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Nondysplastic Crypts in Fission in Nonpolypoid Adenomas and in the Adjacent Mucosa Support Field Cancerization in the Colon

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Abstract. *Background/Aim: We recently noticed in nonpolypoid adenomas (NPA) and the adjacent normal mucosa, nondysplastic crypts in symmetric and asymmetric fission (NDCSAF). Patients and Methods: All NDCSAF found in 80 small NPA and in the adjacent mucosa were registered. Results: A total of 178 NDCSAF (mean, 2.2) were found: 12 (6.7%) interspersed between adenomatous glands, 36 (20.2%) partially replaced by dysplastic epithelium, and 130 (73%) underneath the adenomatous tissue. Of the 61 cases with normal mucosa adjacent to NPA, 40 (65.6%) disclosed NDCSAF, and the remaining 21 (34.4%) normal crypts, exclusively. Conclusion: The accruing of NDCSAF within NPA and surrounding mucosa, are outstanding findings. Given that colonic crypts may undergo only one fission every 30-40 years, the accruing of NDCSAF in and about small NPA reveals mucosal hubs with pathological aberrations of cryptogenesis, probably conveyed by somatic mutations. The findings support the existence of field cancerization in the colonic mucosa.*

The mean total length of the human colon is 160.5 cm (1), and the total mucosal area (2) is 995 cm2. This vast mucosal area is built of anthemic folds (3) and innominate grooves (4, 5). In well-oriented sections, anthemic folds consist of an assemblage of mucosal invaginations called glands or crypts, aligned as parallel "test tubes" (6). The creation of a new crypt is attained by symmetric fission, beginning at the base of the crypt. The bifurcation progresses upwards until two identical individual crypts are finally formed. Crypt fission reaches its peak during early infancy (7), but in adults, crypt fission is rarely seen (8-10). In a previous study of histological sections from 22 normal colonic segments, proximal or distal to surgically removed colonic carcinoma (11), we recorded all crypts present in 15 continuous fields of view, corresponding to 30 mm of colonic mucosa. A total of 8,580 crypts were found in the 22 controls (mean: 390 crypts/segment, range=382-408). Crypts with normal shapes and normal epithelium were present in all 22 colonic segments. Nonetheless, in three of the segments, few crypts with irregular architecture, including occasional crypts in symmetric fission, were present. Importantly, crypts in asymmetric fission were not found (11). This huge colonic mucosal area is relentlessly exposed to a cocktail of risk factors able to set aflame the chain of molecular events that generate epithelial dysplasia and eventually invasive carcinoma. The main risks factors leading to this development are: i) Genetic differences [the risk is higher in patients with germline mutations in one of the DNA mismatch repair genes (MMR)] (12), ii) Gender (the risk is higher in males) (13), iii) Obesity (physical inactivity and obesity are strong independent determinants associated to colon cancer) (14), iv) Type 2 diabetes (hyperinsulinaemia is important in the pathogenesis of colon cancer) (15), v) Environmental carcinogens, such as polycyclic aromatic hydrocarbon (16), vi) Mutant gut microbiome (17), vii) The breakdown of the gut macrophage-barrier (allowing the trespassing of the gut-microbiome into the host, thereby destabilizing host immunity) (18), viii) Epigenetic alterations (addition or deletion of methylated groups or changes in the histones that bind DNA to chromosomes) (19), and ix) Life style factors (alcohol and tobacco, encourage the development of sessile serrated lesions) (20). Given that the

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entire colorectal mucosa is relentlessly exposed to one or several of the aforementioned risks factors, the pertinent questions are: i) Why the aforementioned factors only trigger carcinogenesis in a small mucosal domain, leading to the evolution of nonpolypoid adenomas (as well as sporadic polypoid adenomas, traditional serrated adenomas or sessile serrated adenomas)? In this regard, Konishi and Morson (21) found in a series of 1,241 adenomas, that 50% had only one adenoma, and Griffioen *et al*. (22) found in 124 patients, that 60% had a single adenoma. Colon carcinomas also evolve as solitary lesions; synchronous multiple colon cancers are rare. Drew *et al*. (23) found among 134,305 individuals, 1,981 solitary colorectal cancers (CRC) but only 45 synchronous CRC, and ii) Why the remnant colorectal mucosa in patients harbouring an adenoma or a carcinoma remains refractory to the growth of similar lesions elsewhere in the colon, despite that this mucosa has unremittingly been subjected to the same risk factors?

Exploring that crucial conundrum, Slaughter *et al*. (24) proposed, 67 years ago, the concept of "field cancerization". Field cancerization (also called field defects, field effect, or field of susceptibility) refers to the cellular and molecular alterations triggering initiation, evolution, and progression to neoplastic transformation in the large mucosal domain. In later years, the concept of field cancerization of the colonic mucosa has received much attention (25-27). In the mucosa adjacent to CRC, Filipe found altered mucus secretion (28). That "transitional" mucosa, as it was called, often had increased sialomucins, and decreased or absent sulphomucins, with goblet cells being increased in number and size. Filipe suggested that the changes in the mucin secretion around CRC reflected a transformation to a fetal epithelium, corresponding to an early stage in carcinogenesis (28). TEM studies of the transitional mucosa revealed electron-dense bodies, 0.15-0.3~m in diameter, and an elaborated and enlarged Golgi zone with increased secretory activity (29). It was suggested that mucin (light microscopy) and ultrastructural (TEM) changes in the "transitional" mucosa of CRC, highlighted a failure in the normal process of cell differentiation along the crypt (29). Subsequently, other changes were recorded in the mucosa surrounding CRC, such as loss of the peripheral nuclear heterochromatin (30), deficient expression of DNA repair enzymes (31), increased folate concentrations and reduction of aberrant DNA (27), biological changes (32-34), abnormal gene expression (35), FHL1 on chromosome X (36), mutations of the K-ras codon 12 (37), and Type 2 diabetes mellitus (38). In contrast, there are no previous studies dealing with histologic aberrations occurring in the mucosa bordering small nonpolypoid colon adenomas, except for a substantial crypt elongation of the proximate zone mucosa to flat adenoma, reported by Kristt *et al*. (39).

Recently, while reviewing sections from endoscopically-resected small nonpolypoid adenomas, we observed in the non-dysplastic mucosa adjacent to nonpolypoid adenomas, crypts in symmetric and in asymmetric fission (40).

The purpose of the present survey was to report the frequency of the non-dysplastic crypts in symmetric and asymmetric fission (NDCSAF) found within a cohort of nonpolypoid colon adenomas and in their adjacent nondysplastic mucosa.

Patients and Methods

Histologic sections from 80 consecutive small nonpolypoid conventional adenomas (<1 cm in diameter) were retrieved from the archives of the Department of Pathology. Particular attention was paid to the nondysplastic colon mucosa adjacent to the neoplastic adenomatous tissue. Nonpolypoid lesions were endoscopically removed by mucosectomy, and immersed in formalin for 24 h. At the laboratory, the mucosectomy was cut in approximately two mm broad fractions, and transferred to paraffin wax blocks. Sections were cut at 4 μm and stained with hematoxylin and eosin (H&E).

Histological evaluation. All 80 small nonpolypoid lesions were tubular adenomas: 69 (86.3%) had low-grade dysplasia, and the remaining 11 (13.8%) high-grade dysplasia. The nondysplastic colon mucosa neighbouring nonpolypoid conventional adenomas was present in 61 cases and missing in the remaining 19 cases.

Definitions.
I) Nondysplastic colonic crypts in symmetric fission in well-oriented sections: Twin upright crypts sharing a single luminal opening on top.
II) Nondysplastic colonic crypts in asymmetric fission in well-oriented sections: Two or more upright, disimilar crypts, sharing a single luminal opening on top.
III) Nondysplastic colonic crypts in symmetric fission in cut-across sections: Twin, back-to-back, symmetric "ring-shaped" crypts, joined by a thin epithelial rim. This grouping has been referred to as the back-to-back sign in UC (8).
IV) Nondysplastic colonic crypts in asymmetric fission in cut-across sections: Two or more back-to-back "ring-shaped" crypts varying in diameter and/or shape, joined by a thin epithelial rim.
Ethical approval. The Regional Ethical Review Board in Stockholm approved this study (Dnr 2018-2024/32).

Results

Of the 61 cases having colon mucosa adjacent, 40 NPA (65.6%) revealed 75 NDCSAF (mean: 1.9) interspersed between apparently normal crypts. The mucosa in the vicinity of the remaining 21 NPA (34.4%), showed normal crypts arranged in a "test tube" fashion, lacking NDCSAF.

Nondysplastic crypts in symmetric fission in the vicinity to NPA. All 40 cases (100%) displayed crypts in symmetric fission in the mucosa neigbouring NPA: a single crypt in symmetric fission was seen in 24 cases, two crypts in symmetric fission in 10, and three crypts in symmetric fissision in six cases. In all, a total of 62 crypts in symmetric

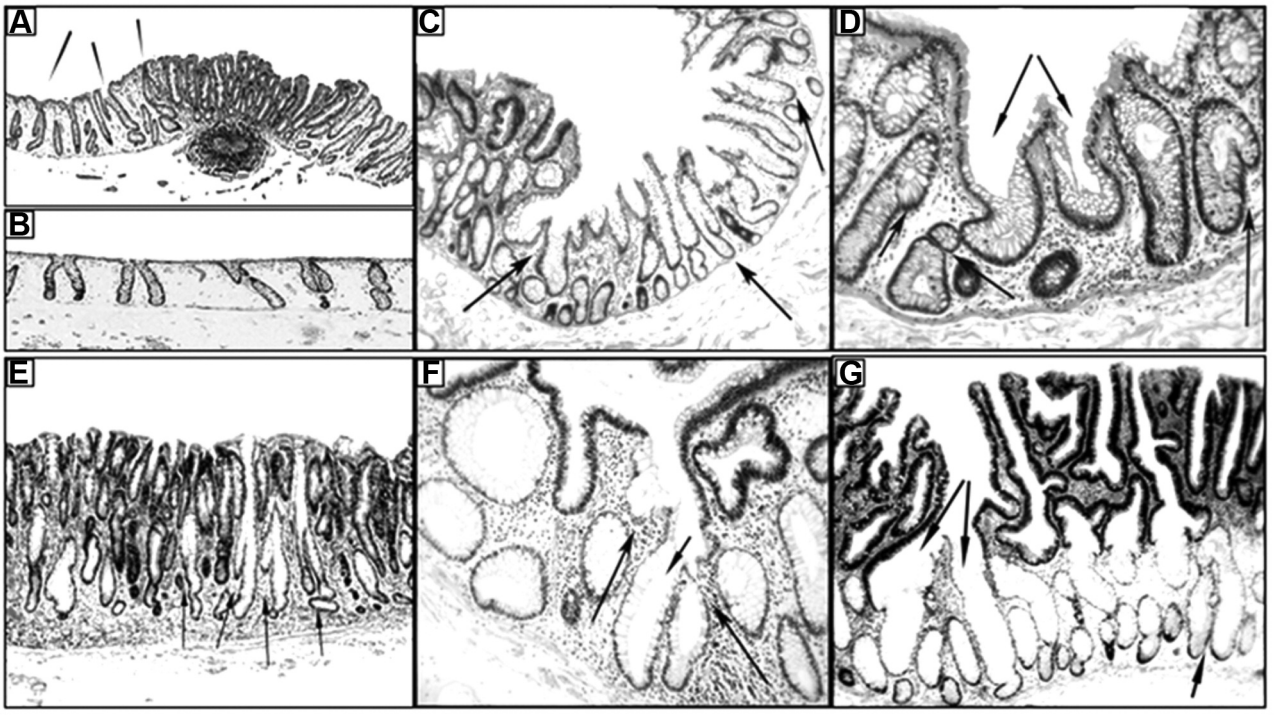

Figure 1. *Images of nondysplastic crypts in asymmetric and symmetric fissions. A: Crypts in fission in the nondysplastic mucosa (pointers) surrounding a nonpolypoid colon adenoma (H&E, original ×4). B: Crypts in symmetric fission in the nondysplastic mucosa surrounding a nonpolypoid colon adenoma (H&E, original ×4). C: Nondysplastic mucosa in the vicinity of a nonpolypoid colon adenoma. Note nondysplastic crypts in asymmetric fission (arrows) (H&E, original ×10). D: Closer view exposing a crypt in asymmetric fission (arrows) in the nondysplastic mucosa neighbouring a nonpolypoid colon adenoma, (H&E, original ×20). E: Interspersed nondysplastic crypts in asymmetric fission (arrows), amid dysplastic crypts of a nonpolypoid adenoma (H&E, original ×10). F: Closer views showing crypts in asymmetric fission (arrows) being replaced by down-growing dysplastic epithelium (H&E, original ×20). G: Crypts in asymmetric fission beneath the neoplastic canopy of a small nonpolypoid colon adenoma (H&E, original ×10).*

Table I. *Total number of crypts in fission in 80 nonpolypoid colon adenomas.*

Crypts in fission	No. asymmetric crypts interspersed with dysplastic crypts	No. asymmetric crypts partially replaced by dysplastic epithelium	No. asymmetric crypts beneath the dysplastic epithelium	Total symmetric fission
Symmetric crypt fission	8 (8%)	21 (21%)	71 (71%)	100 (100%)
Asymmetric crypt fission	4 (5.1%)	15 (19.2%)	59 (75.6%)	78 (100%)
All	12 (6.7%)	36 (20.2%)	130 (73.0%)	178 (100%)

fission were found in the nondysplastic mucosa neigbouring the 40 NPA (mean: 1.6) (Figure 1B).

Nondysplastic crypts in asymmetric fission in the vicinity to NPA. In the neigbouring mucosa, nine cases (22.5%) of the 40 NPA had crypts in asymmetric fission: a single crypt in asymmetric fission was found in six cases, two crypts in asymmetric fission in two, and three crypts in asymmetric fission in the remaining one. All together, 13 crypts in asymmetric fission were recorded in the nondysplastic mucosa adjacent to the 40 NPA (mean: 0.33) (Figure 1A, C, and D).

Nondysplastic crypts in symmetric fission inside NPA (Table I). i) Crypts in symmetric fission interspersed with dysplatic glands were found in six NPA: a single crypt in symmetric fission was found in four NPA, and two crypts in symmetric fission in two NPA. In all, eight crypts in symmetric fission

interspersed with dysplatic glands were recorded in the 80 NPA (mean: 1.3 crypts) (Figure 1E).
ii) Symmetric crypts partially replaced by dysplastic epithelium were detected in 16 NPA: a single symmetric crypt was seen in 11 NPA, and two symmetric crypts in five NPA. In all, a total of 21 symmetric crypts partially replaced by dysplastic epithelium were found in the 16 NPA (mean: 1.3 crypts/NPA) (Figure 1F).
iii) Crypts in symmetric fission were also present beneath the dysplastic compartment in 39 NPA: a sigle crypt in symmetric fission was found in 16 NPA, two symmetric crypts in 14 NPA and three symmetric crypts in nine NPA. Taken together, the total number of crypts in symmetric fission beneath the dysplastic compartment was 71 in the 39 NPA (mean: 1.8) (Figure 1G).

Nondysplastic crypts in asymmetric fission inside NPA (Table I).
i) Nondysplastic crypts in asymmetric fission interspersed with dysplatic glands were found in three NPA: a single crypt in asymmetric fission was found in two NPA, and two crypts in asymmetric fission in one NPA. In all, four crypts in asymmetric fission were recorded interspersed with dysplatic glands in the three NPA (mean: 1.3).
ii) Asymmetric crypts partially replaced by dysplastic epithelium were detected in 12 NPA: a single asymmetric crypt was seen in nine NPA, and two asymmetric crypts, in three NPA. In total, a total of 15 asymmetric crypts partially replaced by dysplastic epithelium were found in 12 NPA (mean: 1.3 crypts/NPA).
iii) Crypts in asymmetric fission were also present beneath the dysplastic compartment in 33 NPA: a sigle crypt in asymmetric fission was found in 14 NPA, two asymmetric crypts in 12 NPA and three asymmetric crypts in seven NPA. Taken together, the total number of crypts in asymmetric fission beneath the dysplastic compartment was 59 in 33 NPA (mean: 1.0).

Discussion

The results of this survey indicate that nonpolypoid adenomas often exhibit NDCSAF, either sandwhiched amongst dysplastic crypts, beneath the neoplastic canopy of nonpolypoid adenomas (40) or are partially replaced by downward-growing dysplastic cells (41, 42). Importantly, NDCSAF were ofen seen in the adjacent, apparently normal mucosa surrounding NPA. A plausible explanation for these findings might be that NPA initially evolved on a restricted mucosal domain consisting of nondysplastic crypts in symmetric and in asymmetric fission. Bearing in mind that in the normal mucosa of adults, crypts in symmetric fission are rarely found (10, 11, 43, 44), and that crypts in asymmetric fission never occur, the present findings revealed abnormally increased cryptogenesis, some with pathologic aberration, as in those with asymmetric fission in and about NPAs.

Today, the histologic pathway of sporadic colon carcinogenesis, also referred to as the adenoma-carcinoma sequence (45), is known to be preceded by cytologic aberrations such as low-grade dysplasia, high-grade dysplasia, and often, by structural villous changes before invasive cancer ensues. These steps are driven by a series of molecular alterations, leading to the accumulation of genomic aberrations, chromosome-instability and microsatellite-instability (46-49). Notwithstandig, it remains elusive why the aforementioned molecular alterations leading to histologic neoplastic changes only affect a limited mucosal domain in the colon and not the entire mucosa.

The repopulation of the crypts is synchronized by stem cells (50-52). The stem cell niche, located at the crypt base, maintains cell number homeostasis in the colonic crypts. Wnt signaling controls cell proliferation, differentiation and apoptosis along the crypts; Wnt concentration is high at the bottom of the crypt (where stem cells reside) and low at the top. In contrast, APC concentration is low at the crypt bottom and high at the top (where differentiated cells reside) (50, 51). Wnt signaling has been linked to crypt fission, being required for the production of colon crypts in infant rats and in mice in vitro (52, 53). Given that colonic crypts may only undergo one fission event every 30-40 years (54), the demonstration of nondysplastic crypts in symmetric fission accruing within the confines of nonpolypoid adenomas and their adjacent mucosa, strongly suggest increased abnormal cryptogenesis in limited mucosal hubs. The finding of nondysplastic crypts in asymmetric fission in the same area suggest that the abnormal cryptogenesis might be possibly generated by putative somatic mutations. The possibility that the pathological cryptogenesis might be linked with the susceptibility of these crypts to develop dysplastic changes cannot be totally rejected. If this is the case, then those small mucosal domains would fulfill the requirements of Slaughter's postulate (24).

In summary, NDCSAF were found: i) In the nondysplastic mucosa adjacent to NPA, ii) Intercalated between the adenomatous glands, iii) Beneath the adenomatous glands and iv) Replacing the dysplastic epithelium in a top-down maner (41). Given that colonic crypts may undergo only one fission every 30-40 years (54), the demonstration of nondysplastic crypts in asymmetric fission, within the confines of small NPA and in their vicinity, strongly suggest pathological aberrations of cryptogenesis, most probably conveyed by somatic mutations.

The present survey supports the concept of field cancerization (24) in the colon.

Conflicts of Interest

The Authors have no conlficts of interest to declare regarding this study.

Authors' Contributions

CAR collected the initial data, reviewed the sections, and wrote the original draft. PTS obtained the permission of The Regional Ethical Review Board in Stockholm, Sweden, revised the original draft and introduced valuable suggestions.

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