Published in final edited form as:

Colorectal Dis. 2011 April ; 13(4): 387–392. doi:10.1111/j.1463-1318.2009.02159.x.

The role of cell proliferation and crypt fission in adenoma aggressiveness: a comparison of ileoanal pouch and rectal adenomas in familial adenomatous polyposis

O. C. C. Will^{*,†}, M. Deheragoda^{†,‡}, R. K. S. Phillips^{*}, S. K. Clark^{*}, and I. P. M. Tomlinson[§]

^{*}The Polyposis Registry, St Mark's Hospital, Harrow, UK

[†]London Research Institute, Cancer Research UK, London, UK

[‡]Department of Histopathology, University College Hospital, London, UK

[§]Wellcome Trust Institute for Human Genetics, Oxford, UK

[#] These authors contributed equally to this work.

Abstract

Aim—In patients with familial adenomatous polyposis (FAP), ileoanal pouch cancer is rare whereas rectal cancer is common, despite polyp initiation at the two sites being similar at the molecular level. This study investigated whether the disparity in adenoma aggressiveness reflects underlying differences in histogenesis.

Method—Normal mucosal biopsies and 2–3 mm adenomas from patients with FAP were dissected into individual crypts. Crypt area, morphology, fission and mitoses were analysed for crypts from pouch, rectum and supra-anastomotic ileum. Immunohistochemistry of similar archival samples was performed for lysozyme, β -catenin and TP53 expression.

Results—The morphology of normal crypts was similar at each site, although crypt area differed. The area of normal pouch crypts was intermediate between rectum and ileum. The area of adenomatous crypts of rectum and pouch was similar, but the latter had increased asymmetrical fission. Crypt mitoses were proportional to area in all tissues, but crypt fission was reduced in adenomatous crypts from the rectum compared with the pouch. Pouch adenomas retained lysozyme expression as seen in normal ileum. Nuclear β -catenin accumulation was similar, but TP53 expression was increased in rectal adenomas.

Conclusion—Diminutive polyps from rectum and pouch differ in morphology and proliferation. Aggressiveness in rectal polyps is not conferred by increased crypt proliferation, fission, or activation of the Wnt signalling pathway. Increased TP53 expression suggests other molecular mechanisms may be responsible. While crypt mitoses are proportional to crypt area, the threshold for fission may be site specific, indicating that tissue origin may influence histogenesis and thus malignant potential.

Keywords

Familial adenomatous polyposis; ileoanal pouch; adenoma-carcinoma progression

Introduction

Patients with familial adenomatous polyposis (FAP) undergo prophylactic colectomy or proctocolectomy to avoid the otherwise inevitable development of colorectal cancer. The ileoanal pouch procedure was developed after it was recognized that nearly one-third of patients eventually developed rectal malignancy after colectomy and ileorectal anastomosis [1]. However, with increasing years of patient follow up, it has become evident that the ileoanal pouch also develops adenomas [2], which can be extremely numerous. Unlike rectal cancer, however, pouch cancer remains rare, with very few cases reported in the literature [3].

There are no data concerning the individual risk of cancer from a single polyp of large bowel vs small bowel origin. However, it is possible that large-bowel adenomas are more aggressive and likely to progress to cancer. There are two main foundations for this hypothesis. First, the clinical experience of the long-term follow up of patients with ileoanal pouch is that unresected polyps in the anorectal cuff progress more frequently to invasion, and frequently recur following polypectomy. This is supported by the literature reporting cancer of the anorectal cuff and canal more frequently than in the true body of the pouch. Secondly, rectal cancers are seen in patients with few polyps in the rectum, in contrast to the rarity of pouch cancer even when there is dense pouch polyposis. As the genetic events triggering adenoma initiation are similar in rectal and pouch polyps [4], it is possible that the subsequent process of tumorigenesis may differ to account for this disparity in the natural history of neoplasms at these sites.

In both locations, neoplasms form when clonal outgrowths develop from mutated stem cells situated near the bottom of intestinal crypts. In normal mucosa, stem cells proliferate to form enterocytes that gradually mature as they move towards the mucosal surface or villus. Once a threshold level of proliferating cells is reached, crypts can undergo fission. Although crypt fission is proposed to be the basis of intestinal development in infancy [5], it is also the mechanism by which a mutated clone of stem cells expands in the gastrointestinal tract [6–9].

Proliferation in the crypt niche is maintained and modulated by cell signalling between mucosal epithelium and underlying mesodermal cells. The most important signalling pathway is Wnt signalling, which controls cell proliferation, differentiation and apoptosis along the crypt–villus axis [6]. Adenomatous polyposis coli (APC) gene is a key mediator of this pathway and is heterozygously mutated in the germline of patients with FAP.

Mesodermal cells, which are specific to the tissue of origin, thus control mucosal cellular proliferation and crypt fission. However, environmental factors also influence crypt proliferation. Mouse studies have suggested that intestinal stem cells respond to segmental bowel resection by undergoing fission [10]. Intestinal cell proliferation and crypt fission

differ in orally or parenterally fed mice, and observed effects vary according to gut locality [11]. In humans, diet (as reflected by obesity) and gastric bypass may increase colonic epithelial cell proliferation and crypt fission [12].

Thus, although ileoanal pouch adenoma initiation is genetically similar to that of colorectal adenomas [4], the interplay between site of origin and environment may affect crypt proliferation and fission, and therefore adenoma progression. This may account for the difference in malignant potential of pouch and rectal adenomas. In order to investigate the disparity, this study examines the histogenesis of these lesions in terms of cell proliferation, crypt morphology and fission, and immunohistochemical characteristics.

Method

For crypt dissection, fresh adenomas and biopsies of normal mucosa were obtained during surveillance endoscopy from 10 patients with mutation-positive FAP after written informed consent. Flexible endoscopy of the rectum or pouch was performed shortly after a phosphate enema by a single experienced endoscopist. Adenomas were 2–3 mm in size and surveyed using magnifying narrow-band imaging for mucosal pit patterns in keeping with mild dysplasia [13]. There were eight pouch body and seven rectal adenomas. Normal mucosa was surveyed by chromendoscopy before biopsy. Seven samples of normal supra-anastomotic ileum (proximal either to the ileoanal pouch or rectum), six of normal pouch mucosa and six of normal rectal mucosa were collected. The tissues were stored immediately in Clark's fluid (three parts absolute ethanol and one part glacial acetic acid) before analysis. Crypt dissection was performed after hydration and acid hydrolysis as described previously [14]. For each biopsy, 100 crypts were counted and the number of crypts undergoing fission recorded. Under $\times 80$ magnification, mitotic figures were counted for 20 microdissected crypts. Outlines of 10 crypts were drawn with a Leitz drawing tube under $\times 16$ magnification, to calculate cryptal area.

For immunohistochemistry, 29 formalin-fixed, wax-embedded archival samples were obtained from 19 mutation-positive FAP patients. All adenomas were mildly dysplastic and 2–3 mm in size. There were nine polyps from the body of the pouch, 10 polyps from the rectum (with matched normal biopsies on the same tissue blocks) and 10 microscopically normal biopsies from the supra-anastomotic ileum. After antigen retrieval from slides (achieved by microwave heating in citrate buffer), immunohistochemistry was performed for TP53 (Dako Denmark A/S, Glostrup, Denmark, antibody concentration 1:100), β -catenin (BD Transduction Laboratories, Oxford, UK, 1:100) and lysozyme (Dako, 1:200). Immunohistochemistry scoring was performed by a gastrointestinal histopathologist (M. Deheragoda), by assessing positivity per 1000 cells in longitudinally orientated crypts.

Statistical analysis was performed using GraphPad Prism software (GraphPad Software, La Jolla, California, USA). Results are given as group means (SEM). Statistical techniques used to calculate *P*-values included two-tailed *t*-tests and logistic regression.

Results

Crypt morphology

Crypt morphology is shown in Figs 1 and 2. Normal crypts were all cylindrical in shape, but the area differed significantly according to their location.

Crypts from the supra-anastomotic ileum were smaller than those from the rectum [areas 0.09 (0.01) and 0.53 (0.06) mm², respectively], as shown in Fig. 3 (graph 1). Interestingly, crypts from normal pouch body were significantly larger than those of supra-anastomotic ileum [0.17 (0.01) mm², $P < 0.001$], and thus were intermediate in area between normal ileal and rectal crypts. Adenomatous crypts arising in the pouch were similar in area to those arising in rectum (means 0.68 and 0.67 mm², respectively), but the shapes of the crypts were different. While most crypts from rectal adenomas were markedly elongated, pouch adenomas were broader, with more asymmetrical and superficial budding (Fig. 1). In the rectum, there was little difference in cryptal area between normal and adenomatous crypts [0.53 (0.06) vs 0.68 (0.09) mm², $P = 0.23$].

Crypt proliferation

Data for cryptal mitoses are shown in Fig. 3 (graph 2). Fewer than 10 mitoses per crypt were observed in normal mucosa at all sites; means (SEM) for ileum, pouch and rectum were 3.27 (0.15), 6.04 (0.73) and 6.76 (0.51), respectively. Normal pouch mucosa had significantly more mitoses per crypt than normal ileum ($P = 0.002$), and did not differ significantly from normal rectal mucosa ($P = 0.4$). Cryptal mitoses were higher in pouch adenomas compared with rectal adenomas: 23.14 (1.25) vs 16.78 (1.48), $P = 0.005$. For all samples, mitoses per crypt were directly proportional to cryptal area ($r^2 = 0.46$, $P < 0.0001$), shown in graph 2b.

Cryptal fission data are shown in Fig. 3 (graph 3). In normal tissue, crypt fission indices (proportion of crypts in fission) for normal pouch and rectal mucosa were similar [5.4 (1.04) and 4.4 (0.56), respectively]. However, the crypt fission index for supra-anastomotic ileum was 9.4 (0.8), statistically greater than those of pouch and rectum ($P = 0.01$ and $P = 0.0006$, respectively). The crypt fission index was 23.4 (2.5) in pouch adenomas, which was substantially higher than in rectal adenomas, at 7.5 (1.8) ($P < 0.001$). In the pouch, there was a strong correlation between cryptal area and fission ($P = 0.03$, $r^2 = 0.32$), shown in Fig. 3 (graph 3b). There was no significant relationship for ileal ($P = 0.78$) or rectal crypts ($P = 0.79$).

Immunohistochemistry

In normal mucosa, expression of nuclear β -catenin and TP53 was less than 1%. There was no significant difference in nuclear β -catenin expression between pouch and rectal adenomas, shown in Fig. 4 ($P = 0.1$). However, TP53 expression was significantly greater in rectal adenomas ($P = 0.001$), shown in Fig. 5. Pouch polyps retained lysozyme expression, which was also present in normal ileal mucosa but absent from rectal tissues (see Fig. 6).

Discussion

The ileal environment is altered when it becomes a reservoir with faecal stasis. This increases bacterial colonization and is likely to change the time the mucosa is in contact with bile acids. Although true metaplasia does not occur in the pouch [15], these results confirm that its mucosa undergoes morphological changes that are presumably adaptive. Crypts enlarge to resemble those from the rectum, which have previously been shown to be deeper than those of the colon [16]. However, ileal mucosal function, as assessed by lysozyme expression, remains present.

Baseline rates of crypt mitoses in normal tissue were similar to those found in previous studies [17]. As in this study, these have also found crypt mitoses to be proportional to crypt area [14], presumably because the stem cell number is proportional to the size of the crypt. A similarly proportional relationship was not, however, seen for crypt fission, in contrast to other studies confined to the colon [14]. It is thought that crypts divide once stem cell numbers exceed a certain threshold, so larger crypts are more likely to be undergoing fission. While the ileal pouch showed a proportional relationship between crypt area and fission, this was not the case for the more proximal ileum or rectum. This finding suggests that the threshold for crypt fission varies according to gut location. In the rectum, the areas of adenomatous and normal crypts were similar; therefore, fission may be initiated only after a significant increase in crypt area. Although macroscopically the pouch and rectal polyps were all less than 3 mm in size, adenomatous crypts from pouches were seen to have already undergone a large change in area from their normal state. This may have initiated their greatly increased and asymmetrical fission. We speculate that a directly proportional relationship between CFI and area in rectal adenomas may be observed only for larger, more advanced lesions.

Diminutive pouch adenomas appear already to be in a state of crypt proliferation and fission, while similar rectal polyps appear to have fewer mitoses and significantly less crypt fission. Possibly counter-regulatory apoptotic processes are activated earlier in pouch adenomas, accounting for their lack of malignant progression. Interestingly, TP53 expression was significantly raised in rectal polyps. Increased nuclear TP53 expression was found to be correlated with TP53 mutation in 68% of colorectal tumours [18]. Mutated TP53 is unable to activate genes responsible for cell cycle arrest and repair, or apoptosis [19]. In contrast, ileal pouch and rectal adenomas showed similar nuclear β -catenin expression, indicating similar Wnt pathway activation.

In conclusion, this study has shown that aggressiveness of rectal adenomas is not due to increased crypt proliferation, fission, or increased Wnt pathway activation. While ileal pouch polyps may resemble those from rectum at a molecular level [4], it is the tissue origin which appears to influence subsequent adenoma histogenesis and thus possibly malignant potential.

Acknowledgements

The authors would like to thank patients from St Mark's Hospital who took part in this study, the nurses in the Polyposis Registry for administrative assistance, Ripple Man, nurse endoscopist, for assistance in sample

collection, and George Elia, Cancer Research UK, who assisted with immunohistochemistry. Olivia Will was funded by St Mark's Hospital Polyposis Registry and Cancer Research UK.

References

1. Nugent KP, Spigelman AD, Phillips RK. Life expectancy after colectomy and ileorectal anastomosis for familial adenomatous polyposis. *Dis Colon Rectum*. 1993; 36:1059–62. [PubMed: 8223060]
2. Thompson-Fawcett MW, Marcus VA, Redston M, Cohen Z, McLeod RS. Adenomatous polyps develop commonly in the ileal pouch of patients with familial adenomatous polyposis. *Dis Colon Rectum*. 2001; 44:347–53. [PubMed: 11289279]
3. Church J. Ileoanal pouch neoplasia in familial adenomatous polyposis: an underestimated threat. *Dis Colon Rectum*. 2005; 48:1708–13. [PubMed: 15937627]
4. Will OC, Robinson J, Günther T, Phillips RKS, Clark SK, Tomlinson I. APC mutation spectrum in ileoanal pouch polyps resembles that of colorectal polyps. *Br J Surg*. 2008; 95:765–9. [PubMed: 18418860]
5. Cummins AG, Catto-Smith AG, Cameron DJ, Couper RT, Davidson GP, Day AS, Hammond PD, Moore DJ, Thompson FM. Crypt fission peaks early during infancy and crypt hyperplasia broadly peaks during infancy and childhood in the small intestine of humans. *J Pediatr Gastroenterol Nutr*. 2008; 47:153–7. [PubMed: 18664866]
6. Yen TH, Wright NA. The gastrointestinal tract stem cell niche. *Stem Cell Rev*. 2006; 2:203–12. [PubMed: 17625256]
7. Greaves LC, Preston SL, Tadrous PJ, 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. [PubMed: 16407113]
8. Leedham SJ, Schier S, Thliveris AT, Halberg RB, Newton MA, Wright NA. From gene mutations to tumours – stem cells in gastrointestinal carcinogenesis. *Cell Prolif*. 2005; 38:387–405. [PubMed: 16300652]
9. Boman BM, Walters R, Fields JZ, Kovatich AJ, Zhang T, Isenberg GA, Goldstein SD, Palazzo JP. Colonic crypt changes during adenoma development in familial adenomatous polyposis: immunohistochemical evidence for expansion of the crypt base cell population. *Am J Pathol*. 2004; 165:1489–98. [PubMed: 15509520]
10. Dekaney CM, Fong JJ, Rigby RJ, Lund PK, Henning SJ, Helmrath MA. Expansion of intestinal stem cells associated with long-term adaptation following ileocecal resection in mice. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293:G1013–22. [PubMed: 17855764]
11. FitzGerald AJ, Mandir N, Goodlad RA. Leptin, cell proliferation and crypt fission in the gastrointestinal tract of intravenously fed rats. *Cell Prolif*. 2005; 38:25–33. [PubMed: 15679864]
12. Sainsbury A, Goodlad RA, Perry SL, Pollard SG, Robins GG, Hull MA. Increased colorectal epithelial cell proliferation and crypt fission associated with obesity and roux-en-Y gastric bypass. *Cancer Epidemiol Biomarkers Prev*. 2008; 17:1401–10. [PubMed: 18559555]
13. East JE, Suzuki N, Bassett P, Stavrinidis M, Thomas HJ, Guenther T, Tekkis PP, Saunders BP. Narrow band imaging with magnification for the characterization of small and diminutive colonic polyps: pit pattern and vascular pattern intensity. *Endoscopy*. 2008; 40:811–7. [PubMed: 18828077]
14. 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. [PubMed: 11788562]
15. Fruin AB, El-Zammer O, Stucchi AF, O'Brien M, Becker JM. Colonic metaplasia in the ileal pouch is associated with inflammation and is not the result of long-term adaptation. *J Gastrointest Surg*. 2003; 7:246–53. discussion 53–4. [PubMed: 12600449]
16. Brunggaard G. Morphological characteristics, epithelial cell proliferation, and crypt fission in cecum and colon of growing pigs. *Dig Dis Sci*. 1997; 42:2384–93. [PubMed: 9398821]

17. Wasan HS, Park HS, Liu KC, Mandir NK, Winnett A, Sasieni P, Bodmer WF, Goodlad RA, Wright NA. APC in the regulation of intestinal crypt fission. *J Pathol.* 1998; 185:246–55. [PubMed: 9771477]
18. Soong R, Robbins PD, Dix BR, Grieu F, Lim B, Knowles S, Williams KE, Turbett GR, House AK, Iacopetta BJ. Concordance between p53 protein overexpression and gene mutation in a large series of common human carcinomas. *Human Pathol.* 1996; 27:1050–5. [PubMed: 8892589]
19. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJ. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut.* 2005; 54:1283–6. [PubMed: 15843421]

Figure 1.
Morphology of representative crypts from ileum, pouch and rectum.

Figure 2.
Photograph of representative dissected crypts from ileal pouch and rectal adenomas.

Figure 3.
Results of crypt dissection (means and standard deviations indicated in graphs 1–3).

Figure 4.
 β -Catenin immunohistochemistry positivity.

Figure 5.
TP53 immunohistochemistry positivity.

Figure 6. Lysozyme expression is retained in ileal pouch adenomas. Rectal adenoma shown on left, ileal pouch adenoma on right.