Human Pathology (2006) 37, 361–368

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Contribution of microdissection for the detection of microsatellite instability in colorectal cancer☆

Marie Danjoux[a,b], Rosine Guimbaud[a,e], Talal Al Saati[c], Fabienne Meggetto[a], Nicolas Carrère[d], Guillaume Portier[d], Georges Delsol[a], Janick Selves[a,b,*]

[a]*INSERM U563, Centre de Physiopathologie de Toulouse Purpan, CHU Purpan, 31059 Toulouse, France*
[b]*Service d'Anatomie et de Cytologie Pathologiques, CHU Purpan, 31059 Toulouse, France*
[c]*Plate-forme d'histopathologie expérimentale, IFR30, CHU Purpan, 31059 Toulouse, France*
[d]*Services de chirurgie digestive, CHU Purpan, 31059 Toulouse, France*
[e]*Département d'oncologie médicale, Centre Claudius Regaud, 20-24, rue du pont St Pierre, 31052 Toulouse, France*

Received 1 January 2005; Accepted 1 June 2005

Keywords:
Colorectal cancer;
Microsatellite instability;
Genetic heterogeneity;
Microdissection;
Loss of heterozygosity

Summary The determination of microsatellite instability (MSI) is an important step in the identification of familial colorectal cancer such as hereditary nonpolyposis colon cancer. It could also be of interest in the therapeutic management of sporadic cancer. International criteria for the determination of MSI have been published, recommending the use of microdissection. The aim of this work was to evaluate the impact of contaminant normal DNA in tumor samples for MSI assessment in colorectal cancer using a microdissection technique. We performed a comparative analysis of the microsatellite status between total DNA (DNA extracted from whole tumor samples) and microdissected DNA in 3 different regions from 23 cases of colorectal cancer. Six microsatellites were amplified using fluorescent polymerase chain reaction. We analyzed 9 cases with MSI and 14 cases without instability, with similar results between total DNA and microdissected DNA. Moreover, within a same tumor, the MSI phenotype was observed regardless of the region analyzed. Thus, this work shows the reproducibility of the MSI phenotype throughout a tumor. However, we observed a regional heterogeneity of the MSI profile, consisting of variations in the number and the size of unstable alleles within different regions. This result reflects the genetic heterogeneity of colorectal cancer with MSI. In the 14 cases without instability, we observed an increase of more than 60% in the loss of heterozygosity detection rate after microdissection. Thus, this work confirms the contribution of microdissection for loss of heterozygosity assessment.

1. Introduction

Two main genetic pathways leading to colorectal carcinoma can be distinguished [1]. The most common pathway is characterized by the sequential inactivation of a series of tumor suppressor genes, such as APC, p53, and DCC. These tumors present with chromosomal instability

☆ This work was supported by a grant from the "Projet Hospitalier de Recherche Clinique" (no. 00-08) and the "Ligue contre le cancer." We acknowledge the "Délégation Régionale à la Recherche Clinique des Hopitaux de Toulouse" (AOL no. 98-36L) for its financial support.
* Corresponding author. Laboratoire D'Anatomie Pathologique, CHU-Purpan Place du Docteur Baylac, 31059 Toulouse Cedex, France.
E-mail address: selves.j@chu-toulouse.fr (J. Selves).

0046-8177/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2005.06.022

with frequent allelic losses. The second genetic pathway is involved in the development of tumors in patients with hereditary non polyposis colon cancer (HNPCC) and in approximately 15% of sporadic colorectal cancers [2,3]. These tumors are much more susceptible to accumulate mutations. This phenomenon has been described as a mutator phenotype. This phenotype is particularly apparent in the microsatellite sequences and is termed microsatellite instability (MSI). Microsatellite instability is a consequence of the inactivation of the DNA mismatch repair system genes required for the correction of DNA mismatches that occur during replication [4-6].

In HNPCC, MSI is associated with germline mutations of DNA mismatch repair genes, mainly *hMLH1* and *hMSH2*. Conversely, DNA mismatch repair gene mutations are rare in sporadic colorectal carcinoma, and MSI is caused mainly by promotor hypermethylation of the *hMLH1* gene [7,8]. Several studies suggest that patients with MSI colorectal carcinomas have a better overall survival rate than patients with microsatellite stable (MSS) tumors and may have a different sensitivity to chemotherapeutic drugs [9-12]. The search for MSI is important to identify patients with HNPCC for genetic counseling and prevention. In addition, it may become equally important to recognize sporadic MSI tumors for therapeutic management.

Microsatellite instability detection using a panel of microsatellite markers is the gold standard method to establish a mutator phenotype. This method requires the comparative analysis of microsatellite sequences between tumor and normal DNA. Usually, a large sample from the resected tumor is analyzed. Despite careful macroscopic sampling protocols, tumoral tissue is always contaminated with normal cells from the stroma. Analysis of tumor-specific genomic alterations could be compromised by the presence of normal cells. Microdissection, controlled by microscopic examination, allows for the selective sampling of tumor cells. Moreover, recent studies suggest a molecular heterogeneity in tumors that could modify MSI detection [13-15]. Thus, microdissection seems to be the choice method for testing variations in genomic alterations within a tumor.

The aim of this study was to evaluate by microdissection the impact of normal cells and of tumoral heterogeneity in diagnosis of MSI. We compared the detection of MSI in DNA extracted from large tumor fragments with DNA extracted from microdissected tumor cells. In each MSI-positive tumor, different regions were microdissected to investigate the heterogeneity of MSI. The results of microsatellite analysis were also compared with the expression of hMLH1 and hMSH2 performed by immunohistochemistry.

Table 1 Comparative analysis of microsatellite status between total DNA and microdissected DNA

Case	BAT 26 total/ microdissected DNA	BAT 25 total/ microdissected DNA	BAT 40 total/ microdissected DNA	D2S123 total/ microdissected DNA	D17S250 total/ microdissected DNA	D18S58 total/ microdissected DNA	MSI total/ microdissected DNA	Tumor cells[a] (%)
19	S/S	S/S	S/S	S/S	S/S	S/S	–/–	75
34	S/S	S/S	S/S	S/S	S/S	S/S	–/–	90
39	S/S	S/S	S/S	S/S	S/S	S/S	–/–	70
50	S/S	S/S	S/S	S/S	S/S	S/S	–/–	40
51	S/S	S/S	S/S	S/S	S/S	S/S	–/–	65
55	S/S	S/S	S/S	S/S	S/S	S/S	–/–	40
58	S/S	S/S	S/S	S/S	S/S	S/S	–/–	75
59	S/S	S/S	S/S	S/S	S/S	S/S	–/–	90
66	S/S	S/S	S/S	S/S	S/S	S/S	–/–	85
72	S/S	S/S	S/S	S/S	S/S	S/S	–/–	75
73	S/S	S/S	S/S	S/S	S/S	S/S	–/–	90
75	S/S	S/S	S/S	S/S	S/S	S/S	–/–	65
84	S/S	S/S	S/S	S/S	S/S	S/S	–/–	45
90	S/S	S/S	S/S	S/S	S/S	S/S	–/–	20
57	U/U	U/U	U/U	U/U	U/U	U/U	+/+	85
65	U/U	U/U	U/U	U/U	U/U	U/U	+/+	85
103	U/U	U/U	U/U	U/U	U/U	U/U	+/+	40
60	U/U	U/U	U/U	U/U	U/U	U/U	+/+	60
64	U/U	U/U	U/U	U/U	U/U	U/U	+/+	80
109	U/U	U/U	U/U	U/U	U/U	U/U	+/+	100
122	U/U	U/U	U/U	U/U	U/U	U/U	+/+	80
130	U/U	U/U	U/U	U/U	U/U	U/U	+/+	75
132	U/U	U/U	U/U	U/U	U/U	U/U	+/+	80

S, stable microsatellite; U, unstable microsatellite.

[a] Percentage of tumor cells in the sample for total DNA extraction as estimated on microscopic examination.