Neuroendocrinology Letters Volume 36 No. 5 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Microsatellite instability analysis in pituitary adenomas

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Submitted: 2015-03-09 Accepted: 2015-06-08 Published online: 2015-11-29

Key words: **pituitary adenoma; nonfuctioning pituitary adenoma; microsatellite instability; MSI; MIN; mismatch repair**

Neuroendocrinol Lett 2015; **36**(5):511–514 **PMID:** 26707053 NEL360515A16

Abstract

OBJECTIVE: Mutator phenotypes with microsatellite instability (MSI) are observed in a subset of solid tumors including those localized in the brain. MSI arises from impaired DNA mismatch repair. It can be a potential marker of resistance to radiation and chemotherapy, as demonstrated for several cancer types. Our study aims are to investigate MSI incidence in pituitary adenomas (PA) with a currently recommended methodology.

METHODS: DNA was obtained from 107 patients with PA of which 83 adenomas were nonfunctioning, 13 somatotrophic, 9 lactotrophic and 2 corticotrophic. These were examined for MSI status by PCR and capillary electrophoresis using five quasimonomorphic microsatellite markers: BAT25, BAT26, NR21, NR24 and NR27; in accordance to current Bethesda guidelines.

RESULTS AND CONCLUSION: No microsatellite instability was detected in the tumor samples thus implying the lack of any clinical usefulness of MSI testing in PA cases.

Abbreviations:

MSI	- microsatellite instability
MIN	- microsatellite instability
MSS	- microsatellite-stable
PA	- pituitary adenoma
NFPA	- nonfunctioning pituitary adenoma
MMR	- mismatch repair
PCR	- polymerase chain reaction
MLH1	- mutL homolog 1
MSH2	- mutS homolog 2
MSH6	- mutS homolog 6

ORIGINAL ARTICLE

INTRODUCTION

Microsatellite repeated sequences are short 2–6 nucleotide DNA motifs scattered in different loci of the human genome. This repetitive character makes them prone to replication errors caused by slippages of the DNA polymerase enzyme. Such small replication errors arise almost ubiquitously in the replication process and, in normal cells, are immediately corrected by the mismatch repair system (MMR). Genetic or epigenetic defects in genes encoding crucial MMR protein components: *MLH1*, *MSH2* and *MSH6* lead to mutations arising within microsatellites. Accumulation of these small genetic changes, referred to as microsatellite instability (MSI, also known as MIN) may be involved in the carcinogenesis process (Umar *et al.* 2004).

Microsatellite instability phenotype has been observed in a subset of solid tumors, including colorectal cancer, where its clinical significance is well-documented (De la Chapelle & Hampel 2010). MSI has been also detected in some patients with brain tumors at a variable frequency (Alvino *et al.* 2000; Eckert *et al.* 2007; Rodríguez-Hernández *et al.* 2013; Viana-Pereira *et al.* 2011; Zhu *et al.* 1996).

Mismatch repair abnormalities not only lead to mutations accumulating that cause mutator phenotypes of tumors, but may also be involved in resistance to radiation (Martin *et al.* 2011) and some cytostatic agents (Claij & Riele 1999). This has been clearly shown in different cancer cell lines and to some extent in cancer patients. A prognostic role of MSI was found in colorectal cancer patients treated with fluorouracil (Jover *et al.* 2009) and in endometrial cancer patients undergoing radiotherapy (Bilbao *et al.* 2010). Its predictive role in glioblastomas, where MSI is infrequent, however remains elusive (Maxwell *et al.* 2008; Pollack *et al.* 2010), despite the very promising *in vitro* results (Zhang *et al.* 2013). It rather seems that MMR defects are involved in acquired temozolomide resistance in gliomas.(Felsberg *et al.* 2011; Nguyen *et al.* 2014; Shinsato *et al.* 2013). This phenomenon was also observed in atypical pituitary adenomas and carcinomas treated with temozolomide (Matsuno *et al.* 2014; Zacharia *et al.* 2014).

Pituitary adenomas (PAs) represent about 15% of intracranial tumors. Based on their secretory activity PAs are classified as endocrinologically inactive – nonfunctioning adenomas (NFPAs) representing about 30% of PAs and secreting tumors. The latter category involves: prolactinomas (50% of PA) somatotroph (15–20%), corticotroph (5–10%) and rare thyrotroph adenomas representing less than 1% of pituitary tumors (Dworakowska & Grossman 2009). Considering the high prevalence of PAs within the general population, their molecular pathogenesis remains poorly understood when compared to other tumors. Currently available evidence indicates that the loss of heterozygosity and copy number variation events, as well as aberrant genes' expression and DNA methylation profiles all play a role in the development of these tumors (Dworakowska & Grossman 2009; Melmed 2011). The role of MSI in pituitary tumors is almost unknown and this issue was addressed in only one previously published work (Zhu *et al.* 1996).

In our study, MSI status was assessed in a group of 107 patients with PA using a currently recommended method.

Tab. 1. Pituitary adenoma patient profiles.

Pituitary adenoma patients (number of patients)	107
Age (years)	
median	60
range	21–84
Gender (number of patients)	14/30
female	40/107
male	67/107
Functional classification (number of patients)	
nonfunctioning PA	83/107
somatotrophic PA	13/107
lactotrophic PA	9/107
corticotrophic PA	2/107
Clinical classification	
new diagnosed	99/107
recurrent	8/107
invasive	62/107
atypical	14/107
macroadenomas	86/107
microadenomas	21/107

MATERIALS AND METHODS

Patients

Subjects were 107 patients with histopathologically confirmed PAs including 83 of those nonfunctioning, 13 somatotrophic, 9 lactotrophic and 2 corticotrophic tumors, and who had undergone surgery at the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw between the years: 2009–2014. The study was approved by the Independent Ethics Committee of the Cancer Centre and the Institute of Oncology, and each patient gave their informed consent. Patient profiles are shown in Table 1.

Methods

Tissue samples were collected during endoscopic endonasal pituitary tumor resection. Part of the resected tissue underwent standard histopathological evaluation whilst the other part was frozen immediately in liquid nitrogen and stored at –70 °C.

Genomic DNA was isolated using the Qiamp DNA mini kit (Qiagen). Five quasimonomorphic microsatellite markers, BAT25, BAT26, NR21, NR24 and NR27, were selected for the study. This marker panel is recommended for MSI screening in the Bethesda guidelines and has been also validated in different worldwide populations (Buhard *et al.* 2006). Previously described PCR primers were used (Buhard *et al.* 2006). Each forward primer was 5' labeled with a fluorescent dye: BAT25 with VIC, BAT26, NR21 with NED, and NR24 and NR27 with FAM. PCR for each marker was performed separately in 12 µl with 0.15 µM of primer, 20 ng of DNA template and 6 µl of KAPA HiFi HotStart ReadyMix (Kapa Biosystems) that include KAPA HiFi HotStart DNA polymerase in an optimized concentration. The following cycling conditions were used: 35 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 40 s; preceded by 3 minutes at 95 °C and followed by 7 minutes at 72 °C. PCR products were pooled and analyzed with the automated capillary DNA sequencer ABI PRISM 3100 Genetic Analyser and Peak Scanner Software v.1.0 (Applied Biosystems). Colon cancer cell lines, HCT-116 and HT29 were respectively examined for MSI with a positive and negative control. MSI status was categorized as being microsatellite-stable (MSS), MSI-low and MSI-high when respectively 0, 1 or ≥3 markers showed instability, according the revised Bethesda Guidelines (Umar *et al.* 2004).

RESULTS AND DISCUSSION

MSI analysis was successfully completed for all the patients enrolled. There was no observed instability of the analyzed markers in any of the tumor DNA samples. DNA samples from 3 colorectal cancer cell lines with different MSI status ie. HCT116, HT29 and SW480 were also included in the analysis, and served as technical controls. HCT116 clearly showed instability in BAT26 and NR21, as described previously, whereas two other cell lines were MSI negative; as expected.

Our results demonstrate that either MSI, as defined with the widely used Bethesda panel of 5 microsatellite marker criteria, does not occur in PAs or it is very rare in such cases.

The proportions of different PA subtypes our study group did not reflect their prevalence in the general population because sampling of adenoma tissue during resection becomes most feasible for macroadenomas that are predominantly nonfunctioning. These tumors are the major subtype in our cohort. Therefore the conclusion that MSI is absent, refers primarily to NFPAs, however it is clear that MSI was not observed in any of the other PAs.

The potential relevance of our study comes from the experience of treating other tumor types, where MSI is considered a predictive marker (Claij & Riele 1999). It's prognostic value has been shown for radiation based treatment (Martin *et al.* 2010) and those using temozolomide (Zhang *et al.* 2013). Both these therapeutic options can be applied for treating PA.

MSI is a consequence of an impaired system of DNA mismatch repair. Previously reported data suggest that MMR components have a role in the response of atypical pituitary adenomas and carcinomas to temozolomide (Hirohata *et al.* 2013; Matsuno *et al.* 2014) and the acquirement of the drug resistance (Murakami 2011). Unfortunately, the lack of MSI in pituitary adenomas found in our study implies its inadequacy as a possible biomarker of these tumors.

The main aim of our study was to plug the gap in evidence regarding the potential role of MSI status in PAs. In doing a Pubmed search, we found only one report, (Zhu *et al.* 1996), published nearly 20 years ago, presenting MSI testing in PAs This study enrolled 31 patients with PA and a comprehensive analysis of MSI in different brain tumors was performed which found instability of one marker in a single patient diagnosed with nonfunctioning PA. This study however used different technical methods compared to those currently used. Namely in the former, different microsatellite markers ie. two dinucleotide markers (D4S251, D9S942), trinucleotide markers (HUMARA) and two tetranucleotide markers (F8VWFP, RB1.20) together with gel electrophoresis and gel silver staining analyses. These types of microsatellite markers, especially tri- and tetranucleotide repeats are currently not recommended for MSI testing (Umar *et al.* 2004). They were shown to have a notably lower sensitivity compared to the currently used mononucleotide markers, which are the most susceptible to replication errors (Bacher *et al.* 2004; Dietmaier *et al.* 1997). Secondly, the previously used gel-based method for MSI analysis is less reliable than capillary electrophoresis, which allows an accurate quantitative measurement of PCR product. A gel-based approach has been previously reported for producing a large ratio of uncertain results and is prone to interpretation mistakes (Dietmaier *et al.* 1997).

The use of obsolete methodology in a previous study on PA patients thereby justifies our attempts of MSI identification by analyzing mononucleotide microsatellite markers with modern methods. We believe that our results provide improved quality/reliability compared to those previously reported by Zhu *et al.* Unfortunately, the results are generally negative and the absence of MSI in the 107 PA patients, in fact suggest a limited role of MMR defects in the pathogenesis of pituitary tumors, as well as the inadequacy of MSI testing in clinics.

ACKNOWLEDGMENT

This work was supported by the research grant from the Polpharma Scientific Foundation.

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