Clinically localised prostate cancer is microsatellite stable

Abdel-Rahmene Azzouzi*†, James W.F. Catto‡, Ishtiaq Rehman†, Stephane Larret†, Morgan Roupret†, Kenneth M. Feeley§, Oliver Cussenot†¶, Mark Meuth° and Freddie C. Hamdy†

*Service d’Urologie, CHU d’Angers, †Centre de Recherche pour les Pathologies Prostatiques, Université Paris VII, Paris, France, ‡The Academic Urology Unit, University of Sheffield, §Department of Pathology, Royal Hallamshire Hospital, Sheffield, UK, ¶Service d’Urologie, Hôpital Tenon, GHU Est, France and °The Institute for Cancer Studies, University of Sheffield, UK

Accepted for publication 12 October 2006

OBJECTIVES
To determine the frequency of microsatellite instability (MSI) change with mono-, di- and tetranucleotide markers in clinically localized prostate cancer, and to correlate those markers with clinical and pathological variables.

MATERIALS AND METHODS
Two forms of MSI have been described in human cancer: MSI typical of hereditary nonpolyposis colon cancer, defined with mono- and dinucleotide repeat MS; and a second variety of MSI is best seen at selective tetranucleotide repeats, i.e. elevated microsatellite alterations at select tetranucleotides (EMAST). Prostate specimens were taken from 50 patients. The MS analysis used the Bethesda consensus panel (BCP) and four tetranucleotide loci shown to detect the presence of EMAST.

RESULTS
All but four tumours were stable for the 14 loci investigated. There were two (4%) cases with adenomatous polyposis coli (APC) instability among the BCP markers and the same instability rate (4%) amongst the EMAST markers. These four tumours were all unstable at one locus of the 10 markers of the BCP that classified them as MS stable.

CONCLUSIONS
The MSI related to a mismatch repair deficiency or to the EMAST does not seem to be important in prostate cancer in the early stages of the disease.

KEYWORDS
microsatellite instability, mismatch repair system, EMAST, prostate cancer

INTRODUCTION
Prostate cancer is a major burden in the industrialized Western world; it is the most prevalent cancer in males and the second leading cause of cancer deaths [1]. Molecular genetic mechanisms involved in the progression of prostate cancer are not well understood, due to extensive tumour heterogeneity and a lack of suitable models. It is suggested that increased genomic instability is associated with decreased androgen responsive and progressive behaviour of human prostate tumours. Genetic instability is important in human carcinogenesis and has traditionally been classified into two categories, according to genomic targets [2]. Chromosomal instability is characterized by frequent chromosomal losses and gains, but to date the cause is unknown. Microsatellite instability (MSI) is characterized by alterations at the individual DNA nucleotide level, and is best seen in the MS regions of DNA. These are ubiquitous repetitive mono-, di-, tri-, tetra- and pentanucleotide repeat sequences, found in both exonic and intronic DNA. Whilst their function is unknown, they are useful markers of genetic instability and for linkage analyses. MSI, as seen in hereditary nonpolyposis colon cancer (HNPCC) is caused by defective DNA mismatch repair (MMR). Loss of MMR can occur by mutation of one of the central genes (either hMLH1 or hMSH2 in HNPCC) or methylation of the promoter (of hMLH1 only, in most sporadic cancers with MSI) [3-5]. MSI, due to deficient MMR, is usually defined with mono- and dinucleotide markers. There are different definitions of MSI at mono- and dinucleotide markers. Honchel et al. [6] classified a tumour as MSI when ≥30% of the loci investigated were unstable, whereas others recommended the use of a panel of 10 MS markers and instability in ≥40% of MS loci was taken to indicate MSI [7]. In addition to those different definitions of MSI, the National Cancer Institute workshop on MSI for cancer-detection recommendations seems to be the most appropriate approach to study the field. They characterized MSI as:

• High-frequency MSI (MSI-H), if two or more of a panel of five markers show instability
• Low-frequency MSI (MSI-L), if only one of the five markers shows instability
• Distinct from MS stable (MSS), none or one of 10 markers shows instability, and MSI-L needs a panel of 10 markers [8].

More recently, a novel form of MSI was described that is best seen in selective tetranucleotide repeats; this was termed EMAST, for ‘elevated MS instability at selected tetranucleotide repeats’ [8] and appears to be distinct from the MSI seen in HNPCC and related tumours [9]. Although the underlying mechanism causing EMAST is unclear, an association with p53 mutations was reported [10,11] and exposure to environmental carcinogens can induce EMAST [12].

Our objectives were to determine the incidence of MSI at mono- and dinucleotide and EMAST markers in clinically localized prostate cancer, and to correlate those markers with clinical and pathological variables.
PATIENTS AND METHODS

Prostate specimens were taken from 50 patients who had radical surgery (radical prostatectomy in 43 and incidental prostate cancer from radical cystoprostatectomy in seven) between March 1996 and March 2002 at the Royal Hallamshire Hospital (Sheffield, UK). The patient age, PSA level, pathological stage (pT), pathological Gleason score, and follow-up and recurrence data, were recorded. Ethics committee approval was granted, and informed consent was obtained from all patients before starting the study.

From each prostate specimen, six slides of 10-µm formalin-fixed, paraffin wax-embedded sections were microdissected to obtain cancerous (>80% tumour) and normal tissue, which was distant from the malignancy. DNA was extracted from the microdissected specimens using the QIAamp® kit (Qiagen, UK) according to the manufacturer’s guidelines. MS was analysed for each prostate cancer using paired normal and tumour DNA. The MSs studied were composed of the standardized Bethesda consensus panel (BCP) consisting of BAT25, BAT26, MFD15 (D17S250), D2S123, APC (D5S346), BAT40, D10S197, MYC1L, D18S58, D18S69 [9]; and four tetranucleotide loci known to detect the presence of EMAST (ACTBP2, CSF1R, D20S82, D11S488) [9,11].

The PCR set up was automated using the RoboAmp® 4200 PE (MWG Biotech, UK). Briefly, PCR using fluorescence-labelled primers (MWG Biotech) was performed for each sample at each locus. The primer sequences were published previously [7,11]. PCR was done in a 12-µL volume reaction, composed of 1 pmol fluorescence-labelled forward and unlabelled reverse primers, 50 ng of DNA template and a pre-made PCR mastermix solution of Taq DNA polymerase, dNTPs, 1.5 mM MgCl₂ and buffers (Abgene, Surrey, UK). For each MS locus a standard reaction was used with 40 cycles of amplification using a ‘Priamus 96’ thermal cycler (MWG Biotech). Each cycle consisted of denaturation (95 °C for 30 s), annealing and extension (72 °C for 30 s). A final extension at 72 °C was done for 5 min. Annealing temperatures varied for each MS locus and are listed elsewhere [13]. The PCR product was analysed on a LICOR automated sequencer (MWG Biotech). The presence of extra or shifted bands in tumours when compared to their normal counterparts was scored as MSI.

For each case of MSI, the PCR reaction was independently repeated to confirm the result. All loci were studied in each tumour. When no product was detected after PCR, the reaction was repeated twice to confirm that no amplification was possible.

The chi-square test was used to examine relationships between the clinicopathological and MS data; P < 0.05 was considered to indicate statistical significance.

RESULTS

From the MS analysis, all but four tumours were MSS for the 14 loci investigated. All four MSI-positive tumours were from patients who had had a radical prostatectomy. There were two (4%) cases with APC instability among the BCP markers and the same instability rate (4%) amongst the EMAST markers (D11S488 and D20S82) (Fig. 1). These four tumours were all unstable at one locus of the 10 markers of the BCP, which classified them as MSS whatever definition was used.

The median (range) age, PSA level and Gleason score at diagnosis were, respectively, 62.3 (36–80.8) years, 9.25 (1.5–24.1) ng/mL and 6 (4–8). Of the 50 tumours 23 (46%) were pT2 and 27 were pT3 (54%). The median follow-up was 41.2 (1–84) months. The biochemical recurrence rate was 25.6%. There was no association between the four MSI-unstable tumours and any clinical or pathological data, including the recurrence status (Table 1).

DISCUSSION

According to the definitions of MSI by previous authors and the Bethesda Consensus Workshop, most of the population in the present study was MSS. Interestingly, the rate...
of instability at mono- and dinucleotide loci (BCP) was equal to that seen at
tetranucleotide (EMAST) loci (4%).

Initially, we planned to use
immunohistochemistry to evaluate the
hMLH1 and hMSH2 protein expression status
on the prostate cancer slides. However, as the
results clearly showed that clinically localized
prostate cancer tumours were all MSS, the
immunohistochemistry became irrelevant.

MSI at mono- and dinucleotide markers was
identified with a frequency of 2.5–65%
in human prostate cancer [14–21]. The
discrepancy in frequencies of MSI in prostate
cancer found by different investigators could
be explained by the use of different MS loci
markers as well as different definitions of the
MSI. The choice of these particular mono- and
dinucleotide markers was based on the
international criteria for determining MSI in
collorectal cancer [8]. Also, the preparation of
the cancer tissue, which could be a possible
cause of differences in MSI incidences among
different studies, should be considered. In
the present study we retained the Bethesda
definition, although the results would be
identical with the Honchel

defined previously [14–21] gives variable results
(Table 2).

Gao et al. [21] published the first report
investigating MSI in prostate cancer, and
found the highest MSI frequency (65%)
published to date. It appears that 44% of
these tumours showed MSI at multiple loci,
although a correlation with the BCP is
difficult. Terell et al. [14] reported a low
instability rate (2.5%) very close to the
present rate (4%). All MS changes were seen
solely in those non-dinucleotide markers that
reduce the instability rate to zero if we only
consider the instability linked to MMR
deficiency.

Crundwell et al. [15] found an instability rate
of 19% in 72 patients with prostate cancer,
using a panel of 21 markers. None of the
internationally recognized definitions showed
an instability rate of >9.5% for that study. Egawa et al. [16] screened 66 patients with
prostate cancer for somatic instability, and
classed 19.7% of the tumours as unstable. In
eight cases, genetic instability could be
detected in at least two MS (12.1% MSI-H
rate according to the BCP definition). Uchida et al. [17] assessed 24 DNA samples from
primary prostate cancer, and detected genetic
instability in nine of them (37.5%); five were
MSI-H [21]. Watanabe et al. [18] found 43% MSI in 21 prostate cancers analysed by 36 MS
markers, 34 of which were dinucleotide loci. In
three tumours there was instability at more
than one locus. Thus the MSI-H rate could be
considered as high as 14.3% according to the
Bethesda definition, but is zero by the
other definitions; notably, the tri- and
tetranucleotide marker instability rate was
zero in that study.

Cunningham et al. [19] analysed 55 prostate
cancers using 135 polymorphic MS markers;
18 tumours had alterations at one or two loci
of the 135. Of the 6803 genotypes, MSI was
detected only at 22. These tumours are all
MSS by any definition. Eventually, Dahiya et al. [20] investigated the genomic instability
associated with prostate cancer using 36 MS
markers. Their results suggest that 45% (18 of
40) of the tumours had genomic instability.
Again, the instability rate was <15% by any of
the internationally recognized definitions.
Overall, in these previous studies, the original
results of MSI fluctuated between 2.5% and
65%. However, applying strict recognized
definitions reduces most of the results to
≈10%, except for the study of Uchida et al.
[17], at 20.8%.

Recently, Sun et al. [22] evaluated the
presence of MSI in six cell lines and 22
xenografts from either high-grade primary

tumours or metastases of prostate cancer.
They found a 14% incidence of MSI in the
xenografts, which suggested a similar
frequency of MSI to that in advanced human
prostate cancer. Therefore, the frequency of
MSI should be even lower than 14% in

localized prostate cancers, because most of
such tumours are mid- to low-grade, and
genetic alterations are much less frequent in

\[
\begin{array}{cccc}
\text{TABLE 1} & \text{The clinical and pathological data of patients with an unstable MS locus} \\
\hline
\text{Patient no.} & \text{Age at diagnosis, years} & \text{Pathological stage} & \text{Gleason score} & \text{Recurrence status} \\
41 & 62.8 & 3b & 3 + 3 & 1^* \\
42 & 63.1 & 2c & 3 + 3 & 0† \\
44 & 53.9 & 3b & 2 + 3 & 0 \\
50 & 57.6 & 3b & 3 + 3 & 1 \\
P & 0.42 & 0.35 & 0.45 & 0.28 \\
\hline
\end{array}
\]

\*1 = recurrence, †0 = no recurrence.

\[
\begin{array}{cccc}
\text{TABLE 2} & \text{Comparison of instability rates according to different definitions of MSI. Each study has been reassessed according strict recognized definitions of MSI.} \\
\hline
\text{Reference} & \text{Original results} & \text{Definitions} & \text{Number of loci studied} \\
& \text{No. of patients} & \text{Instability rates, %} & \text{Bethesda MSI-H, %} & \text{Honchel et al. [6]} & \text{Dietsmaier et al. [7]} & \\
\hline
Terell et al. [14] & 40 & 2.5 & 0 & 0 & 0 & 12 \\
Crundwell et al. [15] & 72 & 19 & 9.5 & 0 & 0 & 21 \\
Egawa et al. [16] & 66 & 19.7 & \leq 12.1 & \leq 12.1 & \leq 12.1 & 8 \\
Uchida et al. [17] & 24 & 37.5 & 20.8 & 20.8 & 20.8 & 9 \\
Watanabe et al. [18] & 21 & 43 & \leq 14.3 & 0 & 0 & 36 \\
Cunningham et al. [19] & 55 & Very low & 0 & 0 & 0 & – \\
Dahiya et al. [20] & 40 & 45 & 15 & 2.5 & 0 & 36 \\
Gao et al. [21] & 57 & 65 & 11–44 & \leq 11 & \leq 11 & 16 \\
\hline
\end{array}
\]
mid- or low-grade tumours. The conclusion of Sun *et al.* is in agreement with the present results.

First reported in HNPCC, the MSI-H phenotype is associated with loss of MMR [5], probably as a result of an exogenous mutagenic agent. In HNPCC it is the mutation of the remaining MMR gene allele that results in defective MMR. In sporadic MSI-H tumours, it is the mutation or methylation of the MMR genes that is responsible for MMR loss. Whilst the cause of abnormal regional hyper- and hypomethylation in cancer is unknown, its anatomical specificity suggests that an exogenous agent is the cause. Loss of MMR results in the mutator phenotype, which allows the malignant cell to accumulate replication errors at a high frequency. These mutations cause most disruption to the cell, and are seen most easily in tumours with a high mitotic activity. Prostate cancer is typically slow growing, and therefore does not appear to be a good candidate for MSI. Two criteria seem to be needed for a tumour to become MSI-H; exposure to carcinogens and a high mitotic index of the tissue exposed. Intestinal, respiratory and urinary tracts and skin match these two criteria, and therefore the MSI-H phenotype is common for tumours arising from these organs. Conversely, tissues poorly or unexposed to carcinogens with low (prostate) or absent (brain) mitotic index rarely have a MSI-H phenotype [14,18,23]. Even gliomas, which are associated with HNPCC in Turcot’s syndrome, have a very low instability rate (<4%) [24]. The liver is even more interesting, as it is highly exposed to carcinogens but has a very low mitotic index. Some studies suggest that MSI is a rare event during hepatocarcinogenesis [25]. Another interesting comparison is with breast cancer, often mentioned as the ‘female prostate cancer’. In agreement with the present results, reports show a low frequency of MSI-H in breast cancer [26]. For prostate cancer, as for breast cancer, a low level of MSI-H is expected, as the natural history suggests the cause is likely to be hormone-related, rather than related to exogenous exposure to carcinogens.

In a recent report by Burger *et al.* [27], HNPCC-type MSI, detected by the National Cancer Institute consensus panel, appeared to be rare in prostate cancer (7.6%). Also, for the first time those authors investigated the role of EMAST in prostate carcinoma and found that only 5% of all cases showed EMAST. This is the lowest frequency of EMAST detected in several cancer types published, and indicates a minor role of this specific form of MSI in prostate carcinogenesis. Both HNPCC type MSI and EMAST results of Burger *et al.* [27] are in agreement with the present findings. In two previous studies, the prevalence of tetranucleotide instability was, respectively, zero and 2.5% [14,17], similar to the present results. Conversely, Perinchery *et al.* [28] found that 25% (10 of 40) of tumours had tetranucleotide instability, although only one marker of five was unstable in each tumour. Whether or not these tumours could be classified as EMAST(+) is unclear.

In the present study we assessed only clinically localized prostate cancer. The frequency of *p53* mutations is reported to be ~5% in organ-confined prostate cancer [29]. These results are in accordance with the 4% of EMAST(+) found in the present study. Indeed, Ahrendt *et al.* [30] suggested that a non-MMR pathway might be involved in the mechanism underlying MS alterations in EMAST, which is probably related to abrogation of a p-53-dependent repair pathway.

In conclusion, consistent published reports, including the present study, suggest that MSI-H is rare in clinically localized prostate cancer. This is supported as follows: (i) most studies of MSI in prostate cancer show <10% of MSI-H tumours when re-assessed using strictly recognized definitions; (ii) unlike cancers with frequent MSI-H phenotype, the prostate is neither exposed to carcinogens nor has a high mitotic index tumour; (iii) in breast cancer, which in many aspects is similar to prostate cancer, MSI-H is uncommon; (iv) *p53* mutations, which might be associated with EMAST, are rare in clinically localized prostate cancer; (v) a higher incidence of prostate cancer is not seen in HNPCC kindreds. In addition, as confirmed by many studies, there is no correlation between the MSI rate and stage, histopathological characteristics or recurrence rate of prostate cancer and therefore identifying MSI seems to have no clinical significance as a biomarker for prostate cancer, at least at an early stage.

CONFLICT OF INTEREST

None declared.

REFERENCES

11 Xu L, Chow J, Bonacum J *et al.* Microsatellite instability at AAAG repeat sequences in respiratory tract cancers. *Int J Cancer* 2001; 91: 200–4
Catto JW, Xinarianos G, Burton JL, Meuth M, Hamdy FC. Differential expression of hMLH1 and hMSH2 is related to bladder cancer grade, stage and prognosis but not microsatellite instability. *Int J Cancer* 2003; **105**: 484–90


Correspondence: Abdel-Rahmene Azzouzi, Service d’Urologie, CHU d’Angers, 4, rue Larrey, 49933 Angers, France.

Abbreviations: MS(S)(I); microsatellite, (stable) (instability); EMAST, elevated microsatellite alterations at select tetranucleotides; HNPCC, hereditary nonpolyposis colon cancer; MMR, DNA mismatch repair; BCP, Bethesda Consensus Panel; MSI-H, high-frequency MSI; MSI-L, low-frequency MSI.