenterology* 2004;126:1270-9.
62. Woolf SH. A smarter strategy? Reflections on fecal DNA screening for colorectal cancer. *N Engl J Med* 2004;351:2755-8.

63. Brenner DE, Rennert G. Fecal DNA biomarkers for the detection of colorectal neoplasia: attractive, but is it feasible? *J Natl Cancer Inst* 2005;97:1107–9.
64. Lagerholm S, Lagerholm S, Dutta S, Nair P. Non-invasive detection of c-myc p64, c-myc p67 and c-erbB-2 in colorectal cancer. *Scand J Gastroenterol* 2005;40:1343–50.
65. Yang SH, Chien CC, Chen CW, Li SY, Huang CJ. Potential of faecal RNA in diagnosing colorectal cancer. *Cancer Lett* 2005;226:55–63.
66. Walsh JM, Terdiman JP. Colorectal cancer screening: scientific review. *JAMA* 2003;289:1288–96.
67. Walsh JM, Terdiman JP. Colorectal cancer screening: clinical applications. *JAMA* 2003;289:1297–302.
68. Smith RA, Cokkinides V, Eyre HJ. American cancer society guidelines for the early detection of cancer, 2006. *CA Cancer J Clin* 2006;56:11–25.
69. Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, Snover DC, Schuman LM. The effect of fecal occult-blood screening on the incidence of colorectal cancer. *N Engl J Med* 2000;343:1603–7.
70. Rennert G. Fecal occult blood testing—trial evidence, practice and beyond. *Recent Results Cancer Res* 2003;163:248–53.
71. Davies RJ, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. *Nat Rev Cancer* 2005;5:199–209.
72. Van Gelder RE, Nio CY, Florie J, Bartelsman JF, Snel P, De Jager SW, Van Deventer SJ, Lameris JS, Bossuyt PM, Stocker J. Computed tomographic colonography compared with colonoscopy in patients at increased risk for colorectal cancer. *Gastroenterology* 2004;127:41–8.
73. Collins JF, Lieberman DA, Durbin TE, Weiss DG. Veterans affairs cooperative study #380 group. Accuracy of screening for fecal occult blood on a single stool sample obtained by digital rectal examination: a comparison with recommended sampling practice. *Ann Intern Med* 2005;142:81–5.
74. Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T, Shiratori Y. A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 2005;129:422–8.
75. Moayyedi P, Achkar E. Does fecal occult blood testing really reduce mortality? A reanalysis of systematic review data. *Ann Intern Med* 2006;101:380–4.