Frequency of Microsatellite Instability in Unselected Sebaceous Gland Neoplasias and Hyperplasias

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Sebaceous gland neoplasias are the cutaneous manifestation of the Muir–Torre syndrome, which is known to be a phenotypical variant of hereditary nonpolyposis colorectal cancer. Both hereditary nonpolyposis colorectal cancer and Muir–Torre syndrome are caused by inherited DNA mismatch repair defects. As a prominent molecular genetic feature, all tumors associated with a DNA mismatch repair defect exhibit high microsatellite instability. So far, the frequency of DNA mismatch repair defects in patients selected solely on the basis of a sebaceous gland tumor has never been determined. In order to estimate this frequency, we assessed microsatellite instability with up to 10 microsatellite markers in a newly collected unselected series of 25 sebaceous gland neoplasias (six sebaceous adenomas, 16 sebaceous epitheliomas, three sebaceous carcinomas) in comparison to 32 sebaceous gland hyperplasias from unrelated patients. As many as 15 of the 25 sebaceous gland neoplasias (60%), but only one of the 32 sebaceous gland hyperplasias (3%), exhibited high microsatellite instability. Thus, in our study, the majority of patients with a sebaceous gland neoplasia in contrast to patients with a sebaceous gland hyperplasia are highly suspicious for an inherited DNA mismatch repair defect. On the basis of the subsequently collected tumor histories, nine of the 15 patients with a high microsatellite unstable sebaceous gland neoplasia were identified to have Muir–Torre syndrome. In none of these cases, however, were the clinical Amsterdam criteria for diagnosing hereditary nonpolyposis colorectal cancer fulfilled. In the sebaceous tumors of the remaining six patients, high microsatellite instability was an incidental finding. In two of these six patients, single relatives were known to be affected with internal cancer; however, their family histories were not suggestive of Muir–Torre syndrome or hereditary nonpolyposis colorectal cancer. In comparison with microsatellite instability screening studies in a variety of other randomly selected tumors, our study identifies sebaceous gland neoplasias as tumors with the highest frequency of high microsatellite instability reported so far, whereas sebaceous gland hyperplasia rarely exhibits high microsatellite instability. Therefore, screening for microsatellite instability in sebaceous gland neoplasias will be of great value in the detection of an inherited DNA mismatch repair defect, which predisposes to various types of internal cancers. ***Key words: DNA mismatch repair/genomic instability/hereditary nonpolyposis colorectal cancer/HNPCC/MSH2/MLH1/Muir–Torre syndrome/MTS/cystic sebaceous tumor. J Invest Dermatol 120:858–864, 2003***

Sebaceous gland tumors are adnexal skin tumors, classified into the rare sebaceous gland neoplasias with their subtypes sebaceous adenoma, epithelioma, and carcinoma and the frequently occurring sebaceous gland hyperplasias.

The sebaceous gland neoplasia is the cutaneous marker tumor for the autosomal-dominant Muir–Torre syndrome (MTS) (OMIM #158320). MTS is defined by the combination of at least one sebaceous gland neoplasia and one internal malignancy (Schwartz and Torre, 1995). In addition, keratoacanthomas and sebaceous gland hyperplasias are a frequent finding in MTS patients. In contrast to sebaceous gland neoplasias, however, neither solitary keratoacanthomas nor sebaceous hyperplasias are skin tumors that point to MTS. MTS is known to be a phenotypic variant of hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) (OMIM #120435 and #120436). Because of the predisposition to cancers of various tissues (especially colon, urothelium, and endometrium), a life-long cancer surveillance program has to be recommended both to an individual affected with MTS and his/her relatives.

Most typical skin tumors of MTS patients exhibit high microsatellite instability (MSI-H) (Honchel *et al*, 1994). MSI-H results from a defective DNA mismatch repair (MMR) system (Alvino *et al*, 2002) and has been shown to be the key feature of tumors related to HNPCC. In patients with HNPCC as well as with MTS, MSI in tumor tissue is caused by an underlying germline mutation in a DNA MMR gene (Fishel *et al*, 1993; Bronner *et al*, 1994; Kolodner *et al*, 1994; Kruse *et al*, 1996; Lynch *et al*, 1999;

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Abbreviations: CST, cystic sebaceous tumor; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability; MTS, Muir–Torre syndrome.

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Swale *et al*, 1999). In the tumor-prone tissue from such predisposed patients, however, the accumulation of replication errors leading to MSI does not develop until somatic inactivation of the corresponding second *MMR* allele ("second hit") has taken place (Kruse *et al*, 2001).

Earlier studies have shown that about two-thirds of patients with the characteristic Muir–Torre phenotype "sebaceous gland tumor plus colorectal cancer" as well as the majority of patients with even isolated cystic type sebaceous tumors (CST) harbor inherited DNA MMR defects (Kruse *et al*, 1998; 1999; Rütten *et al*, 1999). These findings are of clinical relevance as every clinical sign that enables the detection of an inherited cancer predisposition such as HNPCC is of considerable value for cancer prevention (Lynch and Fusaro, 1999).

So far, extensive MSI screening studies of randomly selected tumors of the HNPCC spectrum were focused on colorectal carcinomas (Aaltonen *et al*, 1998; Furlan *et al*, 1998; Cunningham *et al*, 2001). Screening studies for MSI in skin tumors have only been performed in keratoacanthomas (Halling *et al*, 1995; Peris *et al*, 1997a; Langenbach *et al*, 1999) and skin tumors obviously not related to the spectrum of HNPCC or MTS, such as malignant melanomas (Peris *et al*, 1995; Quinn *et al*, 1995; Richetta *et al*, 1997; Talwalkar *et al*, 1998; Hussein *et al*, 2001), nevi (Hussein *et al*, 2001), conventional basal cell carcinomas, squamous cell carcinomas, and Bowen's disease (Quinn *et al*, 1995). Until now, no MSI screening study on unselected sebaceous gland tumors has been published.

The goal of this study was therefore to determine the frequency of MSI-H in a sample of randomly selected sebaceous gland tumors and to correlate these data with the general tumor history of the patient and his/her family. The results of this study will give a first insight into the value of routine MSI assessment in sebaceous gland tumors in view of surveillance recommendations and cancer prevention for patients at high risk for internal malignancies.

MATERIALS AND METHODS

Patients and tumor samples Fifty-eight paraffin-embedded sebaceous gland tumors from 58 different Caucasian patients from Germany ("ST" by RK, AR) and Italy ("SSG" by MB) were consecutively selected, solely on the basis of histopathology without the knowledge of the patient's or his/her family's tumor history. The tumor sample comprised 25 sebaceous gland neoplasias (six sebaceous adenomas, 16 sebaceous epitheliomas, three sebaceous carcinomas) and 32 sebaceous gland hyperplasias.

Tumor histories of the patients and their relatives were subsequently collected, independent of the inclusion of the sebaceous tumors in the study. Sex and age of the patient, histopathology and localization of the sebaceous tumor, as well as the tumor history are given in **Table I**.

All patients had given signed informed consent to the study. The study was approved by the ethical committee of the Medical Faculty, University of Duesseldorf, Germany, and the ethical committee of the "Casa Sollievo della Sofferenza" Hospital in S. Giovanni Rotondo (Foggia), Italy, respectively.

Histopathologic classification of sebaceous gland tumors Sebaceous tumors were subclassified according to the following criteria. A sebaceous hyperplasia was diagnosed if four or more mature sebaceous lobules were attached to the infundibulum of a pilosebaceous follicle connected to the overlying epidermis. Criteria for sebaceous adenomas were well-defined and enlarged sebaceous lobules comprising fully mature sebocytes, frequently demonstrating an attachment to the epidermis with epidermal thinning (Wick *et al*, 1997). A diagnosis of sebaceous epithelioma (synonymous to sebaceoma) was made when more than half of the cells were undifferentiated basaloid cells with rare nuclear atypia in the presence of significant aggregates of sebaceous and transitional cells (Ueda *et al*, 1999). Sebaceous epithelioma is not organized in well-demarcated sebaceous lobules. The diagnosis of sebaceous epithelioma has been rendered by two experienced dermatopathologists using well-established morphologic criteria to differentiate these neoplasms from basal cell carcinomas (Steffen and Ackerman, 1994). Sebaceous carcinomas are characterized by variable atypical polyhedral cells, separated from one another by fibrovascular stroma, sometimes with pagetoid spread of atypical epithelial cells to the epidermis (Wick *et al*, 1997). Cystic sebaceous tumors were defined as large, well-circumscribed cystic sebaceous lesions located deep in the dermis (Rütten *et al*, 1999).

Molecular genetic investigations

Isolation of genomic DNA Tumor DNA was extracted from microdissected paraffin-embedded tumor tissue using the QIAamp tissue kit (QIAGEN, Hilden, Germany). In 47 of the 58 patients, normal DNA was extracted either from surrounding normal tissue with the QIAamp tissue kit or from ethylenediamine tetraacetic acid anticoagulated peripheral blood samples by the salting out procedure (Miller *et al*, 1988). In 11 patients, neither normal tissue nor blood for extracting normal DNA was available.

Assessment of MSI in sebaceous gland tumors Except for the recommendations of the International Collaborative Group on HNPCC (ICG-HNPCC; Loukola *et al*, 2001), there is no unanimity regarding the question as to which microsatellite markers are required for diagnosing MSI. In the case of available normal DNA, two alternative sets of five microsatellite markers were generally used at the same time to analyze paired tumor and normal DNA for assessing MSI. The first set comprises the so-called Bethesda markers BAT25, BAT26, D2S123, D5S346, and D17S250, which are recommended by the ICG-HNPCC. The second set, which was used in the case of unclear MSI results after testing with the first set, comprises the markers BAT40, D3S1298, D3S1611, D6S470, and D18S69. Microsatellite markers were amplified by a polymerase chain reaction (PCR), using the primer pairs described in **Table II**. One oligonucleotide of each pair of primers was labeled with ABI fluorescence dye (Applied Biosystems, Weiterstadt, Germany). To improve specificity, the PCR for each marker was performed as a manual hot-start and touchdown PCR according to standard protocols. In detail, the PCR was performed in a volume of 25 μl containing 4 μl (≈80 ng) genomic DNA, 0.05 μM of each primer, 50 μM of each dNTP, 2.5 μl PCR buffer, 1.5 mM $MgCl_2$, and 1.0 U Taq polymerase (Gibco, Karlsruhe, Germany). Reactions were performed in PCR tubes (Biozym Diagnostik, Hessisch Oldendorf, Germany) by means of a T3 Thermocycler (Biometra, Goettingen, Germany) and consisted of an initial step at 94°C for 15 min, followed by 15 cycles of denaturation at 94°C for 45 s, annealing at primer-dependent temperature T1 (see **Table II**) for 45 s, elongation at 72°C for 60 s, and a subsequent 30 cycles of denaturation at 94°C for 45 s, annealing at primer-dependent temperature T2 (see **Table II**) for 45 s, elongation at 72°C for 60 s. The final step consisted of an additional elongation step at 72°C for 10 min. Whenever possible, after amplification 2–4 μl of each reaction of the five microsatellite markers were pooled. PCR products were separated by electrophoresis on an ABI Prism 310™ Genetic Analyzer (Applied Biosystems). The PCR products of the pooled microsatellite markers were detected by different fluorescence labeling (HEX, TET, or FAM) and the different size of the corresponding microsatellite markers. GeneScan Analysis software (Applied Biosystems) was used to size the PCR products and to analyze the data. MSI is characterized by a length variation of microsatellites in tumor DNA in comparison to normal DNA, and was thus detected as allelic mobility shifts (see **Fig 1**). A tumor was defined to exhibit MSI-H if additional alleles were observed with two or more markers (Boland *et al*, 1998) and with at least 30% of all markers tested; a tumor was defined to exhibit microsatellite stability if none of all the markers examined (at least five) showed additional alleles.

If no normal DNA was available, only the microsatellite marker BAT26 was used for assessing MSI. This method is based on the observation that BAT26, a mononucleotide repeat microsatellite, is quasi-monomorphic in DNA from normal individuals and MSI-negative samples, but shows important size variations in MSI-positive samples (Hoang *et al*, 1997). The efficiency of BAT26 as the only microsatellite marker for the detection of MSI in internal tumors has been evaluated in various studies (Iacopetta and Hamelin, 1998; Zhou *et al*, 1998; Loukola *et al*, 2001). Zhou *et al* (1998) showed that BAT26 could identify the MSI status of tumors from various origins with 99.5% efficiency in a single-step experiment without the requirement for matching normal DNA. According to the results of Loukola *et al* (2001), a tumor was defined to exhibit MSI-H if BAT26 showed an allele shift of three or more base pairs.

RESULTS

An unselected prospective series of 58 sebaceous tumors from different unrelated Caucasian patients was collected in Germany and Italy. The series included 25 sebaceous gland neoplasias (comprising six sebaceous adenomas, 16 sebaceous epitheliomas, and three sebaceous carcinomas) and 32 sebaceous gland hyperplasias.

Table I. Systematic study of consecutively collected sebaceous tumors: MSI data and clinical findings (listed by sebaceous tumor histology)

Patient (Sex)	Tumor histology (Localization; age at diagnosis)	MSI (markers with additional alleles/examined markers)	Tumor history (patient and family)
ST-05 (M)	SA (Forehead, 75 y)	*MSI-H (2/3)*	Patient: three basal cell carcinomas (74–75 y)
ST-09 (M)	SA (CST) (Forearm, 48 y)	*MSI-H (3/4)*	Patient: *Muir–Torre syndrome*: carcinoma of ascending colon (35 y) Family: colorectal carcinoma (brother of father with 72 y)
ST-10 (M)	SA (CST) (Shoulder, 79 y)	*MSI-H (3/3)*	Patient: *Muir–Torre syndrome*: colorectal carcinoma (72 y) Family: no tumors
ST-15 (M)	SA (CST) (Forehead, 57 y)	*MSI-H (3/8)*	Patient: no further tumors Family: carcinoma of the gallbladder (mother with 84 y), hepatic carcinoma (sister of the mother with 72 y)
SSG-20 (M)	SA (Face, 74 y)	MSS (B26–)	Patient and family: no further tumors
SSG-50 (M)	SA (Nose, 69 y)	MSS (B26–)	Patient and family: no further tumors
ST-03 (F)	SE (Capillitium, 60 y)	MSS (0/7)	Patient: no further tumors Family: cancer of the bone (father), lung cancer (sister of mother), colorectal carcinoma (grandmother (m))
ST-08 (M)	SE (Nose, 73 y)	MSS (0/6)	Patient and family: no further tumors
ST-11 (M)	SE (Upper arm, 55 y)	MSS (B26–)	Patient and family: no further tumors
ST-12 (F)	SE (Shoulder, 88 y)	MSS (0/7)	Patient: basal cell carcinoma with 88 y Family: unknown cancer of the lower abdomen (daughter)
ST-13 (F)	SE (Upper lip, 83 y)	MSS (0/6)	Patient: no further tumors Family: colorectal carcinoma (grandfather with 80 y)
ST-17 (M)	SE (Forehead, 65 y)	MSS (0/5)	Patient and family: no further tumors
ST-21 (F)	SE (Forehead, 79 y)	*MSI-H (B26+)*	Patient and family: no further tumors
ST-23 (M)	SE (Back, 66 y)	*MSI-H (6/7)*	Patient: malignant melanoma (31 y), brain metastases of a malignant melanoma (46 y), three extrafacial basal cell carcinomas (63 y) Family: basal cell carcinoma (son with 30 y)
ST-24 (M)	SE (Thigh, 61 y)	*MSI-H (6/6)*	Patient and family: no further tumors
ST-26 (F)	SE (Neck, 76 y)	*MSI-H (4/9)*	Patient: *Muir–Torre syndrome*: colorectal carcinoma (42 y) and breast cancer (68 y) Family: no tumors
ST-28 (F)	SE (Root of the nose, 65 y)	*MSI-H (3/3)*	Patient: *Muir–Torre syndrome*: endometrial carcinoma, cancer of the ureter (60 y), bladder cancer (63 y), squamous cell carcinoma of the skin and malignant melanoma (65 y) Family: colorectal carcinoma (mother 78 y), bladder cancer (sister of the mother 80 y)
ST-33 (M)	SE (Forehead, 64 y)	*MSI-H (2/6)*	Patient: *Muir–Torre syndrome*: colorectal carcinoma (54 y) Family: colorectal carcinomas (mother and sister of the mother 48 y), cancer of the lower abdomen (mother 53 y)
ST-34 (M)	SE (Root of the nose, 86 y)	*MSI-H (4/6)*	Patient: no further tumors Family: colorectal carcinoma (father)
ST-36 (F)	SE (Nose, 88 y)	MSS (0/6)	Patient: basal cell carcinoma
ST-39 (F)	SE (Neck, 78 y)	*MSI-H (3/3)*	Patient: *Muir–Torre syndrome*: colorectal carcinoma (48 y), cancer of the lower abdomen (67 y) and a further sebaceous epithelioma (78 y) Family: cancer of the lower abdomen (mother 48 y), lung cancer (uncle 45 y), leukemia (grandson 17 y)
ST-41 (M)	SE (Cheek, 81 y)	*MSI-H (2/2)*	Patient: *Muir–Torre syndrome*: colon cancer (94 y), basal cell carcinoma (81 y) Family: no tumors
ST-27 (M)	SC (Back, 63 y)	*MSI-H (2/2)*	Patient: *Muir–Torre syndrome*: carcinoma of ascending colon (60 y) Family: no tumors
ST-31 (F)	SC (Back, 71 y)	*MSI-H (3/4)*	Patient: *Muir–Torre syndrome*: colorectal carcinoma, bladder cancer and basal cell carcinoma Family: colorectal carcinoma (brother), colorectal carcinoma, endometrial carcinoma, laryngeal carcinoma, cancer of the stomach (mother, sisters of the mother and their children)
SSG-21 (F)	SC (Face, 85 y)	MSS (0/5)	Patient and family: no further tumors
ST-01 (M)	SH (Cheek, 65 y)	MSS (0/5)	Patient and family: no further tumors
ST-02 (M)	SH (Cheek, 48 y)	MSS (0/5)	Patient and family: no further tumors
ST-04 (M)	SH	MSS (B26–)	Patient: no further tumors Family: malignant melanoma (brother of the mother with 40 y)
ST-07 (M)	SH (Cheek, 44 y)	MSS (B26–)	Patient and family: no further tumors
ST-14 (M)	SH (Root of the nose, 65 y)	MSS (B26–)	Patient and family: no further tumors
ST-16 (M)	SH (Forehead, 47 y)	MSS (B26–)	Patient and family: no further tumors
ST-18 (M)	SH (Cheek, 64 y)	MSS (0/6)	Patient and family: no further tumors
ST-20 (F)	SH (Chest, 60 y)	MSS (0/6)	Patient: no further tumors Family: colorectal cancer (father with 61 y)

Table I. Continued

Patient (Sex)	Tumor histology (Localization; age at diagnosis)	MSI (markers with additional alleles/examined markers)	Tumor history (patient and family)
ST-22 (F)	SH (Cheek, 52 y)	MSS (0/5)	Patient: no further tumors Family: cancer of the gallbladder (sister of the mother)
ST-25 (F)	SH (Cheek, 78 y)	MSS (0/6)	Patient: benign colon tumor (52 y) Family: no further tumors
ST-30 (M)	SH (Infraorbital, 53 y)	MSS (0/6)	Patient: cyst of the hair follicle at 53 y Family: cancer of the stomach (father, mother, and grandmother (m)), breast cancer (mother)
ST-35 (F)	SH (Forehead, 77 y)	MSS (0/6)	Patient and family: no further tumors
ST-38 (M)	SH (Cheek, 46 y)	MSS (0/6)	Patient: no further tumors Family: malignant brain tumor (mother with >60 y)
ST-42 (F)	SH (Cheek, 73 y)	MSS (0/5)	Patient: no further tumors Family: cancer of the stomach (father)
SSG-01 (M)	SH (Face, 56 y)	*MSI-H (3/5)*	Patient: urothelial papilloma Family: colon adenoma (brother), prostate carcinoma (two brothers), CRC and breast cancer (mother and sister), esophageal carcinoma (grandmother (m)), basal cell carcinoma (father), lung cancer (father's sister), prostate carcinoma (mother's brother), breast cancer (sister), cancer of the ovary (sister)
SSG-02 (M)	SH (Face, 61 y)	MSS (0/5)	Patient: no further tumors Family: "abdominal malignoma" (mother)
SSG-03 (M)	SH (Face, 67 y)	MSS (0/6)	Patient: squamous cell carcinoma Family: "skin tumor" (father)
SSG-04 (M)	SH (Face, 60 y)	MSS (0/5)	Patient: prostate carcinoma Family: no tumors
SSG-07 (M)	SH (Face, 60 y)	MSS (0/5)	Patient and family: no further tumors
SSG-08 (F)	SH (Face, 76 y)	MSS (0/5)	Patient: tubular colonic adenoma Family: prostate carcinoma (brother)
SSG-10 (M)	SH (Face, 62 y)	MSS (0/6)	Patient: malignant melanoma
SSG-11 (M)	SH (Face, 58 y)	MSS (0/6)	Patient: no further tumors Family: cancer of the tongue (father), cancer of unknown primary (mother)
SSG-12 (F)	SH (Mons pubis)	MSS (0/6)	Patient: no further tumors Family: colorectal carcinoma (father's brother)
SSG-13 (M)	SH (Face, 44 y)	MSS (0/5)	Patient: no further tumors Family: lung cancer (father)
SSG-14 (M)	SH (Face, 35 y)	MSS (0/5)	Patient: no further tumors Family: malignant astrocytoma (father), testicular cancer (brother)
SSG-15 (F)	SH (Face, 61 y)	MSS (0/5)	Patient: no further tumors Family: laryngeal carcinoma (brother), uterine leiomyoma (daughter)
SSG-16 (M)	SH (Face, 63 y)	MSS (0/5)	Patient and family: no further tumors
SSG-17 (M)	SH (Capillitium, 63 y)	MSS (B26−)	Patient: two basal cell carcinomas Family: no further tumors
SSG-18 (M)	SH (Face, 78 y)	MSS (0/5)	Patient: no further tumors Family: colorectal cancer and lung cancer (father)
SSG-49 (M)	SH (Face, 64 y)	MSS (0/5)	Patient and family: no further tumors
SSG-55 (F)	SH (Cheek, 66 y)	MSS (B26−)	Patient: breast cancer, basal cell carcinoma Family: cancer of the stomach (father)
SSG-59	SH (Face)	MSS (0/5)	Patient: basal cell carcinoma Family: adenocarcinoma of the stomach (father)

ST, sebaceous tumors from Germany; SSG, sebaceous tumors from Italy; SH, sebaceous hyperplasia; SA, sebaceous adenoma; SE, sebaceous epithelioma; SC, sebaceous carcinoma; M, male; F, female; CRC, Colorectal carcinoma; B26+, allele shift of 3 bp or more with the single marker BAT26; B26−, no allele shift with the single marker BAT26; MSI-H, high microsatellite instability (a tumor that shows additional alleles with two or more microsatellite markers and with at least 30% of all markers tested or an allele shift of 3 bp or more with the single marker BAT26): MSS, microsatellite stability (a tumor that shows no additional alleles with at least five microsatellite markers examined or no allele shift with the single microsatellite marker BAT26); m, matrilineal; f, patrilineal.

Molecular genetic analyses with microsatellite markers showed that 60% (15 of 25) of the sebaceous gland neoplasias exhibited MSI-H, pointing towards an underlying DNA MMR defect, whereas only one of the 32 sebaceous gland hyperplasias was highly unstable. The proportion of MSI-H sebaceous gland neoplasias was almost equally distributed between the sebaceous gland adenomas, epitheliomas, and carcinomas (for details see **Tables III, I**). Within the sebaceous neoplasias all three CST exhibited MSI-H.

In a second step, the patients were contacted for their own and their family's history. On the basis of the subsequently collected tumor histories, nine of the 15 patients with MSI-H sebaceous gland neoplasia proved to be affected with at least one additional internal cancer and were therefore diagnosed to have MTS. The clinical Amsterdam criteria for diagnosing HNPCC, (Vasen *et al*, 1991), however were not fulfilled in any of these patients. In the sebaceous tumors of the other six patients, MSI-H was an incidental finding, meaning that no internal cancer typical for MTS

Table II. Microsatellite markers: primer sequences (5′→3′) and their annealing temperatures for microsatellite markers used to assess MSI

Marker (fluorescence labeling)	Forward primer	Reverse primer	Annealing temperature T1→T2 (touchdown PCR)
BAT25 (HEX)	F-tcgcctccaagaatgtaagt	R-tctgcattttaactatggctc	58°C→54°C
BAT26 (TET)	F-tgactacttttgacttcagcc	R-aaccattcaacatttttaaccc	56°C→52°C
D2S123 (FAM)	F-aaacaggatgcctgccttta	R-ggactttccacctatgggac	62°C→58°C
D5S346 (FAM)	F-actcactctagtgataaatcg	R-agcagataagacagtattactagtt	62°C→58°C
D17S250 (TET)	F-ggaagaatcaaatagacaat	R-gctggccatatatatatttaaacc	56°C→52°C
BAT40 (TET)	F-attaacttcctacaccacaac	R-gtagagcaagaccaccttg	60°C→56°C
D3S1298 (TET)	F-agctctcagtgccacccc	R-gaaaaatcccctgtgaagcg	61°C→57°C
D3S1611 (FAM)	F-gcctagcaagatgacttctaag	R-agctgagactacaggcatttg	62°C→58°C
D6S470 (HEX)	F-aagcgatctcaccatatacac	R-acactgcaaaacgattacca	60°C→56°C
D18S69 (FAM)	F-ctctttctctgactctgacc	R-gactttctaagttcttgccag	60°C→56°C

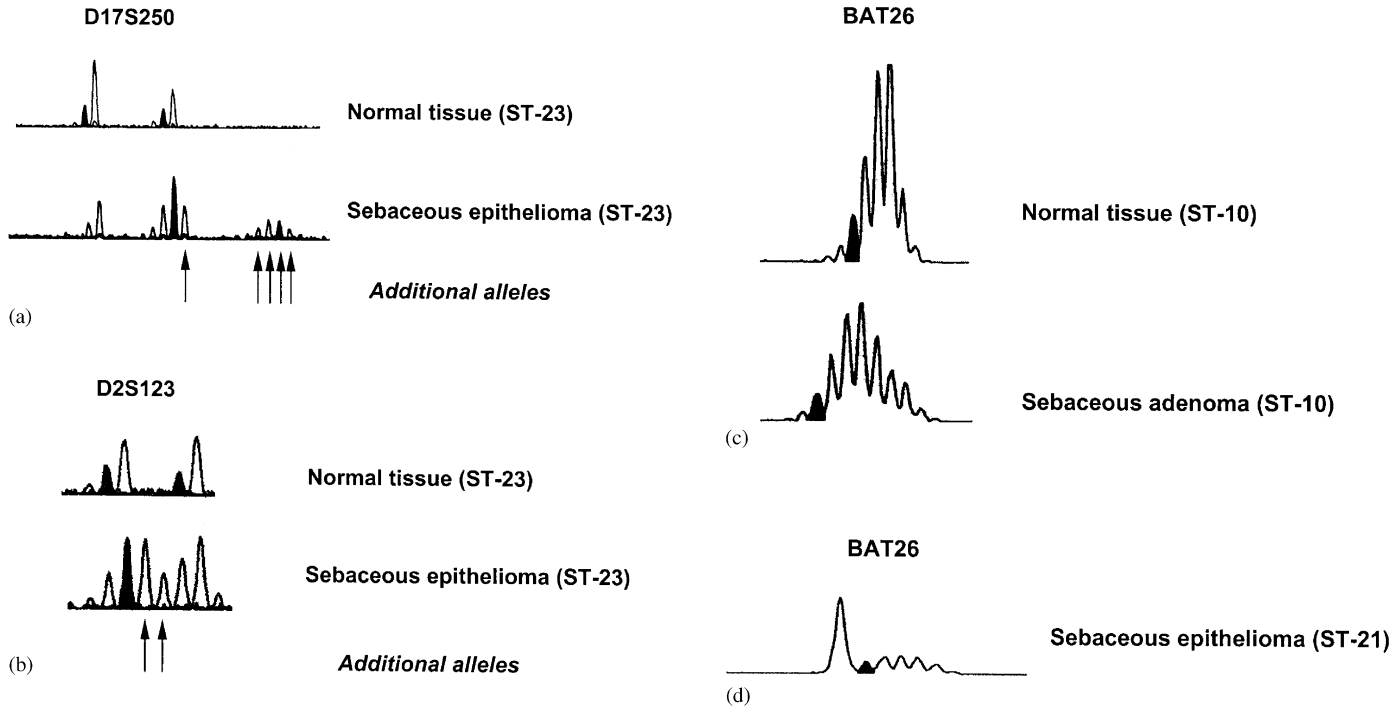

Figure 1. MSI in sebaceous tumors. The electropherograms of PCR-amplified microsatellite markers from normal DNA and from tumor DNA. (*a*), (*b*) Sebaceous epithelioma from patient ST-23: microsatellite markers D17S250 and D2S123 show additional alleles in tumor DNA (*arrows*). (*c*) Sebaceous adenoma from patient ST-10: microsatellite marker BAT26 shows a shift of 3 bp in tumor DNA. (*d*) Sebaceous epithelioma from patient ST-21: microsatellite marker BAT26 shows a shift of at least 5 bp in tumor DNA.

Table III. Results on MSI assessment in sebaceous tumors from different patients

Tumor histology	MSI-H tumors /total number
Sebaceous neoplasias	15/25 (60%)
Sebaceous adenomas	4/6 (67%)
Sebaceous epitheliomas	9/16 (56%)
Sebaceous carcinomas	2/3 (67%)
Sebaceous hyperplasias	1/32 (3%)

or HNPCC had occurred together with the sebaceous tumor. In two of these six patients (ST-15 and ST-34), single relatives had a history of internal cancer; however, their family histories did not meet the clinical criteria for HNPCC.

The patient with the MSI-H sebaceous hyperplasia also had a benign internal tumor (urothelial papilloma). Several of his relatives have been diagnosed with cancer of different organs suspicious for HNPCC.

DISCUSSION

In this study, for the first time we assessed the incidence of MSI-H in a consecutive series of randomly selected sebaceous gland neoplasias and hyperplasias. In our study, 60% of patients with a sebaceous gland neoplasia exhibited MSI-H and are thus at risk for an underlying inherited DNA MMR defect. Alternatively, MSI-H could be caused by a somatic inactivation of both alleles of a DNA MMR gene. It has to be considered, however, that at least two-thirds of MTS patients with MSI-H sebaceous tumors harbor a germline mutation in one of the DNA MMR genes *MSH2* or *MLH1* (Kruse *et al*, 1998). The age at diagnosis proved to be lower in sebaceous gland neoplasias with MSI-H (≈70 y) than in neoplasias with microsatellite stability (≈74 y). This is compatible with the observation that the associated neoplasms in hereditary cancer syndromes such as HNPCC and MTS on average develop earlier than their sporadic counterparts. Interestingly, mean age at diagnosis of MSI-H sebaceous gland neoplasias proved to be significantly higher than that of the initial sebaceous neoplasias in the 120 MTS patients reviewed by Cohen *et al* (1991)

Table IV. Current status: studies on the frequency of MSI-H in randomly selected skin tumors

Skin tumor	MSI-H tumors/ examined tumors (% MSI-H tumors)	MSI-low (L)[a] tumors/examined tumors (% MSI-L tumors)	Reference
Sebaceous neoplasia[b]	*15/25 (60%)*		*This study*
Sebaceous hyperplasia	*1/32 (3%)*		*This study*
Keratoacanthoma	6/53 (11%)		Halling *et al* (1995)
	1/19 (5%)		Peris *et al* (1997a)
	0/12 (0%)	1/12 (8%)	Langenbach *et al* (1999)
Malignant melanoma	0/40 (0%)	8/40 (20%)	Peris *et al* (1995)
	0/41 (0%)	1/41 (2.5%)	Quinn *et al* (1995)
	1/10 (10%)	2/10 (20%)	Richetta *et al* (1997)
	0/22 (0%)	7/22 (31%)	Hussein *et al* (2001)
	1/20 (5%)	4/20 (20%)	Talwalkar *et al* (1998)
Benign melanocytic nevi	0/30 (0%)		Hussein *et al* (2001)
Dysplastic melanocytic nevi	0/60 (0%)	17/60 (28%)	Hussein *et al* (2001)
Basal cell carcinoma	0/47 (0%)	1/47 (2%)	Quinn *et al* (1995)
Squamous cell carcinoma	1/49 (2%)	1/49 (2%)	Quinn *et al* (1995)
Bowen's disease	0/20 (0%)	1/20 (5%)	Quinn *et al* (1995)

[a]MSI-low (L): low microsatellite instability (if additional alleles were observed with only one of at least five examined markers or with less than 30% of the markers tested.)
[b]The 25 sebaceous neoplasias comprise six sebaceous adenomas, 16 sebaceous epitheliomas, and three sebaceous carcinomas.

(53 y). This discrepancy may be due to the unselected recruitment of sebaceous tumors in this study. All four CSTs within our newly collected sebaceous tumors exhibited MSI-H. In our previous studies (Kruse *et al*, 1998; 1999; Rütten *et al*, 1999), each of 10 CSTs examined for MSI showed MSI-H. Thus, our results support the hypothesis that the CST may be a specific marker tumor for an underlying DNA MMR defect.

In addition to the examination of sebaceous gland neoplasias, we also looked for MSI in a series of sebaceous gland hyperplasias. In comparison to sebaceous gland neoplasias, only 3% of the hyperplasias exhibited MSI-H. This result is consistent with the clinical observation that the frequently occurring sebaceous hyperplasia is not a diagnostic criterion for MTS (Schwartz and Torre, 1995).

In contrast to randomly selected sebaceous neoplasias, almost 100% of sebaceous tumors from patients with colorectal carcinomas as part of their underlying MTS (Honchel *et al*, 1994; Peris *et al*, 1997b; Kruse *et al*, 1998; Lynch *et al*, 1999; Ueda *et al*, 1999; Entius *et al*, 2000) or HNPCC (Swale *et al*, 1999), as well as almost 100% of CSTs (Kruse *et al*, 1999; Rütten *et al*, 1999), are known to exhibit MSI. In the study by Harwood *et al* (2001), MSI was demonstrated in three of six sporadic sebaceous carcinomas from immunosuppressed organ transplant patients. The authors hypothesized that immunosuppression might unmask a previously silent MTS phenotype in some cases.

The assessment of MSI in colorectal tumors has been demonstrated to be a useful screening method to identify persons at high risk for HNPCC (Lamberti *et al*, 1999). Accordingly, systematic screening studies in HNPCC-related internal tumors to detect underlying DNA MMR defects have been mainly performed with unselected colorectal carcinomas. These studies revealed MSI-H in 12% (Aaltonen *et al*, 1998), 9% (Furlan *et al*, 1998), or 20% (Cunningham *et al*, 2001) of colorectal carcinomas. Loukola *et al* (1999) designed a study to assess the feasibility of screening benign colorectal adenoma patients for HNPCC. Among these adenoma patients, only 1.6% had at least one MSI adenoma. In endometrial carcinomas, MSI was detected in about 28% of cases (12 of 42) (Catasus *et al*, 1998). These frequencies are significantly lower than the MSI-H frequency of 60% in our series of sebaceous gland neoplasias.

So far, screening studies for MSI in randomly selected nonsebaceous skin tumors have only been performed with keratoacanthomas (Halling *et al*, 1995; Peris *et al*, 1997a; Langenbach *et al*, 1999) and tumors not related to HNPCC or MTS, such as malignant melanomas (Peris *et al*, 1995; Quinn *et al*, 1995; Richetta *et al*, 1997; Talwalkar *et al*, 1998; Hussein *et al*, 2001), nevi (Hussein *et al*, 2001), basal cell carcinomas, squamous cell carcinomas, and Bowen's disease (Quinn *et al*, 1995). The MSI-H detection rates in these skin tumors were in the range of only 0%–11% (for details see **Table IV**).

In conclusion, this is the first systematic MSI screening study in sebaceous tumors selected solely on the basis of their histopathologic diagnosis without previous knowledge of the patients' and their family's history for internal tumors. As more than half of the randomly selected sebaceous neoplasias showed MSI-H, our study demonstrates the clinical relevance of the histopathologic diagnosis and the MSI assessment of a sebaceous gland neoplasia in terms of early detection of patients (and families) at high risk for internal cancer.

The assessment of MSI in a sebaceous gland neoplasia is therefore highly relevant for cancer prevention. For all MTS and HNPCC patients, as well as for patients with MSI-H sebaceous gland tumors and relatives with internal cancer that do not meet the HNPCC criteria but belong to the MTS/HNPCC spectrum (e.g., ST-15 or ST-34), we recommend the same regular cancer surveillance program according to the guidelines of the ICG-HNPCC. This is also recommended for the first-degree relatives of these patients.

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