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Microsatellite instability in a pleomorphic rhabdomyosarcoma in a patient with hereditary non-polyposis colorectal cancer

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Aims: To describe the rare occurrence of a pleomorphic sarcoma with microsatellite instability in a patient with hereditary non-polyposis colorectal cancer (HNPCC).
Methods and results: A soft tissue tumour was removed from the upper leg of a patient who had previously been shown to harbour a germ-line MSH-2 mutation. The tumour was analysed with immunohistochemistry and molecular methods. The morphology and immunohistochemical findings were in keeping with a pleomorphic rhabdomyosarcoma. Microsatellite instability was documented in the tumour with molecular methods and in addition loss of MSH-2 expression in the tumour cells was confirmed by immunohistochemistry.
Conclusions: Although sarcomas do not form part of the HNPCC diagnostic criteria, they may occur in this mismatch repair syndrome and, moreover, may well be caused by the underlying genetic defect.

Keywords: sarcoma, HNPCC, molecular biology, MSI

Abbreviations: HNPCC, hereditary non-polyposis colorectal cancer; MSI, microsatellite instability

Introduction

Hereditary non-polyposis colorectal cancer (HNPCC) is a cancer syndrome in which the underlying genetic defect involves genes functioning in DNA mismatch repair.[1] At the molecular level loss of function of these genes is manifested in the failure to replicate accurately DNA repeat sequences resulting in microsatellite instability (MSI). Several mismatch repair genes are known and mutations in these genes lead to the HNPCC phenotype characterized by the development of colorectal cancer at a young age occurring without the background of multiple polyps as seen in the familial polyposis syndrome.[2] In the HNPCC syndrome cancer may also develop at other sites, including the stomach, endometrium and small bowel.[3,4] The criteria for establishing the diagnosis have been formalized and are referred to as the Amsterdam criteria.[5] Soft tissue sarcomas are not a feature of HNPCC and when these occur in HNPCC patients it has been assumed that these are sporadic rather than part of the syndrome. Conversely, MSI is not a common finding in sarcomas and mutations in mismatch repair genes are rarely described. In this report we describe a HNPCC patient who developed a sarcoma with MSI and in whom loss of the repair gene MSH-2 was documented.

Case report

A 23-year-old female underwent a pancreaticoduodenectomy (Whipple) operation for a duodenal adenocarcinoma. The diagnosis of HNPCC syndrome was suspected due to the early presentation and a family history of large bowel carcinoma. Genetic analysis had revealed a germ-line mutation in the MSH-2 mismatch repair gene in this patient. Eleven years later a fast-growing soft

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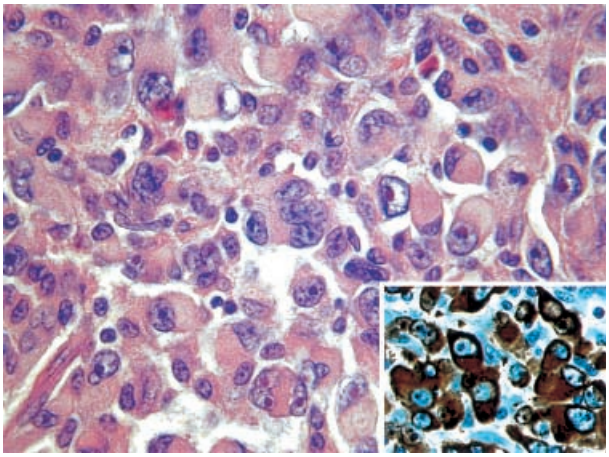

Figure 1. H&E-stained section of the excised tumour. Pleomorphic cells with eccentric nuclei set in copious amounts of eosinophilic cytoplasm imparting a rhabdoid appearance. Inset: strong cytoplasmic desmin positivity in immunohistochemistry.

tissue mass developed in the left upper leg. A diagnostic biopsy preceded excision of the tumour.

Tissue from the resection specimen was submitted for routine histological evaluation. Immunohistochemistry was performed on selected blocks using a standardized procedure (Dako Envision; Dako, Glostrup, Denmark) and commercially available antibodies, including desmin (clone D33; Dako), Myf-4 (clone LO26; Novocastra Laboratories, Newcastle-upon-Tyne, UK) and MSH-2 (clone G219-1129; PharMingen, BD Biosciences, San Diego, CA, USA). For molecular studies DNA was extracted from paraffin-embedded tumour tissue and analysed by radioactive polymerase chain reaction (PCR) with six specific MSI markers Bat25, Bat26, Bat40, D5S346, D2S123 and MSH6.[6,7]

The resection specimen consisted of muscle tissue with a tumour with a maximum dimension of 120 mm. Histology showed a tumour composed of pleomorphic rhabdoid cells which immunohistochemically stained strongly for desmin and with variable nuclear positivity for Myf-4 (Figure 1). The findings were deemed to be consistent with pleomorphic rhabdomyosarcoma. Molecular PCR analysis with markers Bat25 and D5S346 clearly showed an MSI pattern (Figure 2). Immunohistochemical staining with MSH-2 antisera showed nuclear staining in endothelial and stromal cells whilst the nuclei of the tumour cells remained unstained (Figure 3).

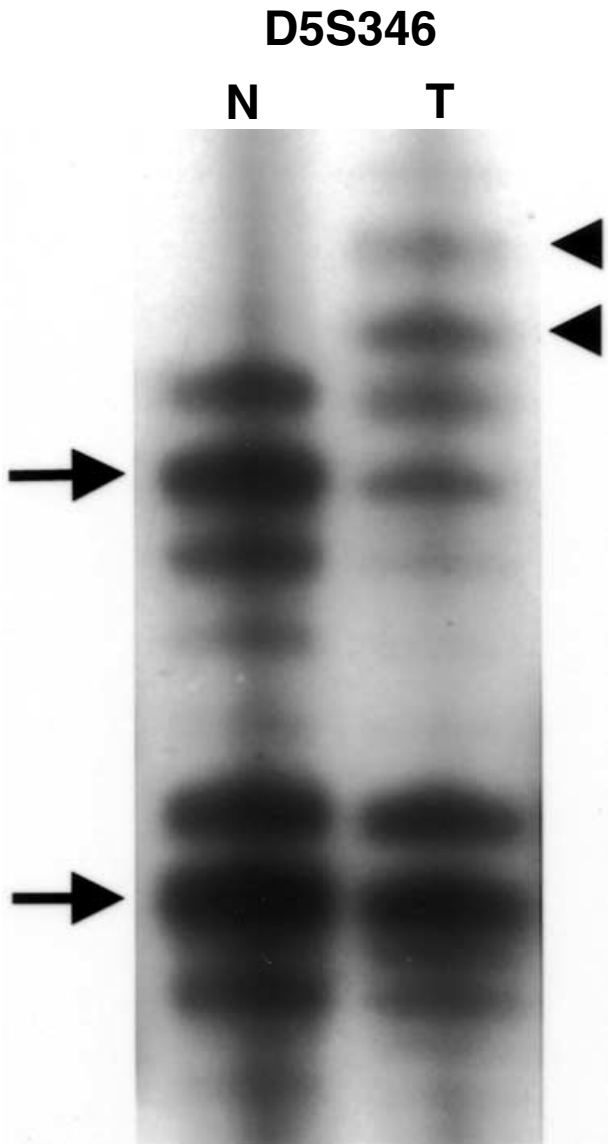

Figure 2. Microsatellite instability analysis with marker D5S346. N, Normal patient DNA; T, tumour DNA. The arrows point to alleles, arrowheads indicate unstable fragments in the tumour DNA.

Discussion

HNPCC is caused by inactivating mutations in DNA repair genes. These genes maintain the integrity of the genetic material by proof-reading and repair in DNA replication and are also known as mismatch repair genes. Loss of function of these genes results in an unstable genome in which aberrations are likely to occur. Because loss of function of mismatch repair genes may secondarily affect many other genes, individuals with this stigma are said to express a mutator phenotype. The functional deficit of these genes is exemplified by the inability accurately to restore DNA synthesis mistakes, resulting in DNA repeat sequence alterations which are the telltale sign of MSI.

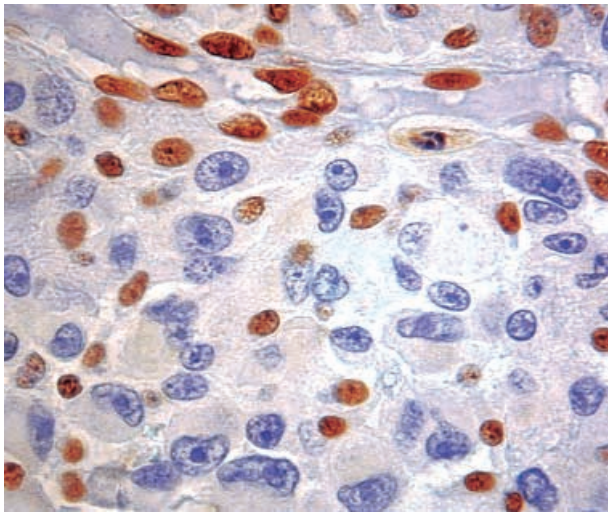

Figure 3. MSH-2 immunostaining. Nuclear staining is seen in endothelial cells, the tumour nuclei do not stain.

In the patient we describe here a germ-line mutation of the mismatch repair gene MSH-2 was established following the diagnosis of duodenal cancer at an early age. Eleven years later a pleomorphic rhabdomyosarcoma was excised from the left upper leg, followed by dose-intensive chemotherapy. In the tumour MSI and loss of MSH-2 expression were observed.

Carcinomas, in particular of the digestive tract, the endometrium and renal pelvis occur in HNPCC, but sarcomas are not a feature of HNPCC. Therefore the observation of MSI coupled with loss of a mismatch repair gene in the sarcoma is highly unusual. It is reasonable to assume that the sarcoma arose because of mutations in target genes caused by replication errors induced by loss of the remaining MSH-2 allele in a mesenchymal stem cell. MSI is uncommon in soft tissue sarcomas.[8–10] Specifically, MSI was not observed in five primary adult rhabdomyosarcomas analysed by Suwa *et al*. In paediatric rhabdomyosarcomas MSI has been shown to occur, appears to be locus specific and is probably a late event.[11] Interestingly, a single case report describes the occurrence of a malignant fibrous histiocytoma (MFH) in a HNPCC patient with a MSH-2 mutation.[12] Because MSI was not observed in additional sporadic MFHs the authors suggest that MFH may form part of the HNPCC spectrum. We conclude that sarcomas may occasionally present as part of the HNPCC phenotype.

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