Mutation Research 686 (2010) 68–73

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Low frequency of somatic mutations in uterine sarcomas: A molecular analysis and review of the literature

S. Murray[a], H. Linardou[b], G. Mountzios[c,*], M. Manoloukos[a], S. Markaki[d], E. Eleutherakis-Papaiakovou[c], M.A. Dimopoulos[c], C.A. Papadimitriou[d]

[a] Department of Molecular Biology and Genetics, Metropolitan Hospital, Athens, Greece
[b] Department of Medical Oncology, Metropolitan Hospital, Athens, Greece
[c] Department of Clinical Therapeutics, "Alexandra" Hospital, University of Athens School of Medicine, Athens, Greece
[d] Department of Pathology, "Alexandra" Hospital, Athens, Greece

ARTICLE INFO

Article history:
Received 1 October 2009
Received in revised form 21 January 2010
Accepted 25 January 2010
Available online 1 February 2010

Keywords:
Uterine sarcomas
Mutation
EGFR
CDKN2A
KIT
RAS

ABSTRACT

Objective: The rarity of uterine sarcomas along with their pathological and molecular heterogeneities render their study particularly challenging. We evaluated a panel of somatic mutations principally centering on the tyrosine kinase gene family and their downstream signaling cascades in an attempt to identify potential candidate markers that may assist in diagnostic or therapeutic decisions in these tumors.
Methods: We performed mutational analysis of 20 exons from 9 genes (*EGFR*, *CDKN2A*, *MET*, *KIT*, *RAS*, *BRAF*, *PI3KCA*, *HER-2* and *PDGFR-α*) on biopsy material from 25 patients who underwent primary surgery for uterine sarcoma between October 1995 and October 2003. Due to the limited number of studies conducted we have also undertaken a literature review of somatic mutations in uterine sarcomas.
Results: A total of 3 different somatic mutations were identified: one KRAS (codon G12D) in a carcinosarcoma and two exon 20 PI3KCA mutations (H1047R and H1047Y) both in carcinosarcomas. Mutational status of all mutations was confirmed using germline DNA extracted from peripheral blood. Consistent with the literature data, no other mutations regarding the rest of the genes of the panel were identified. Due to the low number of somatic mutations in our series, we did not perform further clinicopathological correlations.
Conclusion: The absence of somatic mutations in the majority of genes that are considered critical in neoplastic transformation hampers the identification of potential therapeutic targets in patients with uterine sarcoma.

1. Introduction

Uterine sarcomas are rare neoplasms comprising 1% of all gynaecologic malignancies and 4–9% of all malignant uterine neoplasms [1,2]. It is estimated that the incidence of uterine sarcomas varies between 0.5 and 3.3 cases per 100,000 females per year [3]. Uterine sarcomas usually display aggressive clinical behavior, with a great tendency to local recurrence and even greater to distant spread [4–7]. Due to their low incidence and the fact that they lack a preinvasive stage, there is no established practice for screening these tumours. The rarity of uterine sarcomas and their often aggressive clinical course have resulted in a relatively limited amount of the literature. The staging of uterine sarcomas is based on the International Federation of Gynecology and Obstetrics staging system for uterine corpus cancer [8]. According to WHO classification, uterine sarcomas are classified into four main histological subtypes in order of decreasing incidence: carcinosarcomas, leiomyosarcomas, endometrial stromal sarcomas and "other" sarcomas [9]. Unfortunately, clinical-trial reports and the literature reviews often include a broad range of histological subtypes of sarcoma, which restricts interpretation and application of results. Response rates from protocols with multiple subtypes should consequently be interpreted with caution. Therefore, the effort to tailor the approach to patients with uterine sarcomas by pathological subtype seems mandatory.

Typically, treatment is decided based upon "early" (FIGO stages I/II) versus "late/advanced" (FIGO stages III/IV) stage disease [8]. Total abdominal hysterectomy and bilateral salpingo-oophorectomy represents the standard treatment for non-metastatic uterine sarcomas. Pelvic and para-aortic lymph node dissection in carcinosarcomas is recommended, given their high incidence of lymph node metastases, and may have a role in endometrial stromal sarcomas. Adjuvant radiation therapy has historically been of little survival benefit but it appears to improve

* Corresponding author at: P. Kanellopoulou avenue, 3, P.C. 115 25, Athens, Greece. Tel.: +30 210 74 63 905/698 35 19 989; fax: +30 210 33 81 540.
E-mail address: gmountzios@med.uoa.gr (G. Mountzios).

0027-5107/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrfmmm.2010.01.019

local control and may delay recurrence. Regarding adjuvant chemotherapy, there is little evidence in the literature supporting its use except for carcinosarcomas and the use of the docetaxel–gemcitabine combination in leiomyosarcomas [10]. In advanced uterine sarcomas the effort for optimal debulking may be of value, while chemotherapy remains the main therapeutic option. Drugs with activity in uterine sarcomas include anthracyclines, cisplatin, ifosfamide, taxanes, gemcitabine and trabectedin [11].

More recently, efforts to better characterize soft tissue sarcomas on the basis of immunohistochemical expression patterns and genetic signatures using candidate gene approaches and also multiple gene scanning approaches have been made. Sadly, apart from the recent characterization of GISTs, CD113 and somatic mutations of *c-KIT* and *PDGFR-α*, there has been little headway made against this debilitating disease [12,13]. Notably, uterine sarcomas have scarce expression of *c-KIT*, and even in other sarcomas apart from GISTs the literature is not consistent concerning *c-KIT* expression profiles [14–17]. In light of this and considering their overall rarity, we decided to investigate a panel of somatic mutations principally centering on the tyrosine kinase gene family and their downstream signaling cascades in an attempt to identify potential candidate markers that may assist in diagnostic or therapeutic decisions in these tumors. The majority of the genes included in this study are considered as candidate biomarkers for response and/or resistance to specific chemotherapeutic drugs in the treatment of other solid malignancies such as NSCLC and colorectal cancer [18–20]. Due to the limited number of studies conducted we have also undertaken a literature review of somatic mutations in uterine sarcomas supporting the view that they are a rare event in this tumor type.

2. Materials and methods

2.1. Patient selection

Tumor specimens were obtained from 25 patients who underwent primary surgery for uterine sarcoma in the Department of Oncology in "Alexandra" University Hospital from October 1995 to October 2003. All patients were of Greek origin. The study was conducted according to the Declaration of Helsinki and the guidelines for Good Clinical Practice. The local ethics committees approved the study and the collection of biological material, and all participating patients signed informed consent. Staging was performed according to FIGO classification [8].

2.2. Tissue procurement

Paraffin blocks of tumor were collected retrospectively, and peripheral blood samples were also collected during treatment or follow-up. All tissue samples were formalin-fixed and paraffin-embedded. Hematoxylin–eosin stained slides were available in all cases, and were independently reviewed (SM) to confirm the pathological diagnosis of uterine sarcomas according to WHO criteria [9].

2.3. Mutation analysis

Genomic DNA was derived from paraffin-embedded tumors as previously described [21]. Tumors consisting of >75% tumor cells content (TCC) were eligible for DNA extraction and sequence analysis, otherwise macro-dissection was used to enrich the TCC.

We amplified 20 exons from 9 genes from primary tumor tissue (paraffin-embedded DNA) and germline DNA (peripheral blood DNA extracted with Invisorb® Spin Blood Midi Kit; Invitek GmbH, Berlin, Germany). All PCRs for all genes were conducted as previously described for EGFR with slight modification of annealing temperatures for *CDKN2A*, *KIT* and *PI3KCA* [21,22]. All mutations were reconfirmed by PCR amplification and analysis of an independent DNA isolate. Germline DNA was analyzed on two separate occasions for the corresponding exons for all patients with mutations in order to confirm mutations as somatic or germline in origin.

Additional genes for which analysis was conducted included; *KRAS* mutation analysis of codons 12 and 13 PCR performed using the same conditions as for *EGFR*, using *KRAS* specific primers amplifying exon 2 as previously described [21]; *BRAF* exons 14 and 15 as previously described [22], *CDKN2A* exons 2 and 3 [23], *HER2* exon 20, *PI3KCA* exons 9 and 20, *PDGFR*-α exons 11 and 17, 3′ and 5′ intron–exon splice sites of *MET* exon 14, and *KIT* exons 9, 11, 13 and 17. The primer sequences of all PCR reactions are available in Table 1.

All studied exons were confirmed as previously described above for *EGFR* [21]. All PCR products were purified by solid-phase reversible immobilization chemistry followed by bi-directional dye-terminator fluorescent sequencing. Sequences were analyzed by BLAST and chromatograms by manual review, and compared to the following representative gene accession numbers: *EGFR*, NM_005228 and/or the *EGFR* gene sequence Accession number: AF288738; *KRAS*, gi: 14277199; *KIT*, gi: 180574; *PDGFR-α*, gi: 23463020; *HER2* exon 20, gi: 23462913; *MET*, gi: 212720875, *CDKN2A*, gi: 21886808, *PI3KCA*, gi: 28301920 (www.ncbi.nlm.nci).

The *EGFR* exon 21 mutation L858R which represents approximately 40% of all reported mutations in NSCLC [24] was also analyzed by PCR–RFLP based on the presence of a new Sau96I restriction site created by the mutation [22]. *KRAS* mutations of codons 12 and 13 were also analyzed by PCR–RFLP based on modified versions of the protocols of Boldrini et al. [25], and Kislitsin et al. [26]. *MET* exon 14 5′ and 3′ intron–exon deletions first reported in NSCLC [27] were also analyzed by a mutant allele specific PCR method, that only amplifies in the presence of the given deletions, while *BRAF* V600E was also analyzed by PCR-RFLP based on a modified version of Salvesen et al. [23].

Table 1
Primer sets per gene.

Gene	Exon	Primer sets	Product size (bp)
KIT	9	F gaaggactgcaattcacttgaat	435 bp
		R tagtgagggttaattgagctc	
		IF gtatgccacatcccaagtgt	334 bp
		IR catgactgatatggtagacag	
	11	F gtaatcgtagctggcatgatg	445 bp
		R actcattgtttcaggtggaaca	
		IF ccagagtgctctaatgactg	272 bp
		IR ggaagccactggagttcctta	
	13	F actgtcgctgtaaagatgctc	354 bp
		R ctagcaagagagaacaacagtc	
		IF gtaagttcctgtatggtactgca	254 bp
		IR catgttttgataacctgacagac	
	17	F gccatagttaaaagcagaatgtc	421 bp
		R gatcccaatcacctctgaaatac	
		IF gcaacactatagtattaaaaagttag	302 bp
		IR atttacattagaaagtcacgga	
PDGFR-α	11	F tggcacagagaaggagctca	450 bp
		R gcaatgatccaattaacttactgtc	
		IF atgtggagtgaacgttgttgg	358 bp
		IR ctagttcttactaagcacaagc	
	17	F ctttatatccaggcagacagc	378 bp
		R ctgccacggcagtactgac	
		IF catggatcagccagtcttgc	290 bp
		IR accgaatctctagaagcaacac	
HER-2	20	F ctttgagccttcacaggctg	425 bp
		R gcacccatgtagaccttcta	
		IF gccatggctgtggtttgtgatgg	341 bp
		IR atcctagccccttgtggacatagg	
MET	14	F gttaccttaagaacacagtc	500 bp
		R cataatgtaatatatacatgg	
		5'IF cagtttaagattgtcgtcgattc	217 bp
		5'IR atcgggcacttacaagcctata	
		AS5'IF gtctttaacttaagatctgggcagtga	90 bp
		AS5'IR atcgggcacttacaagcctatc	
		3'IF gataggcttgtaagtgcccgat	243 bp
		3'IR aatgtaattttgtgtcaaatac	
		AS3'IF gaatctgtagacttcagtttattgttc	210 bp
		AS3'IR catgtaattttgtgtcaaatact	
PI3KCA	9	F gattggttctttcctgtctctg	525 bp
		R agcatttaatgtgccacctacc	
		IF tctgtaaatcatctgtgaatccag	281 bp
		IR catgctgagatcagccaaattc	
	20	F aagagaagtgagagaggaatgc	512 bp
		R catggattgtgcaattcctatgc	
		IF tgtctacgaaagcctctctaa	452 bp
		IR caatcggtctttgcctgctga	

The remaining primers pairs for the outstanding genes have previously been reported elsewhere [21,22]. F, forward; R, reverse; IF, internal forward; IR, internal reverse; and AS, allele specific.

2.4. Literature review methodology

The information for the systematic review was obtained by searching the PubMed and MEDLINE databases for articles published until 1st June 2009. Electronic early release publications were also included. We searched journals known to publish information relevant to our topic and cross-referenced the reference