Mol Biol Rep (2012) 39:9307–9310
DOI 10.1007/s11033-012-1729-2

Soft tissue sarcoma and the hereditary non-polyposis colorectal cancer (HNPCC) syndrome: formulation of an hypothesis

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Received: 23 January 2012 / Accepted: 6 June 2012 / Published online: 11 July 2012

Abstract Hereditary non-polyposis colorectal cancer (HNPCC) is a genetic disorder caused by mutation in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) which predisposes to colorectal cancer and other malignances, that not yet include sarcomas. For sustaining that soft tissue sarcomas could be HNPCC related malignances, we report on a HNPCC patient with leiomyosarcoma and review the English literature. Overall, we report on eleven cases of soft tissue malignant tumors involving HNPCC patients, with a mean age of 34 years at diagnosis of sarcomas. In the majority of these tumors loss of MSH2 expression can be found at immunohistochemistry (IHC) and in 10 patients a germline mutation in one of the MMR genes was found (7 cases were MSH2 defective and 3 cases MLH1 defective). Data for supporting our hypothesis are also experimental, epidemiologic, histopathological: excess of sarcomas in PMS2 defective mice; sporadic soft tissue sarcomas are rare, with mean age at onset of 56 years and normal IHC for MMR proteins. In conclusion, the data collected support the hypothesis that soft tissue sarcomas could be included in the spectrum of tumors that, even if rarely, depend on MMR genes deficiency.

Keywords Sarcoma · Hereditary non-polyposis colorectal cancer · *hMSH2*

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Introduction

Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome is an autosomic dominant genetic disorder which predisposes to colorectal cancer and other malignancies prevalent in the same family and/or in the same subject. HNPCC is caused by germline deficiency of one of the DNA mismatch repair (MMR) genes: *hMLH1, hMSH2, hMSH6* and *hPMS2,* the first two responsible of the vaster majority of the mutations identified in HNPCC families [1]. The families that meet criteria for the clinical diagnosis of the syndrome (Amsterdam criteria) [2] show a clustering of one or more of the following cancers: colon, endometrium, small bowel, urothelium, stomach, ovary [3]. In the last years many different malignancies, not included in the spectrum of tumours related to the Lynch syndrome, have been described in HNPCC families. Only rarely definitive evidences to include one of these tumours in the HNPCC syndrome have been reported: this is the case of sebaceous gland tumours or keratoacanthomas, and glioblastomas which are rare tumours of the skin and brain that could occur in kindred with a germline *MMR* genes

mutation, configuring variants of HNPCC, respectively the Muir-Torre syndrome (if presence of such skin tumours) and the Turcot syndrome (if presence of brain cancer) [4, 5]. Soft tissue sarcomas are not yet included in the spectrum of the HNPCC related malignancies. A case of sarcoma in an HNPCC patient was the occasion to collect the available literature on sarcomas and Lynch syndrome, thinking over clinical and molecular aspects of their possible association. Aim of the present study is sustaining the hypothesis that soft tissue sarcomas could be considered in the group of the HNPCC related tumours and to postulate a particular susceptibility to soft tissue malignancies in *hMSH2* deficient kindred.

Report of the case

An Italian male patient born in 1960 developed proximal mucinous colon cancer in 2001, leiomyosarcoma of the right deltoid muscle in 2003, and right kidney cancer in 2004. He underwent surgery at our surgical department.

Cancer family history

Cancer family history fulfils Amsterdam 2 criteria for clinical diagnosis of HNPCC. Pathologic reports of the patient's first degree and second degree relatives were achieved.

MSI analysis

All the three tumours of the patient were analyzed for microsatellite instability. Standard MSI analysis was performed on paired tumour-normal tissue DNA samples using the National Cancer Institute (NCI) panel of microsatellite markers plus an additional panel of mononucleotide markers as recommended and described previously [6, 7]. Tumours were scored as MSI-H (high microsatellite instability), according to the international guidelines [6]. MSI test, revealed MSI-H on colon and kidney cancer, whether sarcoma was stable.

IHC assay

Conventional IHC for MLH1, MSH2, MSH6, PMS2 proteins was performed on all three tumours of the patients as previously described [8]. A case was considered negative for protein expression only when there was a complete absence of nuclear staining of neoplastic cells in the presence of an unquestionable internal positive control in non neoplastic cells. IHC revealed absence of nuclear expression of MSH2 and MSH6 proteins in all the three cancers.

DNA mismatch repair (*MMR*) genes analyses

After genetic counselling and informed consent, *MMR* germline mutation analyses were carried out by bi-directional sequencing on an automatic ABI3100 DNA analyzer (Applera). Point mutations of gene were searched by PCRs of genomic DNA with exon-specific primer pairs and bidirectional sequencing. Multiplex Legation-dependent Probe Amplification (MLPA) was utilized to identify large gene deletion. MLPA detected a germline large deletion of exons 1–16 on *MSH2*. MSH6 sequencing did not revealed pathogenetic mutations. In vivo MSH2 and MSH6 proteins form an heterodimer, and MSH6 is unstable in absence of its counterpart MSH2 [9–11]. So it is well known, that IHC for MSH6 can result negative just because the germline deficiency of MSH2.

Literature search

A Medline search, in the English literature, matching the terms of: HNPCC, Lynch syndrome, mismatch repair genes, microsatellite instability and sarcoma(s), soft tissue tumour(s) was carried out. The Medline search identified 12 cases of soft tissue sarcomas involving patients members of HNPCC families. Results of Medline search are briefly resumed on Table 1. It is to note that in 9 out of the 13 cases collected (included our case), soft tissue sarcoma involved the lower limb. Males are more represented in this series (9 cases) and the median age at diagnosis of soft tissue sarcoma is 38 years (range 15–66 years).

Several different tumour histology were observed; the more frequent hystotypes were liposarcomas (4 cases), malignant fibrous histiocytoma (3 cases), and leyomiosarcomas (2 cases). In 9 cases an IHC study was performed on tumour specimen and in 8 cases loss of hMSH2 protein was found. Microsatellite instability status was investigated only in 7 case (6 of them showing high instability). Medline search identified also a study in animals of Baker et al., which observed an excess of sarcomas and lymphomas in mice defective in one of the *MMR* genes: PMS2 [18].

Discussion

Personal and familial cancer history still represents the milestone of HNPCC diagnosis and is consequently of great relevance to know the spectrum of tumours which, even if to a small degree, could be derived from *MMR* genes deficiency. Cancers not included in HNPCC related tumours, but common in the general population have previously been described in HNPCC families (e.g. female breast and prostate cancer [19, 20]) and there are also

Table 1 Soft tissue tumours described in HPCC patients: results of histology, IHC assay, germline mutation on *MMR* genes

First author	Tumor site	Tumour histology (age, sex of patient)	IHC	*MMR* gene mutation (type of mutation found)
den Bakker [12]	Leg	Rhabdomyosarcoma (23, F)	MSH2 loss	NA
Medina Arana [13]	Paravertebral musculature	Leyomiosarcoma (19, M)	NA	NA
Sijmons [14]	Leg	MFH (45, M)	MSH2 loss	*MSH2 (G429X)*
Hirata [15]	Thigh	Liposarcoma (40, M)	MSH2 loss	*MSH2 (c.677delAT)*
Nilbert [16]	Leg	Sarcoma NOS (27, F)	NA	*MSH2 (c.145-148delGACG)*
	Thigh	Liposarcoma (38, M)	MSH2/6 loss	*MSH2 (c.942+3A>T)*
	Shoulder	Liposarcoma (55, M)	MSH2/6 loss	*MSH2 c.1-?_366+?del*
	Foot	Chondrosarcoma (28, M)	Retained	*MLH1 (c.1204A>T)*
	Leg	Osteosarcoma (15, F)	NA	*MLH1 (c.1276C>T)*
	Thigh	Liposarcoma (66, F)	NA	*MLH1 (c.1,732+?_c.2268del)*
Brieger [17]	Psoas	MFH (43, M)	MSH2 loss	*MSH2 (c.2,038 C>T)*
	Leg	MFH (50, M)	MSH2 loss	*MSH2 (c.942+3 A>T)*
Present case	Shoulder	Leyomiosarcoma (43, M)	MSH2–MSH6 loss	*MSH2 (del.1-16)*

IHC immunohistochemistry for MMR proteins, *MMR* DNA mismatch repair, *NA* not available, *F* female, *M* male, *MFH* malignant fibrous histiocytoma

examples of rare tumours which have appeared in HNPCC kindred such as male breast carcinoma [21], adrenal cortical carcinoma, anaplastic carcinoma of the thyroid [22] and soft tissue sarcomas. The case that we described in this paper is the thirteenth reported in Literature regard the association of soft tissue malignancies and HNPCC patients. There are experimental (in mice), epidemiologic, histo-pathological and molecular data for supporting the hypothesis that sarcomas could be a rare manifestation of *MMR* deficiency (Table 2). An experimental study in mice defective in one of the *MMR* genes, *PMS2*, demonstrates an excess of sarcomas and lymphomas in these animals [18]. In humans, sporadic sarcomas usually are not related to *MMR* genes deficiency: MSI and IHC for MLH1 and MLH2 were recently investigated in 40 unselected sarcomas [21]: only 3 (8 %) tumours showed negative IHC for MSH2. Sarcomas are uncommon tumours 20, so it is difficult to have random clustering in a family of sarcomas with other HNPCC related tumours, as could easily happen for cancers highly prevalent in the general population (e.g. female breast cancer or prostate cancer). It is also known that HNPCC related cancers often appear at younger age if compared to their sporadic counterparts. The mean age of HNPCC patients at diagnosis of sarcoma was 38 years while the mean age of sarcoma diagnosis in the general population is 56 years 20. Moreover in the majority of soft tissue sarcoma in HNPCC patients a germline *MSH2* mutation was found (8 of the 13 valuable cases). Nevertheless, considering the few cases reported these observations suggest that in humans the risk of a soft tissue tumour, even if low in HNPCC kindred, is higher in *MSH2*-deficient families.

In the case that we reported, negative IHC staining for MSH2 and MSH6 proteins was found in all three tumours and germline deletion on *MSH2* was detected but sarcoma did not show MSI-H. The coexistence of microsatellites stable and instable cancers in the same HNPCC patient has been reported by other authors [22–25]. There are different possible explanations for this phenomenon: heterogeneity of instable and stable neoplastic cells in the tumour and, during cancer development, a selection of a stable cellular clone [26, 27], as well as tissue specific differences in genomic instability despite identical germ line disorder, since mechanisms of MMR deficient carcinogenesis could be tissue specific [27]. Muir-Torre and Turcot syndromes are a good example of the importance to point out to the scientific community the possible association between a rare tumour and other clinical situations. Muir-Torre syndrome is caused by mutation in one of the *MMR* genes and it is characterized by association of sebaceous gland tumours and/or keratoacanthomas and other HNPCC related malignancies. Turcot syndrome is defined by familial clustering of brain tumour and colon tumours: more accurately by association of medulloblastomas and polyposis coli (syndrome related to APC gene deficiency) or glioblastomas and colorectal cancer (syndrome related to *MMR* genes deficiency) [13]. Reports on Muir-Torre and Turcot syndromes, even remaining uncommon, are increased in the last years together with the awareness of the physicians that skin tumours and brain tumours could be associated to colorectal cancer, as expression of the same germline disorder. For these reasons it seems important to advertise medical community that soft tissue sarcomas could be related to *MMR* gene(s) deficiency,

Table 2 Data supporting the hypothesis that sarcomas could be a part of the HNPCC related tumours

Experimental data	Excess of sarcomas in *PMS*2 defective mice [18]
Epidemiologic data	Sarcomas are rare tumours: 3 cases/10^5/year (in USA) [24]
	Mean age at diagnosis of sarcomas in HNPCC families is 38 vs 56 years of general population [24]
Pathologic data	Only 3/40 (8 %) sarcomas had IHC- for MMR proteins [23]
Molecular data	Most HNPCC patients with soft tissue sarcoma are *MSH2* defective

IHC immunohistochemistry, *MMR* DNA mismatch repair, *HNPCC* Hereditary Non-polyposis Colorectal Cancer

particularly to mutations on *hMSH2*. We hope that this report would encourage to review HNPCC family registers looking for sarcomas and to consider the presence of soft tissue tumours during counselling for suspected HNPCC.

Conflict of interest None conflict of interest declared.

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