Muir–Torre syndrome or phenocopy? The value of the immunohistochemical expression of mismatch repair proteins in sebaceous tumors of immunocompromised patients

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Abstract Primary and secondary immunodepressive conditions are associated with an increased incidence of sebaceous tumors. Microsatellite instability (MSI) and lack of expression of mismatch repair (MMR) proteins, typical markers of Muir–Torre/Lynch heredo-familial settings, can be recognized also in immunocompromised patients. We aimed to carry on a systematic examination of clinical, immunohistochemical, biomolecular features of sebaceous tumors arising in immunocompromised and immunocompetent patients between 1986 and 2012. Microsatellite screening, immunohistochemical analysis and genetic testing were performed for *hMLH1*, *hMSH2* and *hMSH6*. Methylation status of MMR genes was checked in cases with immunohistochemistry (IHC) loss of MMR proteins expression and no germline mutations. Fifteen patients had a personal history of visceral carcinomas fulfilling diagnostic criteria for Muir–Torre syndrome. In this cohort,

IHC analysis, MSI status and genetic testing were in agreement, showing eight *MSH2* and two *MLH1* germline mutations. Five patients were immunosuppressed and their sebaceous tumors showed a lack of *MSH2/MSH6* expression, although just one case with positive family history for visceral cancer harbored a germline mutation. In immunosuppressed patients, loss of IHC for MMR proteins is not necessarily secondary to MMR germline mutations. IHC false positives are probably due to epigenetic alterations. MSI and lack of expression of MMR proteins can be recognized also in immunocompromised patients without MMR germline mutations.

Keywords Mismatch repair genes · Mismatch repair proteins · Muir–Torre syndrome · Sebaceous tumors · Immunodepression · MMR proteins immunohistochemical expression

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Introduction

It is well known that primary and iatrogenic immunodepression is associated to an increased incidence of skin tumors [1–3]. Among them, significantly higher incidence rates of uncommon sebaceous adenomas and adenocarcinomas were significantly reported in immunosuppressed compared to immunocompetent patients (30 vs. 6 %) [4]. The so-called Muir–Torre syndrome (MTS), a variant of the Lynch syndrome (LS), is characterized by the presence of early-onset sebaceous tumors and keratoacanthomas associated to visceral malignancies. Tumors arising in MTS or LS patients are featured by the presence of a typical instability at microsatellite loci (MSI), which is caused by germline mutations of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, whose loss of

expression can be tested through immunohistochemistry (IHC) [5, 6]. Some primary and secondary immunodepressive conditions are associated to an increased incidence of rare sebaceous tumors [7], in which, beyond the direct pathogenic effect of oncogenic virus [8, 9], a role for genetic aberrations has been hypothesized [10] and among those for MSI and loss of IHC expression of MMR proteins [8]. In immunodepressed individuals, however, the underlying gene alteration causing the loss of MMR proteins expression at IHC is still poorly understood: so far, recent case reports showed that immunodepression can unmask a genetic predisposition linked to germline mutations whose phenotypic silent characteristic is represented cancer development [7, 11]. However, the genetic mechanism determining MSI and/or loss of MMR expression in sebaceous tumors in the absence of a germline mutation could be reconducted to a more general mechanism, like *de novo* methylation of promoters, which has been postulated as a major mechanism of inactivation of tumor suppressor genes [12, 13].

In our single center study, we aimed to carry on systematic examination of clinical, immunohistochemical and biomolecular features of cutaneous appendageal tumors arising in immunocompromised and immunocompetent patients between 1986 and 2012.

Methods

Ethics statement

This study was approved by the Ethical Committee of the University Hospital of Modena, Italy, and was conducted after informed written consent of enrolled patients.

Patients and tumor samples

From 1986 through 2012, a total of 142 sebaceous tumors (embedded in paraffin) from 90 patients (54 males and 36 females) were retrospectively retrieved from the archives of the Pathology Department of the University of Modena. One hundred and eight lesions were sebaceous adenomas and 34 were sebaceous carcinomas. A tumor was classified as sebaceous when well-defined, enlarged, sebaceous lobules were present that comprised fully mature sebocytes, frequently demonstrating an attachment to the epidermis with epidermal thinning. In sebaceous carcinomas, variably atypical, polyhedral cells are present that are separated from one another by fibro-vascular stroma, sometimes with spread of pagetoid epithelial cells into the epidermis.

Family history

Detailed family history was collected for each patient by interviewing the patients and/or their relatives. Verification of cancer occurrence among family members was obtained in the majority of patients through clinical charts, pathologic records, or death certificates. Through the reconstruction of the genealogic tree, 15 patients with MTS were identified (10 males and 5 females).

Microsatellite analysis

For microsatellite analysis, DNA was extracted from micro-dissected neoplastic and paired normal mucosa specimens of dermatologic lesions and visceral malignancies according to standard procedure. MSI was evaluated with 5 microsatellite markers (BAT25, BAT26, BAT40, D2S123, and D5S346) using a fluorescence-based polymerase chain reaction (PCR) method. DNA samples from normal tissues and tumor tissues were amplified in a 10- μ L volume containing 30–50 ng of DNA; 5 ng of dye-labeled forward and unlabeled reverse primers; 200 μ M each of deoxyguanine triphosphate, deoxythymidine triphosphate, deoxyadenosine triphosphate, and deoxycytidine triphosphate; 1.5 mM $MgCl_2$; 50 mM KCl; 10 mM Tris, pH 8.3; and 0.3 U of Taq polymerase. All samples were run on a CEQ 8000 sequencer and were analyzed using a fragment analysis system (Beckman Coulter). MSI-positive tumors were defined as tumors in which instability was detected in at least two microsatellite loci [14].

Immunohistochemistry

Immunohistochemical analysis of MLH1, MSH2 and MSH6 proteins were carried out on paraffin-embedded tumor samples. Immunoperoxidase staining using diaminobenzidine as a chromogen was run with the NEX-ES Automatic Staining System (Ventana, Strasbourg, France). The mouse monoclonal antibodies used were anti-MSH6 (clone 44; Transduction Laboratories) at 1:2,000 dilution, anti-MLH1 (G168-15; Pharmingen) at 1:40 dilution, and anti-MSH2 (G129-1129; Pharmingen) at 1:40 dilution. Nuclei were counterstained with haematoxylin. Adjacent normal tissues from each sample served as positive controls.

Mutational analysis and methylation status analysis of MMR gene promoter regions

Germline mutations in *hMLH1*, *hMSH2* and *hMSH6* were studied by single-strand conformation polymorphism (SSCP) on DNA derived from blood leukocytes. Samples that showed an altered SSCP mobility pattern were

sequenced by means of the Sequenase[®] PCR product sequencing kit (Amersham Life Science, Buckinghamshire, United Kingdom) using a Beckman sequencer (model CEQ 8000).

The probe mix contains thirty-eight probe sequences of which 22 different probes for MMR genes containing one or two digestion sites for the methylation sensitive *HhaI* enzyme. The remains probes are reference ones that are not influenced by *HhaI* digestion. In particular, the ME011 kit includes three testing probes for MSH2 gene, located in the promoter region and in exon 1: the first one is located at nucleotide 269 before ATG, the second one is located at 193 nucleotide before ATG and the last one is at nucleotide 124 after ATG.

In the present study, each experiment was carried out in duplicate, according to the manufacturer's instructions (<http://www.mrc-holland.com>) and normal DNA specimens derived from lymphocytes from healthy controls were included in every assay.

Briefly, for each MS-MLPA reaction, 100 ng of DNA was used. PCR products were run onto an ABI 310 capillary sequencer and analyzed using Gene Mapper v. 4.0 analysis software (Applied Biosystems, Foster City, CA).

Data analysis for evaluating methylation status, has been performed with two different methods: an excel spreadsheet and the Coffalyser.Net software.

A dosage ratio of 0.15 or higher, corresponding to 15 % of methylated DNA, was applied to indicate promoter methylation.

Methylation analysis was performed by the methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay that is a modification of the MLPA technique. It allows the detection of both copy number changes and unusual methylation levels of different sequences in specific regions in one simple reaction.

The MS-MLPA ME011 kit from MRC-HOLLAND company was employed to detect aberrant CpG Island methylation in the MMR promoter region genes.

In general, the MS-MLPA protocol is very similar to the standard MLPA method, except that each MS-MLPA reaction generates two samples: one undigested sample for copy number detection and one digested sample for methylation detection. The MS-MLPA procedure can be divided into five steps: DNA denaturation and hybridization of MLPA probes; ligation and digestion; PCR; separation of amplification products by capillary electrophoresis and data analysis.

MS-MLPA probes for methylation detection resemble other MLPA probes, except that their target sequence contains the restriction site of the methylation-sensitive endonuclease *HhaI*. So, not all the probes provide information on methylation status but only the probes that contain a sequence recognized by the methylation-sensitive restriction enzyme *HhaI*.

After hybridization, the reaction is split into two tubes: one tube is processed as a standard MLPA reaction, providing information on copy number changes. The other is incubated with the *HhaI* endonuclease meanwhile hybridized probes are ligated. Hybrids of probes and unmethylated sample DNA are digested by the *HhaI* enzyme that digests unmethylated DNA from the middle of GCGC sequences but leaves methylated sites intact.

Digested probes cannot be amplified exponentially during PCR and hence will not produce a signal during capillary electrophoresis. In contrast, if the sample DNA is methylated, the DNA-probe hybrids are protected against *HhaI* digestion and the ligated probes will generate a peak.

Results

Clinical features

Between 1986 and 2012, 142 sebaceous tumors of 90 patients (54 males and 36 females) were collected. More specifically, 108 were sebaceous adenomas and 34 sebaceous carcinomas.

Fifteen patients had a history of intestinal or other visceral carcinomas, thus fulfilling the criteria for a clinical diagnosis of MTS (10 males and 5 females) (Table 1). Among the 75 apparently sporadic patients only one (patient 1) had a family history evocative of LS (Fig. 1).

In the MTS group, there were 60 skin lesions, including 48 sebaceous adenomas and 12 sebaceous carcinomas. The average age at onset of the first skin malignancy was 57.87 years (range 38–89 years) compared with 69.35 years (range 45–91 years) in the sporadic group.

In patients with MTS, skin lesions were often multiple, synchronous, or metachronous, and were located predominantly in the head and neck.

In six patients with MTS, sebaceous lesions occurred as the first neoplasm while in the remaining nine patients, these lesions developed after an internal malignancy.

Among the 15 MTS probands, 12 colorectal carcinomas and 3 gastric carcinomas were found.

The average age at onset of the first visceral malignancy was 56.27 years. In addition to skin and visceral malignancies, the tumor spectrum included one kidney carcinoma, one carcinoma of the renal pelvis, one uterus carcinoma and one breast tumor. Finally, in two MTS probands, psoriatic arthropathy was diagnosed. Among first-degree relatives, colon, lung, brain, breast, pancreas, kidney, bladder, gastric, liver, uterus and ovary carcinomas, non-Hodgkin lymphoma and laryngeal tumors were detected.

Five apparently sporadic patients with the only sebaceous tumors were considered immunosuppressed: three

Table 1 Cutaneous and visceral malignancies in patients with MTS, including tumor site, antibodies against *MSH2*, *MSH6* and *MLH1* proteins; microsatellite status; and results of histological diagnosis, and age at onset: results of immunohistochemical staining with mutation analysis of mismatch repair genes

Patient number	Sex	Age at first skin lesion	No. of skin lesions	Skin lesion histology	Site	Visceral tumor in the proband (age in years)	Other tumors in the proband (age in years)	Tumors in the family (age in years)	IHC analysis (loss of)	MS	Mutation analysis
1	M	51	15	SEB CA, SEB AD	Head, chest	Right colon (49)	–	Colon (38), lung (65), colon (59), brain (60)	<i>MSH2/MSH6</i>	MSI	Test refused
2	F	65	3	SEB AD	Head, neck	Right colon (62)	–	Breast (72), pancreas (50), pancreas (60)	<i>MLH1</i>	MSI	<i>MLH1</i> mutation (c.1520–1521 insertion of T)
3	M	47	5	SEB AD	Head, chest	Gastric (55)	–	–	None	MSS	Not tested (proband deceased)
4	M	50	9	SEB AD, SEB CA	Head, neck	Left colon (51), kidney (57)	Melanoma (58)	Kidney (45)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (exon 13, nucleotide 2131, substitution C→T)
5	F	73	4	SEB CA	Head, neck	Colorectal (75)	Breast (73)	Lymphoma (22), larynx (54), lymphoma (46)	None	MSS	Not tested (proband deceased)
6	M	38	2	SEB AD	Head, chest	Right colon (35), renal pelvis (54)	–	Bladder (65), breast (60)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (exon 13, nucleotide 2133 substitution C→T)
7	F	89	1	SEB AD	Head	Gastric (89)	–	Breast (45), gastric (72)	<i>MLH1</i>	MSI	Not tested (proband deceased)
8	M	59	3	SEB CA	Chest	Left colon (56)	–	Liver (55)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (deletion at exon 13)
9	M	79	1	SEB AD	Back	Gastric (82)	–	Larynx (60)	<i>MSH2/MSH6</i>	MSI	Not tested (proband deceased)
10	M	68	4	SEB AD	Head, neck	Right colon (55)	–	–	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (duplication of exon 8)
11	F	46	2	SEB AD	Head	Breast (38), uterus (40), right colon (45)	–	Colon (32), colon (33), colon (39)	<i>MLH1</i>	MSI	<i>MLH1</i> mutation (c.2269–2270 insertion of T)
12	M	54	1	SEB CA	Head	Left colon (48)	–	Colon (38), colon (45), uterus (40)	<i>MSH2/MSH6</i>	MSI	Not tested (proband deceased)
13	M	51	2	SEB AD	Head	Left colon (49)	–	Colon (38), rectum (59)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (large deletion at exon 1)
14	F	46	1	SEB CA	Back	Right colon (52)	–	Uterus (40), ovary (34), ovary (40)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation (deletion TT at 880 exon 5)
15	M	52	7	SEB AD	Head	Right colon (48)	–	Colon (46), colon (44)	<i>MSH2/MSH6</i>	MSI	<i>MSH2</i> mutation [exon 14, c.2300C>G (p.Ser767x) codon 767]

M male, F female, SEB CA sebaceous carcinoma, SEB AD sebaceous adenoma, IHC immunohistochemistry, MSI microsatellite instability, MSS microsatellite stability

Fig. 1 A Muir-Torre syndrome family (renal transplant recipient Patient 1 under immunosuppressive treatment for and gene-carrier of *MSH2* germline mutation c.1216C>T)

patients received a transplant (two had kidney transplantation and one heart transplantation) whereas the remaining two were immunocompromised because of cancer and iatrogenic causes. All of them developed sebaceous tumors after becoming immunosuppressed.

Patient 1

Of the two renal transplant recipients, (RTR) the first patient, RTR1 underwent the surgical intervention at the age of 49, and developed 5 sebaceous adenomas, four located on the face and one in the lumbar region. The first sebaceous adenoma, of the face, was excised at the age of 52. He was diagnosed of a colonic adenoma at the age of 36. After the transplant, he developed a basal cell carcinoma on the nose and one keratoacanthoma on the face. All of his sebaceous tumors were adenomas and shared the same molecular markers, IHC MMR proteins expression, mutation and methylation. Among his relatives, his sister had a positive personal history of colon cancer discovered at the age of 65, while another sister and a niece were diagnosed of endometrial cancer at the age of 54 and 50 respectively. The patient was under immunosuppressive

treatment with prednisone (Deltacortene[®]) and tacrolimus (Prograf[®]) (Fig. 1). However, his family members were not affected by cutaneous sebaceous tumors.

Patient 2

RTR2 underwent kidney transplant in 1998 at the age of 45, and developed a sebaceous adenoma of the left lower eyelid at the age of 58. Later on, two more sebaceous adenomas of the face were found. The patient has not been diagnosed of any visceral malignancies so far, and he is affected by Berger syndrome. In his family, two sisters had uterine fibromas, and one of them also had colon cancer of the sigmoid tract with liver metastasis; moreover, one maternal uncle had colon cancer. The patient was under immunosuppressive treatment with cyclosporine A and switched to prednisone (Deltacortene[®]) and Tacrolimus (Prograf[®]) in 2012.

Patient 3

Patient 3, a heart transplant recipient (HTR) underwent heart transplant at the age of 63; he developed a sebaceous

adenoma of the left cheek 1 year later. He was also diagnosed of one ulcerated basal cell carcinoma (BCC) of the neck. The patient's personal and family history was negative for visceral and skin neoplasms. He was always treated with cyclosporine but denied his consent to genetic testing.

Patient 4

The patient, male, was diagnosed of prostate carcinoma at the age of 55, and non-Hodgkin lymphoma at the age of 67, that was treated with chemotherapy following the R-CHOP scheme. Some months after chemotherapy he developed a sebaceous adenoma of the left cheek (at the age of 68) and a superficial BCC of the face. His family history is negative for either skin or visceral tumors.

Patient 5

Patient 5, a female patient, had a uterine leiomyoma at the age of 26; she was also diagnosed a tubulo-villous adenomas with high-grade dysplasia in the colon at the age 61. Because of her hyperthyroidism, she was treated with radioactive iodine followed by methimazole. She developed a sebaceous carcinoma of the lumbar region at the age of 59. Concerning her family history, her father died of lung cancer while her mother died of brain cancer, respectively.

MSI and IHC features

For all patients, we examined the sebaceous skin tumors for microsatellite status and immunohistochemical expression of the MLH1, MSH2, and MSH6 proteins. The MTS skin lesions showed microsatellite instability (MSI) and loss of MMR expression at the IHC in 13 probands: in 10 cases the lack of expression was related to the MSH2/MSH6 proteins, whereas absence of MLH1 was evident in 3 probands. In two patients the results could not be obtained as the patients died.

The IHC of the sebaceous tumors belonging to the immunocompromised patients showed lack of expression for MSH2/MSH6 in four cases and MLH1 in one (Table 2). No loss of IHC expression of *MLH1* or MSH2/MSH6 proteins were seen in the sebaceous neoplasms of the other 70 sporadic patients.

MMR sequencing

Germline mutation in MSH2 and MLH1 genes were identified in nine MTS probands (Table 1). One MTS patient (Patient 1) denied his consent for blood sampling and five patients were deceased at the time of our investigation.

In the cohort of five immunosuppressed patients, for which IHC showed positivity, direct sequencing for MMR genes was performed for patient RTR1, RTR2, patient 4 and patient 5 (Table 2). One constitutional mutation of the *MSH2* gene has been detected [exon 7, c.1216C>T(p.Arg406x) codon 406] (Table 2). None of the patients analyzed had the promoter hypermethylation of the *MSH2* gene. All the other sporadic lesions were MSI negative and showed expression of all MMR proteins.

Discussion

The examination of clinical, immunohistochemical and biomolecular features of sebaceous tumor patients highlighted that, despite the crucial role of IHC for MMR genes in the selection and identification of MTS, the genetic counselor should consider the possibility of a IHC-bias related to the IHC findings showing loss of MMR proteins expression in sebaceous tumors in both MTS-associated and immunodepressive-related lesions can not be ruled out. This false positivity could lead to an overestimation of "true" MTS since phenocopies (disease subtypes or non-genetic causes of disease) could be mistakenly regarded as MTS and thus the patients subjected to useless direct gene sequencing.

Despite the important correlation between MSI and MMR proteins deficiency, it is generally known that the lacking expression of *MSH2* and *MLH1* might also be unrelated to germline mutations [15, 16]. Moreover, in contrast to colorectal tumors in the context of LS, characterized by clear cut guidelines [17], for determining MSI phenotype and IHC status of MMR proteins, in sebaceous skin tumors the precise modalities for the recognition of MTS are not well delineated, although a huge number of data are available in support of the usefulness, sensitivity and specificity of the IHC of the MMR genes [18–23].

A recent study on the role of the molecular screening of sebaceous adenomas and carcinomas in the diagnosis of LS highlighted the limits of the revised Bethesda guidelines and gave a hint on the possible alternative approaches to the identification of the syndrome, based on other tumors of the syndromic spectrum (i.e. endometrial cancer) [24]. For sebaceous tumors, however, there are no clear data on their role as a screening tool in the ascertainment of MTS.

Even if it is well known that immunodepression can unmask a genetic predisposition linked to germline mutations [7, 11] screening all immunocompromised patients with sebaceous tumors for MSI, IHC and MMR genetic testing may result in a low rate of MTS finding and in the absence of personal or family history clearly suggestive for LS/MTS, additional features should be evaluated to estimate the risk of a genetic syndrome [25–27]. In particular,

Table 2 Clinical, immunohistochemical and biomolecular features of immunosuppressed patients

Patient number	Sex	Age at first skin lesion	No. of skin lesions	Skin lesion histology	Site	Visceral tumor in the proband (age in years)	Other tumors in the proband (age in years)	Tumors in the family (age in years)	IHC analysis (loss of)	Immunological status and therapy	Mutation and methylation analysis
1	M	52	5	SEB AD	Head, back	–	–	Colon (65), uterus (54), uterus (50)	<i>MSH2/MSH6</i>	Glomerulonephritis (35), kidney transplant (49); prednisone, tacrolimus	<i>MSH2</i> mutation [exon 7, c.1216C>T (p.Arg406x) codon 406]
2	M	58	3	SEB AD	Head	–	–	Colon (41), colon (44), uterus (38), uterus (46)	<i>MSH2/MSH6</i>	Berger's disease (27), kidney transplant (45); prednisone, tacrolimus	Negative
3	M	64	1	SEB AD	Head	–	–	–	<i>MSH2/MSH6</i>	Heart transplant (63); cyclosporine A	Test refused
4	M	68	1	SEB AD	Head	Prostate (55)	Non-Hodgkin's lymphoma (67)	–	<i>MSH2/MSH6</i>	Chemotherapy (R-CHOP) (67)	Negative
5	F	59	1	SEB CA	Back	Left colon (61)	Uterus (26)	Lung (86), brain (59)	<i>MLH1</i>	Radioactive iodine and methimazole (30)	Negative

M male, *F* female, *SEB CA* sebaceous carcinoma, *SEB AD* sebaceous adenoma, *IHC* immunohistochemistry

some issues focused on the value of IHC for MMR genes as screening procedure for sebaceous neoplasms in immunosuppressed patients without positive personal and/or family history of visceral cancers, that do not fulfill the clinical criteria for MTS. However, the detection of *MSH2* germline mutation in our transplanted patient presenting a “sebaceous tumors only” phenotype (Fig. 1) supports the hypothesis that immunosuppression may unmask systemic MTS mutations. This experience suggests the effectiveness of testing the mutational status of MMR genes also in immunocompromised patients with cutaneous phenotype MTS compatible and IHC showing the loss of MMR proteins expression, particularly when there is a positive family history for visceral malignancies. This could result in earlier recognition of the MMR gene mutation carrier patients at high risk of developing visceral malignancies, as in the case of Patient 1.

Immunocompromised patients with IHC showing loss of MMR proteins expression in their sebaceous neoplasms and neither germline mutations nor visceral malignancies are much more intriguing from the point of view of pathogenesis, clinical management and follow-up. It is possible that sebaceous tumors carrying IHC loss of MMR proteins

expression in the absence of a germline mutation are related to other mechanisms.

Although our MMR gene promoter methylation in sebaceous tumors with loss of MMR proteins at IHC did not lead to positive results, it is necessary to ascertain the effective limits of the technique used. MS-MLPA kit contains three probes for *MSH2* promoter encompassing the main CpG-rich regions but not the entire gene. Moreover, mosaicism is a common facet of epimutations [28, 29] and mosaic epimutation shows variable degrees of allelic methylation and transcriptional silencing and/or tissue distribution. This semi-quantitative technique could lead to an underestimation of hypermethylation of further specific regions not investigated with this method and it could not detect low-levels of hypermethylation.

Further speculative hypothesis that can somehow explain IHC positivity in the absence of germline mutations are linked to the effects of some immunosuppressive drugs. Immunosuppressors could interact with MMR proteins causing a mutator phenotype and MSI, such as skin cancer. It has been hypothesized that immunosuppressive drugs, in particular azathioprine, may select cells hosting a MMR deficit, as a mechanism of evading its cytotoxic

effects [7]. Moreover, it is also evident that the type, duration and intensity of the iatrogenic immunodepression can influence the singular susceptibility to skin tumors [30, 31]. However, when we consider the common increased predisposition to the development of sebaceous neoplasms with IHC loss of MMR proteins expression in HIV positive individual, it is possible to infer that the above-discussed clinical entity is caused by the immunosuppressive state itself and is not bound to the type of immunosuppressive agents [32].

Although the clinical diagnosis of MTS requires the association of at least one sebaceous skin tumor and/or keratoacanthoma together with a visceral tumor, we must take into account the cases MTS ascertained through sebaceous tumors, in which the clinical tumor spectrum of MTS/LS is not fully expressed in the proband, but only observed in the family history. In order to perform a correct clinical diagnosis of MTS, it is thus necessary to consider the pathological, personal and familial history; there are, in fact, different and complex disease settings as well as sporadic and/or iatrogenic factors potentially responsible for the final clinical MTS phenotype.

In particular, although considering the hypothesis of an unmasked phenomenon while evaluating sebaceous neoplasms in immunocompromised patients, it is necessary to include a potential bias. The latter is linked to the existence of immunocompromised patients with IHC loss of expression of MMR proteins that act as clinical phenocopies and lead to overestimating the potential number of true MTS cases. On the other hand, the analysis of MMR proteins' expression can be considered an efficacious tool, even in immunosuppressed patients with no visceral malignancies but who bear the brunt of a positive family history of cancer.

Future studies focusing on the proteomic profiles of the MMR proteins could help us clarify the role of the epigenetic alterations of the proteins. It is also important to elucidate the role of methylation processes in the expression of MSH2/MSH6 proteins: those cannot be reasonably considered of minor importance with respect to the MLH1 protein, whose silencing mechanisms are already known.

Conflict of interest The authors declare no conflict of interest.

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