Contrasting molecular pathology of colorectal carcinoma in Egyptian and Western patients

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Summary Colorectal cancer is uncommon in Egypt, but a high proportion of cases occurs before age 40 years and in the rectum. We compared the molecular pathology of 59 representative Egyptian patients aged 10–72 to Western patients with sporadic, young-onset, or hereditary non-polyposis colorectal cancer syndrome (HNPPC)-associated carcinoma and found significant differences. Most Egyptian cancers were rectal (51%) and poorly differentiated (58%). High levels of microsatellite instability (MSI-H) were frequent (37%) and attributable in some cases (36%) to methylation of the promoter of the hMLH1 mismatch repair gene, but no MSI-H cancer had loss of hMSH2 mismatch repair gene product of the type seen with germline hMSH2 mutation in HNPPC. K-ras mutation was uncommon (11%). In subset analyses, high frequencies of MSI-H in rectal carcinomas (36%) and p53 gene product overexpression in MSI-H cancers (50%) were found. MSI-H and K-ras mutation in Egyptians under age 40 were unusual (17% and 0%, respectively), and schistosomiasis was associated with MSI and K-ras mutation. Cluster analysis identified 2 groups: predominantly young men with poorly differentiated mucinous and signet-ring cell colorectal carcinoma lacking K-ras mutation; older patients who had wild- or moderately differentiated adenocarcinoma often with MSI-H, K-ras mutation and schistosomiasis. Our findings show that the molecular pathology of colorectal cancer in older as well as younger Egyptians has unique differences from Western patients, and schistosomiasis influences the molecular pathogenesis of some tumours. © 2001 Cancer Research Campaign

Keywords: colorectal cancer; microsatellite instability; Egypt; schistosomiasis

Colorectal cancer is the fourth most common cancer and the second most common cause of cancer deaths in the United States where the vast majority of cases occurs over age 60 years (Greenlee et al, 2000). In contrast, this disease is uncommon in developing countries (Magrath and Litvak, 1993), including Egypt where it is only the fifth most common cause of cancer deaths (Soliman et al, 1999). However, the percentage of young-onset colorectal cancer cases in Egyptians is strikingly high with more than one third of cases occurring under age 40 years, and the age-adjusted mortality rates in young Egyptians are likewise high (Soliman et al, 1997a, 1999). In addition, rectal cancer is frequent (Soliman et al, 1997a, 1999).

We have conducted a series of studies to characterize the distinctive distribution of age and anatomic location and to explore the possible risk factors for colorectal cancer in Egypt. We reported previously that the unusual disease pattern may be related to 2 major groups of risk factors: heavy environmental exposures such as organochlorine pesticides (Soliman et al, 1997b) and inherited genetic factors including familial aggregation (Soliman et al, 1998b), hereditary non-polyposis colorectal cancer (HNPPC), and defective expression of mismatch repair genes (Soliman et al, 1998a). Schistosomiasis, particularly due to S. japonicum, has been implicated as an aetiologic factor in colorectal cancer (Chen et al, 1981; International Agency for Research on Cancer, 1994), and bilharziasis is common in Egypt (El-Khoby et al, 2000).

Recent advances in molecular biology have revealed numerous genetic alterations involved in colorectal tumorigenesis (Kinzler and Vogelstein, 1996; Gryfe et al, 1997; Ilyas et al, 1999). The development of most colorectal carcinomas begins with inactivation of the APC (adenomatous polyposis coli) suppressor gene/β-catenin pathway. Clonal accumulation of alterations then occurs, including activation of proto-oncogenes such as K-ras, and inactivation of additional suppressor genes. The genes commonly inactivated during progression include the p53 gene on the short arm of chromosome 17 (Levine, 1997; Ding and Fisher, 1998). Mutated p53 gene product fails to regulate normally a variety of genes regulated by wild-type p53 (Cox, 1997). For many suppressor genes, including p53, inactivation of one allele is often caused by loss due to chromosomal instability of all or part of the chromosome where the gene resides, and allelic losses throughout the genome are common (Lengauer et al, 1998).

About 15% of sporadic colorectal cancers are distinguished by nucleotide insertions or deletions in numerous, intrinsically unstable repeated sequences in tumour DNA, termed microsatellite instability (MSI), also termed ubiquitous somatic mutations, DNA replication errors/ERR, or nucleotide instability (Jiricny, 1998; Lengauer et al, 1998; Prolla, 1998). A workshop sponsored by the National
Cancer Institute in the United States developed criteria for classification of MSI in colorectal cancers (Boland et al, 1998). Tumours with high levels of MSI (MSI-H) are characteristic of hereditary nonpolyposis colorectal cancer syndrome (HNPCC), and MSI testing provides a sensitive diagnostic indicator of patients with the syndrome, although most MSI-H tumours in older patients are sporadic (Boland et al, 1998). MSI-H also occurs at high frequency in young patients with presumably sporadic colorectal cancer (Liu et al, 1995; Lukish et al, 1998; Chan et al, 1999).

HNPCC is caused by germline mutation of one of a group of DNA mismatch repair genes followed by somatic inactivation of the other allele (Lynch and Lynch, 2000). Abnormalities of the hMLH1 gene or hMSH2 gene are responsible for most HNPCC cases, and loss in tumours of immunohistochemical nuclear expression of the products of these genes often indicates which gene is mutated in the germline (Dietmaier et al, 1997; Fujiwara et al, 1998; Dieumegard et al, 2000; Muller et al, accepted). By contrast, sporadic MSI-H tumours usually occur due to somatic inactivation of both alleles of the hMLH1 mismatch repair gene, most commonly via transcriptional silencing which results from aberrant methylation of cytosines in the cytosine- and guanine-rich promoter region with loss of nuclear immunohistochemical expression of the gene product (Kane et al, 1997; Herman et al, 1998; Kuismanen et al, 2000). The genetic alterations which accumulate during progression of both hereditary and sporadic neoplasms characterized by MSI-H include mutations in genes with microsatellites within the coding region, such as the type II receptor for transforming growth factor β1 (TGFβ1RII) (Parsons et al, 1995; Fujiwara et al, 1998; Akhurst and Balmain, 1999; Abe and Masuda, 2000; Calin et al, 2000). Allelic deletions which are frequent in microsatellite-stable cancers are rare in MSI-H cancers (Fujiwara et al, 1998; Lengauer et al, 1998). Colorectal carcinomas with low levels of MSI (MSI-L) are uncommon and appear to arise through a pathway which differs from MSI-H and microsatellite-stable tumours, possibly involving hyperplastic polyps as a precursor (Iino et al, 1999; Jass et al, 1999).

The molecular pathology of colorectal carcinoma in Egypt has not been reported. We, therefore, studied the profile of pathological and molecular characteristics of colorectal cancers in patients from this country where adverse environmental exposures are high and genetic factors are known to be important (Soliman et al, 1997b, 1998a, 1998b). We analysed specimens from 59 Egyptian colorectal cancer cases for MSI status, methylation of the hMLH1 promoter, loss of expression of hMSH2 gene product, K-ras gene mutation in codons 12 and 13, and p53 gene product overexpression of the type seen with p53 gene mutation. We compared the molecular pathology to Western patients with sporadic, young-onset or HNPCC-associated colorectal cancer previously published from our laboratory (Jen et al, 1994a; Fujiwara et al, 1998; Lukish et al, 1998).

**MATERIALS AND METHODS**

**Egyptian tumour specimens and patients**

Paraffin-embedded blocks of unbuffered formalin-fixed colorectal carcinomas from 59 consecutive patients were included in this study. These patients participated in our previous study on familial aggregation of colorectal cancer in Egypt (Soliman et al, 1998b) and had their resection in 1996 and 1997 at the National Cancer Institute in Cairo (n = 37) or Tanta Cancer Center in the mid Nile River Delta region (n = 22). The study was approved by the institutional review boards at the MD Anderson Cancer Center and The Johns Hopkins Medical Institutions and was performed in accordance with filed institutional assurances. Information on demographics, residence, family history, tumour site and stage were retrieved from the medical records, pathology reports and our previous epidemiologic studies. Patients with familial adenomatous polyposis were excluded.

Patient age ranged from 10 to 72 years (mean 42.8 ± SD 15.1), and the series included 34 males (58%) and 25 females. All patients had stage II or stage III colorectal carcinoma. The residence of most patients was rural (78%). 27% of the patients were offsping of consanguineous marriages of first cousins. The study group was representative of our previous national study of 1608 colorectal cancer patients in Egypt (Soliman et al, 1997a), with no statistically significant differences in age, gender, residence or frequency of rectal cancer.

For each case, the histopathologic diagnosis of colorectal cancer was confirmed, and tumour differentiation and mucinous (colloid) and signet-ring cell histology were recorded based on criteria used in our previous studies (Jen et al, 1994a; Kim et al, 1994). All blocks of tumour tissue, nearby mucosa and distant surgical margins were serially sectioned (5 serials, 20 μm apart) for evaluation of schistosomal infection. Criteria included the presence of intact or calcified ova, active granulomas or worms. Speciation of schistosoma on the basis of morphology was not attempted.

**Comparison groups of Western patients**

Previously published data for comparison with the Egyptian carcinomas were available from 3 groups of colorectal carcinomas analysed in our laboratory: (1) sporadic colorectal cancers (n = 145) from stage II and III patients at The Johns Hopkins Hospital enrolled in a study of prognostic markers (Jen et al, 1994a); (2) young-onset colorectal cancers in patients under age 40 years (n = 36) from a study at the National Naval Medical Center in Bethesda, Maryland (Lukish et al, 1998); and (3) HNPCC-associated colorectal cancers (n = 39) from American, Canadian, and New Zealand patients in 20 families (Fujiwara et al, 1998) that met the International Collaborative Group criteria for HNPCC (Vasen et al, 1991) and/or had germline mutation of hMSH2 or hMLH1 mismatch repair genes (Wijnen et al, 1997; Genuardi et al, 1998).

**Microdissection and DNA extraction**

For all 59 Egyptian tumours, areas of carcinoma and non-neoplastic tissue were microdissected from numbered tissue sections. Cellular tumour that contained few stromal and inflammatory cells was selected. Multiple areas with high cellularity from each slide were combined to enhance the detection of clonal abnormalities. DNA was extracted as in our previous studies (Jen et al, 1994a; Moskaluk and Kern, 1997; Fujiwara et al, 1998; Lukish et al, 1998).

**Assay for microsatellite instability (MSI)**

All molecular and immunohistochemical analyses were done without access to clinical data. MSI was evaluated by polymerase chain reaction (PCR) amplification of 8 non-coding polymorphic dinucleotide repeat sequences, 2 non-coding rarely polymorphic polyadenine tracts (BAT26 and BAT25), and 1 coding rarely polymorphic
polyadenine tract in the transforming growth factor beta 1 type II receptor gene (TGFβRII), as in our previous studies (Jen et al., 1994a, 1994b; Parsons et al., 1995; Fujiwara et al., 1998). The 8 dinucleotide repeat sequences included 5 on the long arm of chromosome 18 (D18S69, D18S64, D18S55, D18S61, and D18S58 from centromere to telomere), 2 on the long arm of chromosome 5 (D5S107 and D5S346), and 1 on the short arm of chromosome 2 (D2S123).

The normal alleles were represented by 1 (non-polymorphic) or 2 (polymorphic) major bands accompanied by a few minor bands. The mobility shift of PCR products from tumour DNA as compared with corresponding non-neoplastic tissue DNA was determined for each marker independently. Each tumour was classified from the aggregated marker results as: (1) high levels of MSI (MSI-H) if at least 2 markers representing at least 30% of informative dinucleotide or mononucleotide markers were shifted (Boland et al., 1998) and/or mutation was present in TGFβRII (Dietmaier et al., 1997; Perucho, 1999); (2) low levels of MSI (MSI-L) if only 1 marker among a minimum of 5 dinucleotide and mononucleotide markers was shifted; (3) microsatellite-stable if no marker among a minimum of 5 informative markers was shifted; (4) indeterminate for MSI if 3 or fewer markers without shift were informative.

**Assay for methylation of hMLH1 promoter**

Somatic and germline mechanisms leading to MSI were evaluated. Somatic methylation of the promoter of the hMLH1 mismatch repair gene is associated with transcriptional silencing of this gene and resultant MSI-H. The methylation status of hMLH1 in Egyptian carcinomas with known MSI status was determined by bisulfite treatment of DNA followed by methylation-specific polymerase chain reaction (MSP-PCR) as described (Herman et al., 1996) with modification of primers for methylated and unmethylated alleles and cycling conditions. Detailed protocols are available at the website www.mdanderson.org/leukemia/methylation/. In brief, 2 μg of genomic DNA from microdissected tumour were denatured with sodium hydroxide, incubated with sodium bisulfite, purified with DNA Cleanup Kit (Promega), incubated with sodium hydroxide, precipitated with ammonium acetate and ethanol, washed with ethanol and resuspended in distilled water. The primers were: methylated allele, sense primer: 5′-GATAGCGATTTTTAACGC-3′; methylated allele, antisense primer: 5′-TCTATAAATATACCTCTTCG-3′; unmethylated allele, sense primer: 5′-AGAGTGTTTATAATTTTTCAATG-3′ unmethylated allele, antisense primer: 5′-ACTTATAATTACCTCTTCCA-3′. Cycling conditions were 95°C for 10 cycles and 40 cycles of 95°C for 30 seconds and 53°C for 45 seconds. PCR products for methylated and unmethylated reactions were electrophoresed on acrylamide gels and visualized by ethidium bromide staining. Gel photographs were digitized using a BioRad imager and evaluated by densitometry using the manufacturer’s software.

**Immunostaining for hMSH2 and hMLH1 gene products**

Loss of nuclear expression of hMSH2 gene product is strongly associated with germline mutation of the hMSH2 gene in patients with HNPPC (Dietmaier et al., 1997; Fujiwara et al., 1998; Dieuemgard et al., 2000; Muller et al., accepted). Loss of hMLH1 gene product can occur with germline mutation of the gene in HNPPC patients (Muller et al., accepted) and with transcriptional silencing by somatic methylation (Kane et al., 1997; Herman et al., 1998; Kuismanen et al., 2000). The subset of Egyptian carcinomas previously characterized for MSI status was evaluated by immunohistochemistry after antigen retrieval with citrate buffer. hMSH2 antibody (clone FE11, Oncogene Research Products, Boston, MA) was used at 1:100 dilution and hMLH1 antibody (clone G18–15, Pharmingen, San Diego, CA) at 1:30 dilution in the TechMate 1000 automated staining system (Ventana/BioTek Solutions, Tucson, AZ), similar to our previous studies (Fujiwara et al., 1998). Each stained slide was evaluated for positive-control staining of nuclei in epithelial cells at the bases of colorectal crypts and/or germinal centres of lymphoid nodules. Tumour cell nuclei in the immediate vicinity of internal positive controls were then interpreted for the presence or absence of nuclear staining. As in our and other previous studies (Fujiwara et al., 1998; Muller et al., accepted), hMLH1 staining was inconsistent and therefore not evaluated further.

**Assay for K-ras proto-oncogene mutations**

All possible mutations in codons 12 and 13 of the K-ras proto-oncogene were determined as in our previous studies (Jen et al., 1994b; Nucci et al., 1997). Exon 1 was amplified, and the resulting PCR product from each tumour was sequenced using the SequiTherm EXCEL II™ DNA sequencing kit (Epiconcept Technologies, Madison, WI).

**Immunostaining for p53 gene product overexpression and mutation analysis**

Detection of p53 gene product overexpression by immunohistochemistry was used as an indicator of p53 gene mutation. This method provides about 75% overall accuracy in colorectal neoplasms owing to the prolonged half life of most mutant proteins in such tumours (Baas et al., 1994). DO7 mouse monoclonal antibody against p53 (Dako, Carpinteria, CA) was used at 1:100 dilution in the TechMate 1000 automated staining system (Ventana/BioTek Solutions, Tucson, AZ), as in our previous studies (Moskaluk et al., 1996). p53 labelling index of >40% in a discrete region of or entire tumour was considered positive. Exons 5 through 9 of the p53 gene were amplifiable and sequenced as in our previous studies (Moskaluk et al., 1996) in 3 MSI-H cancers with p53 gene product overexpression and high-quality DNA.

**Statistical analysis**

Differences in frequencies between the Egyptian group and the Western groups and between subsets within the Egyptian group were evaluated by simple contingency table analysis (Fisher’s exact probability test) using the SAS statistical package (Statistical Analysis System User’s Guide, 1990) and true Epistat. P values are two-sided.

Cluster analysis was performed to identify subgroups of Egyptian patients who had similar demographic, pathologic and molecular attributes (Anderberg, 1973). Variables that were included in this analysis were age (categorized into 2 groups younger or older than 40 years), sex, residence (urban versus rural), schistosomiasis (presence or absence of histopathologic
gene mutation, and not significantly different from the young-onset group (47%). However, the Egyptian cases were heterogeneous, as MSI-H was somewhat more commonly seen in older than younger Egyptian patients (17% vs 46% \( P = 0.15 \), Table 3), and in those from urban areas (50% vs. 22%, \( P = 0.002 \)). A family history evidence), tumour site (colon versus rectum), tumour histology (4 variables defined by glandular, mucinous, signet-ring cell or other histopathologic type), MSI status (one variable contrasting MSI-H versus MSI-L and microsatellite-stable cancers, and another variable contrasting MSI-L versus MSI-H and microsatellite-stable tumours), \( K ras \) gene mutation, and \( p53 \) gene product overexpression. We first re-scaled the data as a distance measure using one minus the Jaccard coefficient (Anderberg, 1973). We used the average linkage cluster analysis method and chose the number of clusters by evaluating the pseudo \( R^2 \). In order to create clusters in a parsimonious manner, we performed the cluster analysis, evaluated the resulting clusters to identify any variables that did not vary significantly among the clusters, and then removed those variables.

## RESULTS

The Egyptian patients were young, with ages ranging from 10–72 years and with 44% of patients under age 40 (Table 1). The pathologic features of the Egyptian colorectal cancers were unusual as compared to Western patients. About half of the 59 Egyptian carcinomas (51%) were located in the rectum (Table 1), as contrasted with less than 25% in all 3 Western groups (\( P = 0.009 \) to < 0.0001, Table 2). Most Egyptian tumours (58%) were poorly differentiated, and about a third (37%) were mucinous (Figure 1); by contrast, among Western cases poorly differentiated tumours were frequent only in HNPCC patients (Table 2). Tumour histopathology differed between younger and older Egyptians as well: a significantly larger proportion of tumours from patients under 40 years of age were mucinous and poorly differentiated (\( P = 0.03 \) and \( P = 0.009 \), respectively, Table 3). Schistosomiasis was evident in tumour and/or adjacent tissue of 32% of patients and was unrelated to clinical and other pathologic features.

Consistent with the unusual tumour pathology, the molecular characteristics of the Egyptian cancers also differed from Western patients studied in our laboratory (Table 2). MSI-H occurred in 37% of Egyptian carcinomas. Among the 10 MSI-H cases evaluated with dinucleotide and mononucleotide markers, the percentage of altered markers ranged from 33% to 100% with a mean of 61 ± 8% (standard error) and a median of 47%. 8 of these 10 MSI-H cancers had informative mononucleotide markers, and all had at least 1 shifted. 4 MSI-H cases were evaluated with mononucleotide markers only. 3 had at least 2 mononucleotide markers shifted, and the fourth had a mutation in \( T G F \beta 1 R I I \). MSI-H was explained by methylation of the promoter of the \( h M L H 1 \) mismatch repair gene in 36% of the cancers (Table 4, Figure 2), but no loss of nuclear \( h M S H 2 \) mismatch repair gene product indicative of germline \( h M S H 2 \) mutation was found (Table 4, Figure 3). MSI-L was found in 16% of cancers, based on shift of 1 to 5 of 11 informative dinucleotide and mononucleotide markers.

The frequency of MSI-H tumours in the Egyptian group as a whole was higher than in the Western sporadic group (37% vs. 13%, \( P = 0.002 \)) but much lower than the HNPCC group (95%, \( P < 0.0001 \)) and not significantly different from the young-onset group (47%). However, the Egyptian cases were heterogeneous, as MSI-H was somewhat more commonly seen in older than younger Egyptian patients (17% vs 46% \( P = 0.15 \), Table 3), and in those from urban areas (50% vs. 22%, \( P = 0.002 \)). A family history of a first-degree relative with colorectal cancer was present in only 1 patient with an MSI-H cancer, who was in the older age group (48 years old). The Egyptian patients had by far the highest proportion of MSI-H tumours located in the rectum as compared to the 3 groups of Western patients (36% vs. 0% to 6%, Table 2).

\( K ras \) mutation was unusual in Egyptian colorectal cancers as compared with Western patients (11% vs. 28–48%, \( P = 0.05 \) to 0.0004, Table 2 and Figure 2). There was a trend toward higher prevalence of \( K ras \) mutation in older than younger Egyptian patients (17% vs 0%, \( P = 0.14 \), Table 3). \( p53 \) overexpression was somewhat more common in cancers of younger Egyptian patients compared with older Egyptian patients (57% vs. 39%, Table 3 and Figure 4), but the difference was not statistically significant.

When profiles of multiple alterations were considered, the frequency of MSI-H cancers with \( K ras \) mutation was comparable in the Egyptian and Western groups, but the frequency of MSI-H tumours with \( p53 \) overexpression of the type seen with \( p53 \) gene mutation was markedly higher among Egyptians (50% vs 0% in the sporadic group and 5% in the HNPCC group, Table 2). Sequencing of exons 5–9 of the \( p53 \) gene in 3 available MSI-H Egyptian cancers with \( p53 \) gene product overexpression confirmed a mutation in 2 (T to A transversion in codon 137, nucleotide 410 of exon 5, CTG to CAG, leu to gln; G to A transition in codon 238, nucleotide 713 of exon 7, TGT to TAT, cys to tyr). These mutations have been reported previously in colorectal and other tumours (Hainaut et al, 1998).

### Table 1 Clinical characteristics of 59 colorectal cancer patients in Egypt

| Characteristic         | Frequency in % (No.) |
|------------------------|----------------------|
| Age (years)            |                      |
| < 40                   | 44 (28)              |
| 40 +                   | 56 (33)              |
| Gender                 |                      |
| Male                   | 58 (34)              |
| Female                 | 42 (25)              |
| Residence              |                      |
| Rural                  | 78 (46)              |
| Urban                  | 22 (13)              |
| Tumour site            |                      |
| Ascending colon        | 25 (15)              |
| Transverse colon       | 5 (3)                |
| Descending colon       | 15 (9)               |
| Rectosigmoid colon     | 3 (2)                |
| Rectum                 | 51 (30)              |
Schistosomiasis was unequally distributed relative to MSI and K-ras gene mutation status. Ova were identified in 50% (8/16) of the patients with MSI-H cancers and in 67% (4/6) of the MSI-L patients, but in only 22% (7/32) of the patients with microsatellite-stable cancers ($P = 0.002$). Schistosomiasis was also more frequent among patients whose tumours showed K-ras gene mutation (80% of the 5 patients whose tumour had K-ras mutation vs. 31% of the 42 patients whose tumour was negative for K-ras mutation, $P = 0.05$).

Complete data including K-ras mutation analysis were available for 46 patients. We therefore performed cluster analyses in this subset of patients including and excluding those with K-ras mutation data, and the results were qualitatively similar. Initial analysis showed no effect of p53 and tumour site in differentiating the clusters, so these variables were dropped from further analysis. The pseudo $t^2$ statistics for 1 versus 2 clusters were 19.3 and 8.5, suggesting that a model with 2 clusters would adequately describe the data. The first cluster was composed predominantly of young men with poorly differentiated carcinomas including mucinous or signet-ring cell histologies (Table 5). No patient in this cluster had K-ras mutation. MSI-H was infrequent, although MSI-L was evident in about 20% of patients, especially those with schistosomiasis. By contrast, the second cluster included predominantly older patients with well- or moderately differentiated gland-forming adenocarcinoma and a higher frequency of MSI-H, K-ras mutation and schistosomiasis than the first.

| Pathology | Egyptians | Sporadic | Westerners | Young (< 40 yrs) | HNPCC |
|-----------|-----------|----------|------------|-----------------|--------|
| Rectal cancer | 51 (38–64) | 19 (13–26) | 22 (10–39) | 3 (0–13) | 0.0001 |
| Poor differentiation | 58 (44–70) | 15 (10–22) | 22 (10–39) | 56 (40–72) | 0.0001 |
| Mucinous type | 37 (25–51) | 11 (6–17) | 14 (5–30) | Data not available | 0.001 |

| Molecular pathology | Egyptians | Sporadic | Westerners | Young (< 40 yrs) | HNPCC |
|---------------------|-----------|----------|------------|-----------------|--------|
| MSI-H | 37 (22–54) | 13 (8–20) | 47 (30–65) | 95 (83–99) | 0.002 |
| K-ras mutation in codon 12 or 13 | 11 (4–23) | 28 (21–37) | 48 (30–67) | 28 (15–45) | 0.02 |
| p53 gene product overexpression | 46 (33–60) | 36 (24–50) | Not done | 8 (2–21) | 0.34 |
| MSI-H in rectal cancers | 36 (13–65) | 0 (0–19) | 6 (0–29) | 3 (0–14) | 0.01 |
| MSI-H and K-ras mutation | 23 (5–54) | 18 (4–43) | 33 (13–59) | 31 (17–49) | 0.99 |
| MSI-H and p53 overexpression | 50 (23–77) | 0 (0–19) | Not done | 5 (1–18) | 0.001 |

Table 2  Comparison of pathology and molecular pathology in colorectal cancers of patients in Egypt and 3 Western groups

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Table 3  Age-related differences in pathology and molecular pathology in colorectal cancers of Egyptian patients

| Tumour characteristic | < 40 | Age (years) 40+ |
|-----------------------|------|-----------------|
|                       | %    | Fraction        | %    | Fraction    | P    |
| Differentiation       |      |                 |      |             |      |
| Poor                  | 77   | 20/26           | 42   | 14/33       | 0.009|
| Moderate or well      | 23   | 6/26            | 58   | 19/33       | 0.009|
| Mucinous carcinoma    | 54   | 14/26           | 24   | 8/33        | 0.03 |
| MSI-H carcinoma       | 17   | 2/12            | 46   | 12/28       | 0.15 |
| K-ras gene mutation   | 0    | 0/18            | 17   | 5/29        | 0.14 |
| p53 overexpression    | 57   | 13/23           | 39   | 13/33       | 0.28 |

Table 4  Evaluation of colorectal cancers from Egyptian patients for mechanisms producing microsatellite instability

| Mechanism                  | MSI-H (95% CI) | MSI-L (95% CI) | MS Stable (95% CI) |
|----------------------------|----------------|----------------|--------------------|
|                           | n positive/n | examined     | n positive/n | examined     | n positive/n | examined     |
| Loss of nuclear hMHS2 gene product (germ line) | 0 (0–31) | 0/10 | 0 (0–4) | 0/14 | 0 (0–23) | 0/14 |
| Methylation of MLH1 promoter (somatic) | 36 | 0/11 | 0 | 0/5 | 0 | 0/18 |

DISCUSSION

We report here that the molecular pathology of colorectal carcinoma in Egypt differs from Western patients, and between younger and older Egyptians. These findings reinforce the conclusion from our previous epidemiologic studies (Soliman et al, 1997a, 1999) that the pathogenesis of colorectal carcinoma in Egypt has unique features. Although Arabic countries share some cultural background and environmental exposures (Tentamy et al, 1994; Al-Saleh et al, 1998), rates of young-onset disease are higher in Egypt than in Jordan (Al-Jaberi et al, 1997), Lebanon (Adib et al, 1998), Algeria (Parkin et al, 1992), and Saudi Arabia (Ishbister, 1992).

Occurrence of colorectal carcinoma at young age in Egypt could reflect the presence of clinically inapparent inherited syndromes. We excluded patients with history of or morphologic evidence of familial adenomatous polyposis. HNPCC is unlikely because tumours with loss of hMHS2 gene product of the type seen with germline hMHS2 mutation were not identified in our study. MSI-H cancers in young patients suggestive of HNPCC were rare, and the frequency of family history of colorectal or other HNPCC-associated cancers was low. In addition, some of the MSI-H cancers were explained by methylation of the hMLH1 promoter. There is a high prevalence of consanguinity in Egypt, reflected in our study group, because of the tradition of interfamilial marriages (Tentamy et al, 1994), and this cultural characteristic could contribute to non-syndromic inherited predisposition.

Poorly differentiated carcinomas with mucinous and signet-ring cell histology occurred in our first cluster (Table 5) of young Egyptian patients with low frequency of schistosomiasis. Studies in Western countries with high risk of colorectal cancer and in low-risk populations such as the black populations of South Africa and East Africa have also shown increased frequency of mucinous histology and rectal cancer in young patients (Bedikian et al, 1981; Adloff et al, 1986; Peters et al, 1989; Heys et al, 1994; Steele, 1994; Rubin et al, 1998; Boytchev et al, 1999). Epidemiologic studies indicate that tumour site may be related to specific risk factors affecting an anatomical segment of the colorectum (Williams et al, 1977; Weisburger et al, 1980; Peters et al, 1989). We found, however, that the rectal predominance in Egyptian cases occurred in both younger and older patients and was unrelated to urban/rural residence, which is associated with very different environmental exposures.

Both microsatellite-stable and MSI-H carcinomas in our series had unusual molecular genetic characteristics in comparison to Western tumours. Mutation of the K-ras proto-oncogene was uncommon in Egyptian colorectal cancers in general, in contrast to Western cases (Gryfe et al, 1997; Ilyas et al, 1999), and was not found in any patient under age 40 years in our study. Low frequency
of K-ras mutation has also been found previously in colorectal carcinomas in the low-incidence population in Singapore, with increasing mutation frequency associated with increasing incidence in the population in recent years (Tang et al, 1999). K-ras mutation is also uncommon in rectal carcinomas of Finnish women (Servomaa et al, 2000). MSI-H cancers are rarely located in the rectum in Western populations (Boland et al, 1998; Lukish et al, 1998), but fully half of Egyptian MSI-H cases were at that site. MSI-H carcinomas in our Egyptian series had frequent overexpression of p53 gene product of the type seen with p53 gene mutation, and sequencing confirmed the presence of a p53 gene mutation in 2 of 3 informative cases. By contrast, p53 overexpression is uncommon in MSI-H cancers in a large number of Western series, including our own (Ionov et al, 1993; Konishi et al, 1996; Breivik et al, 1997; Losi et al, 1997; Olschwang et al, 1997; Ruschoff et al, 1997; Forster et al, 1998; Lukish et al, 1998; Yagi et al, 1998).

Schistosomiasis with S. japonicum is associated with risk of colorectal cancer in studies correlating incidence, prevalence and/or mortality rates of the 2 diseases and in case–control studies done mainly in China and Japan (Chen et al, 1981; International Agency for Research on Cancer, 1994). Studies in Egypt showed an average prevalence of S. haematobium of 7.8% in Upper Egypt and 36.4% for S. mansoni in Lower Egypt in the respective endemic areas (El-Khoby et al, 2000). Infection rates were higher in children and adolescents and in males, 21% of young children in the Nile Delta region had schistosomiasis in another study (Curtale et al, 1998). We identified by histopathology a prevalence of 32% in our resection specimens of colorectal carcinoma from 2 cancer centres in Cairo and Lower Egypt, a rate comparable to the general population. We also found associations between molecular characteristics of colorectal carcinomas and infection. Although our patient numbers are small, our results suggest that schistosomiasis is associated with microsatellite instability and with K-ras mutation in Egyptian colorectal carcinomas (cluster 2 in Table 5), favouring schistosomiasis as a factor influencing pathogenesis in some cancers. This finding also indicates different molecular mechanisms for these genetic changes than in Western patients. A previous study has related the type of p53 mutation in rectal cancer to schistosomiasis with S. japonicum (Zhang et al, 1998), but the mechanisms resulting in the differing profiles of accumulated genetic alterations in Egyptian and Western cancers remain unexplained.

The morphologic precursors to colorectal carcinoma in Egypt have not been studied extensively. Flat adenomas have been characterized as an important precursor to colorectal carcinoma (Rembacken et al, 2000). These lesions have a low frequency of K-ras gene mutation (Jass, 1995), as does dysplasia complicating chronic inflammatory bowel disease in the form of ulcerative colitis and Crohn colitis, in which MSI-H is relatively common (Umetani et al, 1999). Our finding that K-ras gene mutation is infrequent in Egyptian colorectal carcinomas unassociated with schistosomiasis raises the possibility that the flat adenoma could be an important precursor.

Westernization of Egypt is occurring as the country develops and is affecting young people first because they are more likely to change lifestyle than older people (Rockhill and Giovannucci, 1999). The remarkable differences in molecular pathology as compared to Western patients could result from inherent differences
in sensitivity and molecular responses to Western lifestyle in Egyptians (Rockhill and Giovannucci, 1999). Schistosomiasis appears to be important in colorectal carcinogenesis in some cases. The outcome of the search for germline predispositions, environmental factors and gene-environment interactions that are responsible for our observations will impact strategies for prevention as well as advance knowledge of the pathways involved in the molecular pathogenesis of colorectal carcinoma.

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REFERENCES

Abe Y and Masuda H (2000) Genetic alterations of sporadic colorectal cancer with microsatellite instability, especially characteristics of primary multiple colorectal cancers. J Surg Oncol 74: 249–256
Addb SM, Mufarrij AA, El-Omari N and Al-Omari M (1997) Colorectal adenocarcinoma in a well-defined Jordanian population from 1990 to 1995. J Royal Coll Surg Edin 42: 323–325
Ahmed S, Aleem M, Majeed F, Khan NA, Javaid M, Arain MA, et al. (2000) H. pylori infection and colorectal cancer in young adults. South Med J 93: 920–924
Boland CR, Thiodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Faderre P, Ranzani GN and Srivastava S (1998) A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248–5257
Boyechko V, Jhiang S, Oettle GJ (1999) The characteristics of large bowel cancer in the low-risk population of the Wittwatersrand. J Royal Coll Surg Edin 44: 366–370
Brenvik I, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL and Gaudernack G (1997) Different genetic pathways to proximal and distal colorectal cancer influenced by sex related factors. Int J Cancer 74: 664–669
Calin GA, Gafa R, Tihitere MG, Herlea V, Becheanu G, Covazirici L, Barbantti-Brodano G, Nenciu I, Negruzzi M and Lanza G (2000) Genetic progression in microsatellite instability high (MSI-H) colon cancers correlates with clinicopathological parameters: A study of the TGFBetaRII, BAX, IxMISH, 1vMSH6, RFIIR and BLM genes. Int J Cancer 89: 230–235
Chen TL, Vien ST, Chang LP, Ho JW, Kwan ASY, Ho JCY, Leung SY and Wylie AH (1999) Frequent microsatellite instability and mismatch repair gene mutations in young Chinese patients with colorectal cancer. J Natl Cancer Inst 91: 1221–1226
Chen MC, Chang PY, Chuang CY, Chen YJ, Wang FP, Tang YC and Chou SC (1991) Colorectal cancer and schistosomiasis. Lancet 1: 971–973
Cox LS (1997) Multiple pathways control cell growth and transformation: Overlapping and independent activities of p53 and p21WAF1/CIP1. J Pathol 183: 134–140
Curtale F, Nabil M, El-Wakeel A and Shamy MY (1998) Anemia and intestinal parasitic infections among school age children in Beheira Governorate, Egypt. J Trop Ped 44: 323–328
Dietmaier W, Wallinger S, Backer T, Kullmann F, Fishel R and Ruschoff J (1997) Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 57: 5248–5257
Drozdeur G, Grandjouan S, Sabourin J-C, Le Bihan M-L, LEfretre I, Belfegh1,ignon J-P, Rougier P, Lasser P, Benard J, Couturier D and Bressac-de Paillerets B (2000) Extensive molecular screening for hereditary nonpolyposis colorectal cancer. Br J Cancer 82: 871–880
Ding HF and Fisher DE (1998) Mechanisms of p53 mediated apoptosis. Crit Rev Oncol Hematol 9: 83–98
El-Khoby T, Galal N, Fenwick A, Barakat R, El-Hawey A, Nooman Z, Habb M, Abdel-Wahab F, Gahr NS, Hamman HM, Hussein MH, Mkhial NH, Cine BL, and Strickland GT (2000) The epidemiology of schistosomiasis in Egypt. Summary findings in nine governorates. Am J Trop Med Hyg 62(2 Suppl): 88–89
Forster S, Satller HP, Hack M, Romanakis K, Rohde V, Seitz G and Wullich B (1998) Microsatellite instability in sporadic carcinomas of the proximal colon: association with diploid DNA content, negative protein expression of p53, and distinct histopathological features. Surgers 123: 13–18
Fujitawa T, Toikek MF, Watanabe T, Rashid A, Longo P, Eshleman JR, Booker S, Lynch HT, Jass JR, Green JS, Kim H, Jen J, Vogelstein B and Hamilton SR (1998) Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. Am J Pathol 153: 1063–1078
Genuardi M, Antt M, Capozzi E, Leonardi F, Fornasarig M, Novella E, Bellacosa A, Iino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y and Watanabe H (1999) DNA microsatellite instability in human milk samples from women living in Amman, Jordan. Crit Rev Oncol Hematol 31: 81–98
Gryfe R, Swallow C, Bapat B, Redston M, Gallinger S and Couture J (1997) Microsatellite instability in hyperplastic polyps, serrated adenomas, and colorectal cancer in the low-risk population of the Witwatersrand. J Clin Pathol 50: 825–834
Hajnal S, Hamman HR, Melis MM, Mkhial NH, Hussein MH, Fodde R, Ranzani GN, Gin F and Esteller M (1998) MLH1 and MSH2 constitutional mutations in colorectal cancer families not meeting the standard criteria for hereditary nonpolyposis colorectal cancer. Int J Cancer 75: 835–839
Greenlee RT, Murray T, Bolden S and Wingo PA (2000) Cancer statistics, 2000. CA Cancer J 49: 8–31
Gryfe R, Swallow C, Bapat B, Redston M, Gallinger S and Couture J (1997) Molecular biology of colorectal cancer. Curr Prob Cancer 21: 233–300
Hainaut P, Hernandez T, Robinson A, Rodriguez-Torres P, Fournes T, Holstein M, Harris CC and Montesano R (1998) IARC database of p53 mutations in human tumors on cell lines: Update compilation, revised formats and new visualization tools. Nucleic Acids Res 26: 205–213
Herman JG, Graff JR, Mysakhan S, Nelkin BD and Baylin SB (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93: 9821–9826
Herman JG, Graff JR, Mysakhan S, Nelkin BD and Baylin SB (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93: 9821–9826
Heyes SD, O’Hanrahan TJ, Britenden J and Eeremine O (1994) Colorectal cancer in young patients: a review of the literature. Eur J Surg Oncol 20: 225–231
Iino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y and Watanabe H (1999) DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: A mild mutator pathway for colorectal cancer? J Clin Pathol 52: 5–9
Ilyas M, Straub J, Tomlinson IP and Bodmer WF (1999) Genetic pathways in colorectal cancer: mixed polyps: A mild mutator pathway for colorectal cancer? J Clin Pathol 52: 5–9
Iliev SI, Batin T, Banfeski S, Mitrovski G, Sekulic S, et al. (1999) NOD1 genotyping in young patients with colorectal cancer. J Clin Pathol 52: 5–9
Ilyas M, Straub J, Tomlinson IP and Bodmer WF (1999) Genetic pathways in colorectal and other cancers. Eur J Cancer 35: 1986–2002
International Agency for Research on Cancer (1994) Schistosomases, Liver Flukes and Helicobacter pylori. IARC Monogr Eval Carcin Risk Hum 61: 68–100
Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM and Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, Papadopoulos N, Lengauer C, Kinzler KW and Vogelstein B (1998) Genetic instabilities in human colorectal cancer. Nat Genet 21: 23–28.

Lynch HT and Lynch JF (2000) Hereditary nonpolyposis colorectal cancer. In Cancer: Principles & Practice of Oncology, 6th edn, eds. H. J. Weisburger, J. H. Reddy and N. E. Spingarn, Philadelphia: Lippincott-Raven, pp. 1552–1556.

Lynch HT and Lynch JF (2001) Hereditary nonpolyposis colorectal cancer. In Cancer: Principles & Practice of Oncology, 6th edn, eds. H. J. Weisburger, J. H. Reddy and N. E. Spingarn, Philadelphia: Lippincott-Raven, pp. 1552–1556.

Olschwang S, Hamelin R, Laurent-Puig P, Thuille B, DeRycke Y, Li Y-J, Muzeau F, Girerdot J, Salmon KJ and Thomas G (1997) Alternative genetic pathways in colorectal carcinogenesis. Proc Natl Acad Sci USA 94: 12122–12127.

Parikin DM, Mui CS, Whelan SL, Gao YT, Ferlay J and Powell J (eds.) (1992) Cancer incidence in five continents. IARC Scientific Publ 120: 182–185.

Parkin DM, Mui CS, Whelan SL, Gao YT, Ferlay J and Powell J (eds.) (1992) Cancer incidence in five continents. IARC Scientific Publ 120: 182–185.

Perucho M (1999) Correspondence re: Boland CR et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 59: 5548–5550.

Peters RK, Garabedian DH, Yu MC and Mack TM (1998) A case-control study of occupational and dietary factors in colorectal cancer in young men by subtype. Cancer Res 58: 5459–5468.

Prolla TA (1998) DNA mismatch repair and cancer.Curr Opin Cell Biol 10: 311–316.

Rembacken BJ, Fuji T, Cairns A, Dixon MF, Yoshida S, Chalmers DM and Aoxon AT (2000) Flat and depressed colorectal neoplasms: A prospective study of 1000 colonscopies in the UK. Lancet 355: 1211–1214.

Richter ED and Saji F (1997) Pesticide use, exposure, and risk: a joint Israeli-Palestinian perspective. Environ Res 73: 211–218.

Rocken B and Giovannucci E (1999) Epidemiology of colorectal cancer mortality rates in Menofeea, Egypt: comparison with US mortality rates. Cancer Causes and Control 10: 345–347.

Rubin BM, Domian DB, Goldberg HJ and Goldberg Ml (1998) Colon cancer in young persons of East African descent. Am J Gastroenterol 93: 1014–1015.

Sachs J, Jemal A, Bobbitt CL, Weiss BM, Modlin IM, Ries LA, Heeren T, Krapcho M, Howlader N, Noone AM (1999) SEER Cancer Statistics Review, 1973–1997. National Cancer Institute, Bethesda, MD, www.seer.cancer.gov/csr/1973_1997/

Saland BM, Levy BL, Hamza MR, Ismail K, Ismail S, Hammam HM, el-Hattab HO, Kamal SM, Soliman AG, Dorgam LA, McPherson RS and Beasley RP (1997a) Colorectal cancer in Egyptian patients under 40 years of age. Int J Cancer 71: 26–30.

Soliman AS, Bondy ML, Levin B, Hamza MR, Ismail K, Ismail S, Hammam HM, el-Hattab HO, Kamal SM, Soliman AG, Dorgam LA, McPherson RS and Beasley RP (1997b) Sequence analysis of p53 and K-ras genes in carcinoma of the rectum among Finnish women. J Clin Pathol Mol Pathol 50: 23–30.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.

Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Soliman AS, Bondy ML, Goh H-S, Elnatan J, Lee Y-S, Tang W-Y, Bondy ML, Levin B, El-Badawy S, Khaled H, Ismail S, McPherson RS, Seifeldin IA and Bondy ML (1997b) Serum uric acid concentrations in patients with colorectal cancer in Egypt. Arch Environ Health 52: 409–415.
Wijnen J, Khan PM, Vasen H, van der Klift H, Mulder A, van Leeuwen-Cornelisse I, Bakker B, Losekoot M, Moller P and Fodde R (1997) Hereditary nonpolyposis colorectal cancer families not complying with the Amsterdam criteria show extremely low frequency of mismatch repair gene mutations. *Am J Hum Genet* **61**: 329–335

Williams PR, Stegens NL and Goldsmith JR (1977) Associations of cancer site and type with occupation and industry from the Third National Cancer Survey Interview. *J Natl Cancer Inst* **59**: 1147–1185

Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M and Yuasa Y (1998) Proapoptotic gene BAX is frequently mutated in hereditary nonpolyposis colorectal cancer but not in adenomas. *Gastroenterology* **114**: 268–274

Zhang R, Takahashi S, Orita S, Yoshida A, Maruyama H, Shirai T and Ohira N (1998) p53 gene mutations in rectal cancer associated with schistosomiasis japonica in Chinese patients. *Cancer Lett* **131**: 215–221