Frequent Microsatellite Instability in Sporadic Tumors of the Upper Urinary Tract


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Abstract

Urothelial carcinoma of the renal pelvis and ureter may develop sporadically or as a manifestation of hereditary nonpolyposis colorectal cancer. The majority of hereditary nonpolyposis colorectal cancer is caused by mutation of the human DNA mismatch repair (MMR) genes and is detected by associated microsatellite instability (MSI). Seventy-three unselected urothelial carcinomas of the ureter and/or renal pelvis were screened for MSI using the National Cancer Institute-designated reference panel (plus BAT40). Instability of at least two microsatellite markers (MSI-high) was detected in 15 samples (21%). Immunohistochemical staining of the MMR proteins (hMSH2, hMLH1, or hMSH6) was absent in 13 of 15 (87%) MSI tumors, and alteration of coding sequence microsatellites (TGFβRII, Bax, hMSH3, and hMSH6) was found at frequencies of 7–33% in these samples. Tumors with MSI had significantly different clinical and histopathological features including higher prevalence in female patients, low tumor stage and grade, and a papillary and frequently inverted growth pattern. Our results suggest a molecular pathway of tumorigenesis that is similar to MMR-deficient colorectal cancers and consistent with the notion that the site distributions of hereditary or sporadic MSI-high tumors may reflect tissue-specific susceptibility to lesions processed by the MMR machinery.

Introduction

HNPCC is a dominant genetic predisposition to colorectal cancer (for review, see Ref. 1). The vast majority of HNPCC is caused by an alteration of one of the human MMR genes hMSH2 or hMLH1 (2). Mutations of the other MMR genes are either absent (hMSH3 and hPMS1), very rare (hPMS2 and hMLH3), or largely associated with atypical families [hMSH6 (3, 4)]. Although the precise mechanism of carcinogenesis is not fully understood (5), colorectal tumors of HNPCC patients exhibit clinical and molecular characteristics that are different from those of non-HNPCC colorectal carcinomas (6–8). It is also remarkable that HNPCC families display an increased risk for developing well-defined extracolonic cancers, particularly tumors of the endometrium, stomach, ovary, small bowel, brain, hepatobiliary tract, and UUT (9, 10).

Instability of short tandem repeat sequences (MSI) appears to be associated with the majority of HNPCC. The observation of MSI in human tumors is the phenotypic foundation for the hypothesis that a mutator phenotype may drive carcinogenesis (5, 11–14). Examination of a panel of five microsatellite sequences has been shown to be highly effective at diagnosing MSI in HNPCC or sporadic colorectal tumors (15, 16). The use of this or a similar panel also appears to be effective for endometrial, ovarian, and gastric tumors (17–19). The frequency of MSI in sporadic colorectal, gastric, and endometrial carcinomas varies from 10–15% (15, 17–19). Whereas germ-line deletions, splice-site mutations, and pathogenic missense mutations of the MMR genes are the primary cause of HNPCC tumors, methylation of the hMLH1 promoter appears to be the dominant mechanism leading to MSI in sporadic tumors (20, 21).

Secondary frameshift mutations in target genes that contain repetitive sequences within the coding region (coding sequence microsatellites) appear to be a hallmark of MMR-deficient tumors (12). The growth control and apoptosis genes TGFβRII, IGFRII, and Bax appear to contain coding sequence microsatellite alterations in a significant proportion of MSI colorectal and gastric cancers (22–25). Similar patterns of coding sequence microsatellite mutations appear less frequently in endometrial carcinomas with MSI (26).

Whereas some UUT tumors are associated with HNPCC, on the whole, they are relatively rare and account for approximately 8% of all urinary tract tumors (4% renal pelvis and 4% ureter; Ref. 27). Similar to urinary bladder cancer, smoking and occupational exposure to arylamines are well-established risk factors accounting for more than half of the cases (28). In addition, epidemiological studies have suggested that familial urothelial carcinoma, which is independent of HNPCC, may exist as a unique entity (29).

A number of studies have suggested a low frequency of MSI (<10%) associated with urothelial carcinoma (30–34). Similarly, loss of MMR protein expression, MMR mutations, or hMLH1 promoter hypermethylation was found to be a rare occurrence in urothelial carcinoma (35–38). Taken together, these results suggested that MMR pathway alterations did not significantly contribute to the development of urothelial carcinomas. Whereas most molecular studies of urothelial carcinomas focus on tumors of the urinary bladder, one report has suggested widespread MSI in two of three tumors of the ureter (31).

Here we have examined the prevalence of MSI and of the loss of MMR protein expression in UUT tumors. The frequency of coding sequence microsatellite alterations in the genes TGFβRII, Bax, IGFRII, MSH3, and MSH6 was assessed to elucidate a pathway of carcinogenesis analogous to MMR-deficient colorectal cancers. Finally, the association between these alterations and clinicopathological features was characterized. We find that a significant portion of apparently sporadic UTT tumors are associated with MMR defects. Together with other studies, our results are consistent with the notion that sporadic tumors caused by MMR defects are likely confined to a subset of tissue types that are largely identical to the extracolonic tumor spectrum of HNPCC. We suggest the possibility that the tumor spectrum of MMR defects reflects tissues in which DNA lesions are...
generated that are uniquely recognized and processed by the MMR machinery.

**Materials and Methods**

**Tumors.** Sixty-two consecutive unselected tumors of the renal pelvis and/or ureter diagnosed between 1990 and 1998 were retrieved from the archives of the Institute of Pathology, University of Regensburg (Regensburg, Germany). Three cases were excluded from the study because of insufficient tumor tissue (one case) or because the extracted DNA could not be successfully amplified by PCR (two cases). The median age at diagnosis was 71 years. The family history and smoking history for these patients were not available. Fourteen additional tumors of the renal pelvis and ureter from patients 50 years or younger at diagnosis (median age, 42.8 years) were selected from the archives of the Department of Laboratory Medicine and Pathology, Mayo Clinic (Rochester, MN). Family history, history of secondary tumors, and smoking history were available for these patients. Thirteen of 14 patients were smokers with an average amount of 40 pack-years.

Overall, there were 22 tumors of the ureter, 40 tumors of the renal pelvis, and 11 multifocal tumors both in the ureter and renal pelvis. Seventy-one tumors were urothelial carcinomas, one was a squamous cell carcinoma, and one was an adenocarcinoma. In 19 patients with spatially separate multifocal tumors of the UUT and coexisting bladder cancers, all lesions were investigated separately for MSI. Age and gender of the patients, location, stage and grade of the tumor, and the histological growth pattern are given in Table 1. Staging, grading, and histological typing of the tumors were performed according to the tumor-node-metastasis (TNM) classification (39) and the WHO classification of urothelial neoplasms (40). Tumors not fulfilling the minimal criteria for diagnosis of urothelial carcinomas as defined in the new WHO classification (e.g., papillomas and papillary tumors of low malignant potential) were not included in the study.

**Microsatellite Analysis.** For MSI analysis, matched normal tissue DNA was extracted from paraffin-embedded tissue as described previously (7). Renal parenchyma without tumor infiltration or microdissected lamina muscularis propria of the ureter and surrounding adipose tissue was used as normal tissue. The tumor was separated from stromal cells by microdissection with a needle (22-gauge) under an inverted microscope (×40 magnification). The microdissected areas contained at least 80% urothelial cells. A panel of six microsatellites was used, including the recognized reference panel (recommended by Dietmaier et al. (15) and endorsed at a National Cancer Institute workshop on MSI diagnostics in cancer detection and familial predisposition) plus BAT40 as an additional mononucleotide marker (15, 16). In cases with only one unstable marker, additional markers were analyzed ([D10S197, D18S58, D18S69, and Mycl1 (15)]. The primer sequences have been published previously (15). PCR amplifications were performed with 100 ng of purified genomic DNA in a final volume of 20 μl in a MJ Research Thermocycler (PTC100; MJ Research, Watertown, MA). Subsequently, PCR products were analyzed by 6.7% polyacrylamide/50% urea gel electrophoresis as described previously (41).

MSI was defined by the presence of novel bands after PCR amplification of tumor DNA that were not present in the PCR products of the corresponding normal DNA. All gels were evaluated by two observers (A. H. and L. Z.). A tumor was classified as MSI-H if 2 of the 6 markers (>30%) of the first panel were found to be unstable or if at least 3 of the 10 markers of both primer sets showed MSI. If <30% of the investigated markers revealed MSI, the tumor was designated as having a low-level instability (MSI-L). All instable markers were verified in a second PCR amplification. Losses of heterozygosity were not counted as MSI.

**Detection of Frameshift Mutations.** Frameshift mutations in repetitive sequences in the coding region of the genes **MSH3, MSH6, BAX, TGFβRII, and IGFRII** were also analyzed using a PCR-based assay as described previously (22, 23, 26, 42, 43). Primers were labeled with TET (Bax and MSH3), HEX (TGFβRII and IGFRII) and FAM (hMSH6). PCR amplifications of the five loci were performed with 100 ng of DNA in a final volume of 20 μl (2.5 mm MgCl2, 200 mM deoxynucleotide triphosphates, 0.33 μM primers, and 0.04 unit/μl AmpliTaq Gold). After a denaturation step at 95°C for 12 min, PCR was carried out at 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s for 10 cycles; followed by 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s for 25 cycles; and a final elongation step of 72°C for 30 min. Five μl of the PCR loading mix containing 1.5 μl of PCR mix of the five separate amplifications (1 μl of 6-FAM product, 1 μl of each TET product, and 2.5 μl of each HEX product in a final volume of 20 μl), 2.5 μl of denatured formamide, 0.5 μl of blue dextran/50 mM EDTA (50 mg/ml blue dextran), and 0.5 μl of size standard (GeneScan-350 or GeneScan-500, labeled with TAMRA) were loaded on an ABI 373 sequencer. The chromatograms were analyzed with GeneScan software.

**IHC for MSH2, MLH1, and MSH6.** All tumors were subjected to immunohistochemical analysis using the streptavidin-biotin-peroxidase method as described previously to determine MMR protein expression (15). The primary antibodies used were a polyclonal antibody against the MSH2 protein (0.5 μg/ml; Oncogene Science, Cambridge, MA), a mouse monoclonal antibody against the MLH1 protein (clone GI68-728; 1 μg/ml; PharMingen, San Diego, CA) and a monoclonal antibody against the hMSH6 protein (clone 44; 1:50 working solution; Becton Dickinson). Tissue lymphocytes served as internal positive controls.

**Statistical Analyses.** The frequencies of events in all clinicopathological features were compared between patients with and without MSI using the χ2 test. All Ps resulted from two-sided tests. The age distributions between both groups were compared by the Mann-Whitney test.

**Results**

MSI, MSI of at least two microsatellite markers (MSI-H) was found in 15 tumor samples (21%; see Table 1). MSI-H was infrequent in patients with tumors of the renal pelvis (3 of 40, 7.5%). In contrast, MSI was observed in 9 of 22 (41%) cases of ureteral (only) cancer (Table 1). Three of 11 (27.5%) patients with simultaneous tumors of the ureter and renal pelvis were MSI-H with marked instability in both tumors. Overall, 12 of 32 (38%) patients with at least a ureteral tumor showed MSI-H. In contrast, only 3 of 39 (8%) patients with tumors restricted to the renal pelvis displayed MSI-H (P = 0.002). The frequency of MSI in tumors of the ureter and UUT tumors in general (combined renal pelvis and ureter) is the highest found in any unselected series of tumors of any location and histological type to date.

Nine tumors showed MSI with only one of the markers of the initial reference panel (Table 1). All of those tumors were screened with the second well-characterized primer panel (15). Only one of these tumor samples showed an additional MSI marker (Myc1l; patient R52). In all cases, these tumors were classified as MSI-L. Similar to colorectal cancer, the mononucleotide microsatellite markers (BAT25, BAT26, and BAT40) were most often affected by MSI in UUT tumors (see Table 1). At least one mononucleotide repeat showed MSI in all tumors with more than one unstable marker. Interestingly, BAT40 was the most sensitive marker, detecting 14 of 15 MSI-H tumors in this series (sensitivity, 93%). The detection rate of the other markers was considerably lower (BAT25, 8 of 15 [53%]; D2S123, 7 of 15 [47%]; BAT26, 6 of 15 [40%]; D17S250, 6 of 15 [40%]; and D5S346, 5 of 15 [33%]). Use of the three mononucleotide markers (BAT25, BAT26, and BAT40) resulted in 100% detection of MSI-H tumors (Table 1). These results underline the limited usefulness of the BAT26 marker alone in MSI diagnostics (44).

**MMR Protein Expression.** IHC analyses revealed a strong expression of MSH2, MLH1, and MSH6 in normal urothelium (Fig. 1). The proteins appeared to be homogeneously expressed with strong nuclear staining in the basal cell layer and fainter nuclear expression in the upper matured cells (Fig. 1c). All three MMR proteins showed a very strong nuclear staining in >80% of the cells in the majority of the tumors investigated (Table 1 and Fig. 1g). In 5 of 15 (33%) MSI-H tumors, loss of MSH2 expression (nuclear staining in <5% of the tumor cells) was found (Fig. 1f). One additional tumor (R54) reproducibly demonstrated MSH2 loss of expression yet displayed MSI-L (2 of 28 MSI markers including BAT40 and hMSH6). In 7 of 15 (47%) MSI-H tumors, loss of MLH1 staining was observed (Fig. 1h). Three tumors with MSI-H (R48, M10, and M12) demonstrated strong nuclear staining with both MSH2 and MLH1. In case R48, loss of...
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Patients with MSI in at least 2 of 6 initial panel markers (MSI-H)

- R2 58m pT2G2 RP pap 2/6 BAT25,40 pos neg MSH6
- R15 75m pT2G2 UR pap 2/6 BAT40, D2S123 pos neg
- R16 59f pT2G2 UR pap 3/6 BAT25,26,40 neg pos
- R17 70f pT4G2 RP/UR pap/inv 6/6 BAT25,26,40, D2S123, D5S346, D17S250 neg neg TGF, RII
- R20 62f pT1G2 RP pap 6/6 BAT25,26,40 pos neg
- R21 78f pT2G2 RP pap/inv 6/6 BAT25,40, D5S346, neg neg
- R23 63m pT3G2 RP pap 2/6 BAT40, D5S346, neg neg
- R24 63m pT3G2 UR pap 2/6 BAT26, D2S123 neg pos TGF, RII
- R25 59f pT2G2 UR pap 3/6 BAT40, D2S123, D17S250 pos pos
- R26 81f pT4G2 UR pap/inv 4/6 BAT25,40, D5S346, pos neg
- R27 71m pT4G2 RP pap 2/6 BAT40, D2S123 pos neg
- M4f 45f pT2G2 UR pap/inv 5/6 BAT25,26,40, D2S123 pos neg
- M10f 48m pT4G2 UR pap 3/6 BAT25,26,40 pos pos
- M12 49m pT2G2 UR pap/inv 2/6 BAT40, D2S123 pos pos
- M13 46m pT1G2 UR pap 4/5 BAT25,40, D2S123, D17S250 neg pos

Patients with MSI in one of 6 initial panel markers (MSI-L)

- R9 64f pT3G3 RP sol 1/10 D17S250 pos pos
- R33 69m pT3G3 RP/UR sap/sol 1/10 D5S346 pos pos
- R41 70m pT1G2 RP sol 1/10 D17S250 pos pos
- R42 79m pT1G2 RP pap 1/10 D17S250 pos pos
- R52 70m pT3G3 RP sol 1/10 BAT40, Myc11 pos pos
- R54 84m pT2G2 UR pap/inv 1/10 D2S123 pos pos
- M11 50m pT3G3 RP pap 1/10 D2S123 pos pos
- M14 30f pT3G3 RP pap 1/8 D2S123 pos pos

Patients without MSI in 6 initial panel markers (MSS)

- R1 68m pT3G2 UR pap/sol 0/6 pos pos
- R3 73m pT3G3 RP pap 0/6 pos pos
- R4 78m pT3G3 RP pap 0/6 pos pos
- R5 82m pT3G3 RP sol 0/6 pos pos
- R6 72m pT3G3 UR sol 0/6 pos pos
- R7 75m pT2G3 RP sol 0/6 pos pos
- R8 70m pT3G3 RP sol 0/6 pos pos
- R9 70f pT2G2 UR pap 0/6 pos pos
- R11 59f pT3G3 RP pap 0/6 pos pos
- R12 64m pT3G3 RP/UR pap 0/6 pos pos
- R13 71m pT1G2 RP/UR pap 0/6 pos pos
- R14 72f pT1G2 RP pap/inv 0/6 pos pos
- R15 75m pT2G3 RP pap 0/6 pos pos
- R16 75m pT2G3 RP pap 0/6 pos pos
- R17 75m pT2G3 RP pap 0/6 pos pos
- R18 75m pT2G3 RP pap 0/6 pos pos
- R19 55m pT3G3 UR sol 0/6 pos pos
- R20 74m pT1G2 RP/UR pa 0/6 pos pos
- R21 72f pT1G2 RP pap/inv 0/6 pos pos
- R22 72f pT3G3 RP pap/inv 0/6 pos pos
- R23 71m pT1G2 RP pap 0/6 pos pos
- R24 62f pT4G3 RP sol 0/6 pos pos
- R25 80m pT4G3 RP/UR pap 0/6 pos pos
- R26 64m pT4G2 UR pap/inv 0/5 pos pos
- R28 85f pT3G3 RP pap 0/6 pos pos
- R29 69m pT1G1 UR pap 0/6 pos pos
- R30 75m pT3G3RP pap 0/6 pos pos
- R31 88f pT1G3 UR pap 0/6 pos pos
- R32 63m pT3G2 RP pap 0/6 pos pos
- R33 75m pT3G3 RP/UR sol/CIS 0/6 pos pos
- R34 75m pT3G3 RP/UR sol/CIS 0/6 pos pos
- R35 78m pT1G2 RP pap 0/6 pos pos
- R36 70m pT3G3 RP pap 0/6 pos pos
- R37 70m pT3G3 RP pap 0/6 pos pos
- R38 51f pT4G3 RP sarc 0/6 pos pos
- R39 66f pT3G3 RP sol 0/6 pos pos
- R40 74f pT3G3 RP adeno 0/6 pos pos
- R41 69m pT3G2 RP pap/inv 0/6 pos pos
- R42 75m pT3G2 RP pap 0/6 pos pos
- R43 75m pT3G2 RP pap 0/6 pos pos
- R44 73m pT3G2 UR pap/inv 0/6 pos pos
- R45 67m pT1G3 UR pap 0/6 pos pos
- R46 67m pT3G3 RP pap 0/6 pos pos
- R48 78m pT3G3 RP sol 0/6 pos pos
- R49 73m pT3G3 RP sol 0/6 pos pos
- R50 75m pT3G3 RP sol 0/6 pos pos
- R51 55m pT4G3 RP pap/inv 0/6 pos pos
- R52 87m pT4G3 RP SCC 0/6 pos pos
- R53 87m pT4G3 RP SCC 0/6 pos pos
- R54 76m pT1G1 RP pap 0/6 pos pos
- R55 81m pT2G2 RP pap 0/6 pos pos
- R56 81m pT1G2 RP pap 0/6 pos pos
- R57 47m pT1G2 RP pap 0/6 pos pos
- R58 47m pT1G2 RP pap 0/6 pos pos
- R59 47m pT1G2 RP pap 0/6 pos pos
- R60 47m pT1G2 RP pap 0/6 pos pos
- M1 87m pT4G3 RP pap 0/6 pos pos
- M2 47m pT1G2 RP pap 0/6 pos pos
- M3 47m pT1G2 RP pap 0/6 pos pos
- M4 47m pT1G2 RP pap 0/6 pos pos
- M5 47m pT1G2 RP pap 0/6 pos pos
- M6 47m pT1G2 RP pap 0/6 pos pos
- M7 47m pT1G2 RP pap 0/6 pos pos
- M8 47m pT1G2 RP pap 0/6 pos pos
- M9 47m pT1G2 RP pap 0/6 pos pos
hMSH6 was demonstrated (Fig. 1j). Strong nuclear staining with hMSH6 was observed in the remaining two hMSH2/hMLH1 expression-positive MSI-H samples as well as in all cases with instability of only one marker. Interestingly, 6 of 8 (75%) tumors with a frameshift mutation of hMSH6 revealed loss of hMSH6 expression. In 5 MSI-H tumor samples, IHC showed loss of MSH2 (n = 3) or MLH1 (n = 2), in the dysplastic urothelium and histomorphologically incoincisuous urothelium adjacent to the tumor in the renal pelvis or ureter (Fig. 1r). These results are consistent with the MMR defect occurring early in the carcinogenesis process.

Alterations in Coding Mononucleotide Repeats. Analysis of coding sequence microsatellite alterations was successful in 13 MSI-H tumors and 57 MSI-L + MSS tumors. We observed coding sequence microsatellite alterations of TGFβRII in 3 of 13 (23%) tumors with MSI, Bax in 3 of 13 (23%) tumors with MSI, hMSH6 in 5 of 13 (38%) tumors with MSI, and hMSH3 in 2 of 13 (15%) tumors with MSI (Table 1). Coding sequence microsatellite alterations of IGFRII were not detected in any of the tumors investigated. Only three tumors showed coding sequence microsatellite alterations in more than one target gene. Interestingly, there was a coding sequence microsatellite alteration mutation of hMSH6 in case R48 that contained intact expression of both MSH2 and MLH1. R48 also showed instability in three of six primary panel microsatellite markers and complete loss of hMSH6 expression (Fig. 1l). There were no coding sequence microsatellite alterations in TGFβRII, IGFRII, Bax, or hMSH3 in the 57 MSI-L + MSS tumors. However, a MSI6 coding sequence microsatellite alteration was found in two MSI-L tumors and one MSS tumor (Table 1).

Clinicopathological Characteristics of Tumors with MSI. The clinicopathological features of MSI-H tumors displayed highly significant differences compared with MSS tumors (Fig. 2). All 15 MSI-H tumors showed low histological grade (G1 to G2; Fig. 1, a–d) and low pathological stage (≤PT2). In contrast, 32 of 58 (55%; P = 0.0012) MSS carcinomas were low grade, and 34 of 58 (59%; P = 0.0024) tumors showed low stage. MSI-H tumors frequently showed spindle cells with only slight nuclear polymorphism, homogeneous chromatin, and rare basal mitoses. However, 14 of 15 tumors were classified as moderately differentiated (G2) because the cells demonstrated loss of polarity and maturation in a disordered urothelium. In addition, there were scattered tumor cells with enlarged pleomorphic nuclei, but no hyperchromasia (Fig. 1d). All MSI-H tumors showed a predominance of papillary growth, whereas in 18 of 58 (31%) MSS tumors, a solid growth pattern without evidence of papillary differentiation was present (P = 0.012). Most interestingly, in 8 of 15 (53%) MSI-H tumors, an inverted tumor growth (defined as at least 50% of the tumor) could be demonstrated. In contrast, only 5 of 58 (9%) MSS tumors displayed the inverted tumor growth pattern (P = 0.00005). There was no difference in the number of inflammatory cells or in the occurrence of lymph follicles (Crohn’s-like lesions) between MSI-H and MSS cases (data not shown). These results contrast the frequent observation of Crohn’s-like lesions in MSI-H colorectal carcinoma (7). We found that 7 of the 15 (48%) MSI-H tumors occurred in male patients. The MSS group showed the expected male predominance (43 of 58, 74%; P = 0.04). There was no statistically significant difference between the frequency of MSI-H in the selected cohort of patients with tumors occurring before the age of 45 years (4 of 14, 29%) and the unselected patients (11 of 59, 19%; P = 0.41). The median age of patients with MSI tumors was 5 years younger than the age of MSS patients (61.6 ± 11.8 years versus 66.6 ± 14.1 years). However, this difference did not reach statistical significance (P = 0.097). There were no differences in the synchronous or metachronous occurrence of bladder cancer in both patient groups. The smoking history did not differ between both groups in the cohort of young patients. Interestingly, the only nonsmoker (M13) was MSI-H and negative for MSH2 staining. There was a positive family history of cancer in 5 of 15 (33%) patients for whom data were available (see the footnotes of Table 1). Three patients (R20, M4, and M13) fulfilled the clinical criteria (Amsterdam I) for diagnosis of HNPPC (12). All three patients demonstrated MSI-H in at least four markers. In patient R20, a germ-line mutation in MSH2 was found (exon 5, double missense mutation, ATGCAG—>ATAGAG, amino acid M492I and Q493E).

Discussion

Clinical and epidemiological evidence supports the hypothesis that urothelial carcinomas of the upper and lower urinary tract share many similarities (28). However, only UUT tumors show a strong association with the HNPPC syndrome (9, 45). Although the lifetime risk to develop UTT tumors in HNPPC families does not exceed 10%, the relative risk is increased 14-fold over that of the general population (45, 46). Moreover, carriers of germ-line hMSH2 mutations have a 75-fold elevated relative risk of UUT tumors (47). Thus, small bowel and UUT carcinomas represent the sites of highest relative risk for extracolonic tumors in HNPPC carriers (47). In this study, we show that unselected UUT tumors display among the highest frequency of MSI-H (21%) of any tumor site. Remarkably, 38% of the ureteral tumors were MSI-H. A predisposition to HNPPC could only be

Table 1 Continued

| R1-R59, consecutive cases from the files of the Institute of Pathology Regensburg 1990–1998; M1-M14, cases of patients <50 years of age from the files of the Mayo Clinic, diagnosed between 1985 and 1992. | m, male; f, female; age at diagnosis of the first upper urinary tract tumor. | RP, renal pelvis; UR, ureter; RP/UR, tumors both in the renal pelvis and ureter. | pap, dominating papillary growth pattern; pap/sol, solid, nonpapillary tumor component is dominating (>50%), but papillary growth can be clearly demonstrated; sol, solid, nonpapillary tumor growth without papillary tumor component in serial sectioning; adenocarcinoma; SCC, squamous cell carcinoma; sarcoma, sarcomatoid urothelial carcinoma; pap/lymphoplasmacytoid, papillary urothelial carcinomas with inverted tumor growth (58) in at least 50% of the tumor, CIS, carcinoma in situ. | neg, negative granular nuclear staining in less than 5% of the tumor cells. | Frameshift mutations in coding nucleotide repeats. ND, not done. | Six of eight tumors with frameshift mutations of MSH6 (R2, R16, R17, R20, R48, and R54) showed loss of MSH6 expression detected by IHC. | Five patients had a family history of cancer. R20, three colorectal cancers and endometrial carcinoma before the diagnosis of ureteral carcinoma in the patient and three relatives in three generations with colorectal carcinoma. M4, metastasizing ovarian carcinoma in the patient simultaneously to the ureter tumor and colorectal carcinoma in grandmothers, mother, and sister. M6, breast and endometrium carcinoma in the grandmother, M10, prostate cancer in the father and carcinoma of the gallbladder in the grandmother. M13, colorectal carcinoma in the mother, the father, and the grandmother. | The positive immunohistochemical staining in tumors with MSI was confirmed at least twice. However, tumor R48 showed a reproducible loss of expression of MSH6 by immunohistochemistry (Fig. 1). The other MSI-H, hMSH2/hMLH1 expression-positive tumors (M10 and M12) showed a normal expression of MSH6. | All tumors with MSI in at least one marker were investigated for MSH6 expression and for MSI using four additional markers (see “Materials and Methods”). All tumors showed normal nuclear MSH6 expression. Only one additional MSI could be detected. R52 was screened with the panel of 10 recommended microsatellite markers suggested by Deitmaier et al. (15). Two of 10 displayed MSI, categorizing it as MSI-L (15,16). | The negative staining for MSH2 was confirmed twice. Immunoreactivity of the tissue was confirmed by strong staining with MLH1, the proliferation marker Ki67, and the epithelial marker CK20. Besides instability in BAT40 and MS6H0, no other change could be detected in a total of 28 investigated microsatellite markers (data not shown). |
identified in 3 of 15 of these unselected MSI-H UUT tumors. These results suggest that a large fraction of MSI UUT tumors are sporadic and subject to tumor promotion and selection processes similar to those of HNPCC tumors. Based on these and other studies, it appears that bona fide MSI-H is confined to well-defined tissue types in both hereditary and sporadic tumors.

Mononucleotide repeats appear to be most affected in MSI-H UUT tumors. Instability of mononucleotide markers in tumors that displayed MSI-L was significantly less likely. There was a significantly higher frequency of MSI in tumors of the ureter (38%) in comparison with tumors of the renal pelvis (8%). In contrast, data from several sources suggest that MSI in more than one marker is infrequent in urothelial carcinoma of the bladder (12 of 524, 2.2% (30–34, 38, 48)). Interestingly, a single study has suggested an extremely high frequency of MSI in bladder tumors (49). However, we regard it likely that the selection of microsatellite markers, the criteria for the diagnosis of MSI, and the inclusion of LOH may explain these latter results. Importantly, extended studies from our group have confirmed a high frequency of MSI-H in a large cohort of unselected Caucasian patients with UUT tumors (31%; Ref. 50).

We found coding sequence microsatellite alterations of TGFβRII (20%), Bax (20%), hMSH3 (7%), and hMSH6 (33%) in MSI-positive UUT tumors. Coding sequence microsatellite alteration of hMSH6 resulted in loss of protein expression in six of eight cases. Loss of hMSH2 expression was observed in three of six of these cases (R16, R20, and R54), suggesting that the destabilization of hMSH6 was due

Fig. 1. Histopathological characteristics and immunohistochemical staining for MMR proteins MSH2, MLH1, and MSH6 in urothelial carcinomas of the UUT. a–d, papillary, mostly inverted urothelial carcinomas with MSI in patients R17 (a and b) and M4 (c and d). H&E staining, ×25 (a and c) and ×400 (b and d). Note the predominant inverted growth pattern (arrows) and the papillary tumor component. The tumors have disordered urothelium and scattered cells with enlarged pleomorphic nuclei, but no hyperchromasia (arrowheads). e, normal urothelium with strong nuclear staining for MSH2. ×400. f, papillary urothelial carcinoma with negativity for MSH2 in case R16 (arrow). ×200. Note the strong staining in inflammatory cells and in adjacent normal urothelium as internal positive controls (arrowheads). g, solid invasive poorly differentiated urothelial carcinoma without MSI with strong nuclear staining for MSH2 (case R8). ×200. h, papillary urothelial carcinoma with negative staining for MLH1 in case R13 (arrow). ×400. Note the strong nuclear staining in stromal and inflammatory cells as internal positive control (arrowhead). i, normal urothelium with strong nuclear expression of MSH2 (arrow) and adjacent urothelial dysplasia with loss of MSH2 expression (arrowhead), ×400. j, papillary urothelial carcinoma with MSI and loss of expression of MSH6 in case 48. ×400. Note stromal and inflammatory cells with strong nuclear staining as internal positive control (arrowhead).

Fig. 2. Clinicopathological features of UUT tumors with MSI.
to the loss of its heterodimeric partner (13). Of the remaining three hMSH6-negative tumors, one was likely a bona fide primary hMSH6 alteration (R48), and the remaining two are likely to be a secondary consequence of a primary hMLH1 alteration (R2, R17). These latter numbers are not sufficient to determine whether hMSH6 is a true secondary target that enhances carcinogenesis. However, there is no experimental evidence that any combination of double MMR mutation leads to a synergistic increase in mutation rate or resistance to damage-induced apoptosis, the two likely enhancers of tumorigenesis (4). The frequency of coding sequence microsatellite alterations in MSI UUT tumors is considerably lower than that found with colon and gastric tumors, where TGF/βRII was found in up to 90% of the samples (23, 24, 26). We found no coding sequence microsatellite alteration of IGFR1 (25). These data indicate that there are at least some similarities in the molecular carcinogenesis pathway of MMR-deficient UUT tumors compared with colorectal and gastric tumors. However, it is likely that there are as yet unknown downstream mutations in MSI-positive UUT tumors that play an important role.

The correlation between MSI and loss of MMR protein expression supports the role of these genes/proteins in UUT tumors. We found loss of hMSH2 expression in 33% of MSI-H UUT tumors. Our experience with colorectal tumors would suggest that these are most likely due to germ-line and/or somatic gene mutations. In two of the five IHC hMSH2-negative patients (R20 and M13), the family history fulfilled the Amsterdam criteria for diagnosis of HNPCC (12). An apparent germ-line mutation of hMSH2 was identified in one of these patients. The majority of the patients with MSI displayed loss of hMLH1 (54%). One of these patients also had a family history diagnostic for HNPCC. Although untested here, we regard it likely that promoter methylation was responsible for inactivation of hMLH1 in the majority of UUT tumors, a result that would be similar to sporadic colorectal, gastric, and endometrial tumors (19, 20, 51). We also detected loss of expression of either hMSH2 or hMLH1 in normal urothelium or in urothelial dysplasias of five patients with MSI-H. These observations are consistent with the notion that the MMR deficiency is an early and likely the initiating event in the development of MSI-H UUT tumors. Similar findings have been reported for colorectal, gastric, and endometrial carcinomas with detection of MSI and loss of MMR protein expression in premalignant lesions in both HNPCC patients and sporadic cases (52–54).

We were unable to obtain family histories for all patients analyzed in this study and could not perform sequence analyses of the MMR genes to unequivocally identify germ-line mutations. Nevertheless, our data provide strong evidence that there exists a substantial subset of sporadic UUT tumors that are characterized by MMR deficiencies. This observation is further supported by the statistically significant overrepresentation of women in the MSI-H patient cohort (8 of 15, 53%) compared with all patients (23 of 73, 31%; male:female ratio, 2.2:1; P = 0.04). Large epidemiological studies (50,000 patients with sporadic bladder cancer and 5,000 patients with sporadic UUT cancers) showed that there is a male:female ratio of 3:1 in urothelial carcinoma of the bladder and of 1:1.7:1 in UUT tumors (27). Smoking and occupational exposure to several androgen and estrogenic compounds, which have been shown to increase the risk of developing cancer (28–30), are associated with an increased risk of developing UUT cancer (31), but the exact mechanisms are unknown. This association may be explained by the fact that both smoking and occupational exposure and gender differences in the metabolism and processing of DNA lesions that are ultimately recognized by the MMR machinery may account for the tissue distribution of hereditary and sporadic MSI tumors.

Finally, an effective clinical program in patients with HNPCC and germ-line mutations in one of the MMR genes has been suggested that includes annual screening for urothelial carcinomas by urine cytology (32). This is important for early detection of MSI-H UUT cancers: This is an infrequent finding in urothelial cancers and in most cases is associated with a low tumor grade and stage and an excellent prognosis. The frequent occurrence of MSI-H in UUT cancers and the fact that most, if not all, urothelial carcinomas from HNPCC patients will display MSI may provide a tool for more sensitive and specific urine screening tests that will result in early detection of cancer in these families.

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References


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