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Molecular Genetics of Dysplasia in Ulcerative Colitis

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Numerous molecular genetic events occurring in the development of sporadic colorectal neoplasia have been previously defined. The most frequent genetic alterations are mutations of the *APC*, *KRAS*, and *TP53* genes, as well as loss of the *DCC* gene and of the second *TP53* allele. The data from several groups indicate that these genes play an important role in ulcerative colitis-associated dysplasias and cancer, as they do in sporadic colorectal adenomas and carcinomas. *KRAS* and *TP53* mutations were detected in dysplasia, but also in villous regeneration and active colitis, and affect a subpopulation of the cells composing these lesions. We conclude that in histologically defined dysplasia, clones can be found that genetically represent precancerous lesions in ulcerative colitis. Seen in this way, part of the active colitis and villous regeneration lesions might be considered as preneoplastic. When present, *KRAS* mutation is an excellent genetic marker to map populations of preneoplastic cells.

Key words: *KRAS*, *TP53*, colorectal, ulcerative colitis, dysplasia
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INTRODUCTION

CHRONIC ULCERATIVE colitis (UC) is associated with an increased risk of colorectal carcinoma. Dysplasia is a precursor of carcinoma; epithelia containing high-grade or severe dysplasia are more likely to develop cancer. Villous regeneration occurs after repeated mucosal damage, and results in structural changes in the colon mucosa with or without the cytological features of dysplasia. It is important to determine which genetic parameters are related to and possibly predictive of increased carcinoma risk in UC.

Like sporadic colorectal carcinoma, the development of cancer in UC is hypothesised to evolve by a multistep process involving genetic instability and clonal expansion. However, the critical events in tumorigenesis seem to be activation of oncogenes and deactivation of tumour suppressor genes. These genes play a critical role in differentiation and regulation of cell proliferation, and thus alterations to the expression or structure of these genes may result in perturbation of some essential functions of the cell.

MOLECULAR GENETICS OF COLONIC CANCER IN INFLAMMATORY BOWEL DISEASE

Numerous molecular genetic events occurring in the development of sporadic colorectal neoplasia have been previously defined [1]. The most frequent genetic alterations are mutations of the *APC* tumour suppressor gene, mutations of the *KRAS* proto-oncogene, loss of the *DCC* tumour suppressor gene on chromosome 18q, mutations of the *TP53* tumour suppressor gene and loss of the second *TP53* allele on chromosome 17p. These genetic changes apparently occur sequentially during tumour progression. Different groups have analysed these genetic alterations in UC-associated cancer to determine whether these lesions represent the major pathway for colorectal tumorigenesis or whether UC-associated cancers are specific.

In the literature, most reports comprise small series of cases. Thus, it is difficult to have a clear idea of which genetic alteration is important in UC-associated neoplasm. Compilation of the results obtained to date is shown in Table 1. Unfortunately, there are not enough studies on the *APC* and *DCC* tumour suppressor genes to draw definitive conclusions [2, 3]. The data from several groups indicate that *KRAS* [3–7] and *TP53* [3, 4, 8–10] play an important role in UC-associated carcinomas. However, the results indicate that the prevalence of *KRAS* and *TP53* genetic alterations found in UC-associated carcinomas is slightly lower than in sporadic carcinomas.

Nevertheless, *KRAS* and *TP53* genetic alterations remain important parameters for UC-associated colonic carcinogenesis. Their role and use as molecular genetic markers during neoplastic progression is thus important to evaluate.

Table 1. Somatic mutations in oncogenes and tumour suppressor genes in colorectal cancer

Gene	Genetic alteration	Sporadic cancers	UC-associated cancers
APC	Point mutation/LOH	60%	NS
KRAS	Point mutation	40%	30%
DCC	LOH	65%	NS
TP53	Point mutation/LOH	70%	50%

LOH, loss of heterozygosity; NS, not significant.

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KRAS MUTATIONS IN DYSPLASTIC FIELDS IN ULCERATIVE COLITIS

One important genetic alteration identified in sporadic colorectal adenomas and carcinomas is *KRAS* gene mutation. Point mutations in codons 12, 13 and 61 have been detected in 40–50% of colorectal carcinomas and in a similar percentage of adenomas [11, 12]. In UC-associated neoplasia, *KRAS* mutations are either common or infrequent depending on the study. Seventeen of the 58 (30%) carcinomas analysed contained a *KRAS* mutation [3–7]. Though *KRAS* mutation rates are lower in UC-related carcinomas than in sporadic cases, this genetic alteration must be considered as a relatively important genetic event in the tumorigenic pathway followed by UC-associated neoplasms.

Several groups have examined small numbers of dysplasia and carcinomas associated with UC. Dysplastic lesions in UC are generally localised in different areas of mucosa, and it has become evident that in each area the genetic alterations present are not clonal. Thus, in the foci analysed, the different genetic alterations may or may not be detected depending on the region and on the sample size. For this reason, the choice of the method of detection is important, and the results can be divergent. Two groups have used relatively insensitive methods of detection: sequencing [7] and sequencing after cell sorting [6]. Only 2 of 18 (11%) dysplastic lesions harboured a *KRAS* mutated gene. More sensitive methods, such as cloning followed by sequencing [3], allele-specific polymerase chain reaction [4] and restriction fragment length polymorphism [5], have been developed to detect small subpopulations of genetically mutated cells. Using these techniques, detection of cells harbouring *KRAS* mutations were found in 11 of 25 (44%) foci within dysplastic lesions.

Even though relatively few foci of dysplasia have been analysed, interesting observations can be made.

(1) In the majority of dysplastic foci examined, only part of the dysplastic cell populations have been found to contain mutated *KRAS*.

(2) Very often carcinomas and adjoining dysplastic lesions have the same *KRAS* mutations [3–5, 7]. One group found a *KRAS* mutation in a tumour as well as in the dysplastic epithelium 9–10 cm from the cancer [5]. These results suggest that the cancer evolves from dysplastic lesions without destroying their precursor lesions. This is not the case in advanced sporadic colorectal cancers which do not contain residual adenomatous precursor tissue.

(3) In few cases, it has been observed that the absence of *KRAS* mutations in the cancer does not exclude the presence of clonal mutated *KRAS* cells in the adjacent mucosa [4, 5]. This result suggests that the cancer evolved from a different dysplastic clone. The possibility that *KRAS* mutations may be lost during the dysplasia–carcinoma sequence is unlikely in view of data suggesting that *KRAS* mutations are fairly stable throughout the natural history of sporadic colorectal carcinomas [13].

TP53 GENE ALTERATIONS IN DYSPLASTIC FIELDS IN ULCERATIVE COLITIS

The normal product of the *TP53* tumour suppressor gene is a phosphoprotein that plays a critical role in cell proliferation. Inactivation of the *TP53* gene by mutation or loss may lead to loss of cell growth regulation. In sporadic colorectal carcinogenesis, the *TP53* gene was found mutated in more than 70% of carcinomas, but infrequently in adenomas [14, 15].

The role of the *TP53* gene in UC-associated carcinomas seems to be important. Detection of *TP53* gene alterations in dysplastic lesions has involved, as noted above, sensitive techniques [3, 8], and the results show that:

(1) Mutations and loss of the *TP53* gene are common events in UC-associated dysplasia, but infrequent in sporadic colorectal adenomas.

(2) In some instances, the dysplastic areas adjacent to a *TP53* mutated carcinoma contain cells with the same *TP53* mutation [3, 5, 8]. This event is less common than for *KRAS* mutations. However, when *KRAS* and *TP53* mutations are both observed in tumour, some cells of the adjacent dysplasia harbour the same *KRAS* mutation, but none contain a *TP53* mutation [3, 4].

(3) Dysplastic tissues with *TP53* mutations are more often aneuploid [8].

(4) *TP53* mutation precedes *TP53* loss, and is probably a relatively early event in the process of UC-associated tumorigenesis.

KRAS AND TP53 MUTATIONS IN NONDYSPLASTIC AREAS IN ULCERATIVE COLITIS

Villous regeneration may occur after repeated mucosal damage, and results in structural changes in the colon mucosa with or without the features of dysplasia.

KRAS and *TP53* mutations can be present in histological lesions not considered as preneoplastic, i.e., regenerative (villous regeneration) and even inflammatory (active colitis) mucosae [4, 8, 9]. *TP53* mutations have only been observed when the nondysplastic fields analysed are adjacent to dysplastic regions [8, 9]. In certain foci of nondysplastic mucosae, a few per cent of the cells contained mutated *KRAS* or *TP53* alleles, and it is clear that these mutated cells represent authentic clones with a yet unknown biological significance.

CONCLUSION

Some progress has been made in identifying some of the genetic alterations that occur during the process of UC-associated tumorigenesis. Importantly, mutations in both the *KRAS* oncogene and *TP53* tumour suppressor gene have been identified. However, more work is necessary for a better understanding of the tumorigenesis process, and possibly identifying additional oncogenes and tumour suppressor genes which may play an important role.

Less is known about the molecular genetics of dysplasia in UC, however, the main results of the different research groups are: (1) similar genetic alterations can be found in the dysplastic lesions surrounding the carcinomas; (2) distant dysplastic lesions can exhibit a different genotype from the carcinoma; and (3) from a genetic point of view, at least part of the inflammation of active colitis and villous regeneration must be considered as preneoplastic.

The presence of *KRAS* or *TP53* mutations indicates that a patient has a very high risk of developing colorectal cancer; unfortunately the converse is not true. However, further research is necessary to clarify the molecular basis of the pathogenesis of UC-associated dysplasia and carcinomas, which will certainly improve diagnosis and management of patients at risk.

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**, 759–767.
2. Greenwald BD, Harpaz N, Yin J, *et al.* Loss of heterozygosity affecting the *p53*, *Rb*, and *mcc/apc* tumor suppressor gene loci in dysplastic and cancerous ulcerative colitis. *Cancer Res* 1992, **52**, 741–745.

3. Chaubert P, Benhattar J, Saraga E, Costa J. K-ras mutations and p53 alterations in neoplastic and non-neoplastic lesions associated with longstanding ulcerative colitis. *Am J Path* 1994, **144**, 767–775.
4. Kern SE, Redston M, Seymour AB, *et al.* Molecular genetic profiles of colitis-associated neoplasms. *Gastroenterology* 1994, **107**, 420–428.
5. Chen J, Compton C, Cheng E, Fromowitz F, Viola MV. c-Ki-ras mutations in dysplastic fields and cancers in ulcerative colitis. *Gastroenterology* 1992, **102**, 1983–1987.
6. Burmer GC, Levine DS, Kulander BG, Haggitt RC, Rubin CE, Rabinovitch PS. c-Ki-ras mutations in chronic ulcerative colitis and sporadic colon carcinoma. *Gastroenterology* 1990, **99**, 416–420.
7. Meltzer SJ, Mane SM, Wood PK, *et al.* Activation of c-Ki-ras in human gastrointestinal dysplasias determined by direct sequencing of polymerase chain reaction products. *Cancer Res* 1990, **50**, 3627–3630.
8. Brentnall TA, Crispin DA, Rabinovitch PS, *et al.* Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology* 1994, **107**, 369–378.
9. Yin J, Harpaz N, Tong Y, *et al.* p53 point mutations in dysplastic and cancerous ulcerative colitis lesions. *Gastroenterology* 1993, **104**, 1633–1639.
10. Burmer GC, Crispin DA, Kolli VR, *et al.* Frequent loss of a p53 allele in carcinomas and their precursors in ulcerative colitis. *Cancer Commun* 1991, **3**, 167–172.
11. Bos JL, Fearon ER, Hamilton SR, *et al.* Prevalence of ras mutations in human colorectal cancers. *Nature* 1987, **327**, 293–297.
12. Vogelstein B, Fearon ER, Hamilton SR. Genetic alterations during colorectal tumor development. *N Engl J Med* 1988, **319**, 525–532.
13. Losi L, Benhattar J, Costa J. Stability of K-ras mutations throughout the natural history of human colorectal cancer. *Eur J Cancer* 1992, **28A**, 1115–1120.
14. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991, **253**, 49–53.
15. Baker SJ, Preisinger AC, Jessup JM, *et al.* p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 1990, **50**, 7712–7722.