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**Absence of mutations of the *BRAF* gene in malignant melanoma of soft parts
(clear cell sarcoma of tendons and aponeuroses)**

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Running title: No *BRAF* mutations in malignant melanoma of soft parts

Keywords: malignant melanoma of soft parts, clear cell sarcoma, translocation, *EWS* gene, *ATF1* gene, *BRAF* gene

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Abstract

Malignant melanoma of soft parts (MMSP, also called clear cell sarcoma of tendons and aponeuroses) is cytogenetically characterized by the translocation t(12;22)(q13;q12) resulting in the chimeric *EWSRI/ATF1* gene. MMSP shares a number of morphologic, histologic and immunohistochemical features with malignant melanoma of the skin, causing diagnostic difficulties in the distinction between MMSP and metastatic malignant melanoma with an unknown primary site. Recently, a high incidence of activating mutations in the kinase domain of the *BRAF* gene has been reported in malignant melanoma of the skin. The most common mutation (V599E) is the T1796A substitution in exon 15, leading to an exchange of valine for glutamic acid at position 599. Because of the extensive clinical, histologic and immunohistochemical similarities with melanoma, we decided to analyze whether MMSP also has mutations in the *BRAF* gene. Eight MMSP with an *EWSRI/ATF1* chimeric transcript, one soft tissue metastasis of a malignant melanoma of the skin and one malignant melanoma cell line were examined. Both conventional melanomas had the exon 15 T1796A (V599E) mutation, but none of the MMSP was found to harbor any mutation in exon 11 or 15 of the *BRAF* gene. Our data further emphasize that MMSP and conventional malignant melanoma develop through different genetic pathways.

1. Introduction

Malignant melanoma of soft parts (MMSP, also called clear cell sarcoma of soft tissue) is a rare malignant soft tissue tumor first described by Enzinger [1] and has since been accepted as a distinct clinicopathological entity. The tumor is particularly associated with tendons and aponeuroses but other tumor locations have also been reported including the head and neck, ear, penis, kidney and colon [2]. MMSP is most commonly found in young adults between the age of 20 and 40 years, but has also been described in children and elderly [3,4]. Repeated local relapse is very common and with time, most tumors metastasize to lymph nodes, the lungs, skeleton, brain or liver [2]. Clonal chromosome abnormalities have been described in MMSPs and the translocation $t(12;22)(q13;q12)$ seems to be pathognomonic [5,6]. The $t(12;22)$ results in rearrangements of the *EWSRI* gene on chromosome 22 and the *ATF1* gene in 12q13 creating a chimeric *EWSRI/ATF1* gene in which the 3'-terminal part of *EWSRI* is replaced by the 3'-terminal part of *ATF1* [7].

Histologically, the tumor shows a characteristic architecture of nests or short fascicles of epithelioid or spindle-shaped cells with clear to granular eosinophilic cytoplasm [2]. Immunohistochemically, the tumor cells express S-100 protein, HMB-45, vimentin, and the microphthalmia transcription factor, and synthesize melanin [2]. In a recent study, Segal et al. [8] used cDNA microarray to show that MMSP has a gene expression profile closely related to melanoma. Because MMSP shares a number of morphologic features with conventional malignant melanoma, there are diagnostic difficulties in the distinction between MMSP and metastatic malignant melanoma with an unknown primary site [2].

Recently, a high incidence of activating mutations in the *BRAF* gene has been reported in melanoma cell lines, melanoma short term cultures, primary and metastatic

melanomas and nevi [9-12]. All the mutations were detected in the kinase domain of the *BRAF* gene and found in exons 11 and 15 [9]. By far, the most common mutation (V599E) is the T1796A single base substitution in exon 15, leading to an exchange of valine for glutamic acid at position 599 [9]. Mutated BRAF proteins possess ten fold higher basal kinase activity and are more than 100 times as efficient in transforming NIH3T3 as compared to the wild-type *BRAF* gene [9].

Because of the extensive clinical, histologic and genetic similarities with melanoma, we decided to analyze whether MMSP also has mutations in the *BRAF* gene.

2. Materials and methods

The material consisted of eight MMSP with an *EWSRI/ATF1* chimeric transcript, the melanoma cell line SK-MEL-28 and a soft tissue metastasis from a malignant melanoma of the skin. The clinical data and the cytogenetic and molecular identification of the fusion gene have been reported [13].

Tumor tissue pieces adjacent to those used for histological examination had been frozen and stored at -80°C . A one-step PCR was then performed using the primer combination BRAFEx11For: ATCCCTCTCAGGCATAAGGTAATG and BRAFEx11Rev: GCGAACAGTGAATATTTTCCTTTGA and BRAFEx15For: TGCTTGCTCTGATAGGAAAATGAG and BRAFEx15Rev: TCTCAGGGCCAAAATTTAATCA for amplification of exons 11 and 15 of the *BRAF* gene, respectively. The 50 μL reaction volume contained 20 mM Tris-HCl pH 8.4 (at 25°C), 50 mM KCl, 1.5 mM MgCl_2 , 0.2 mM of each dNTP, 1 unit Platinum*Taq* DNA polymerase (Invitrogen), 0.5 μM of each of the forward and reverse primers and 300 ng of the genomic DNA. After an initial denaturation at 94°C for 5 minutes, 30 cycles of 1 minute at 94°C , 1 minute at 58°C , and 1 minute at 72°C were run using a

PCT-200 DNA Engine (MJ Research), followed by a final extension for 10 minutes at 72°C. For sequence analysis, the amplified fragments were run on 1.5% agarose gels, purified using the Qiagen gel extraction kit (Qiagen), and directly sequenced using the dideoxy procedure with an ABI Prism BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems) and the same primers as for PCR on the Applied Biosystems Model 3100-Avant DNA sequencing system.

3. Results and discussion

None of the MMSP was found to harbor any mutations in exon 11 or 15 of the *BRAF* gene whereas both malignant melanomas showed the previously described [9] exon 15 T1796A (V599E) mutation (Figure 1). Sequencing of the entire PCR products from the MMSP confirmed a wild-type sequence over the entire exons 11 and 15. Although the present study was limited to eight samples, it seems safe to conclude that *BRAF* mutations are rare in MMSP. Consequently, if *BRAF* is activated in MMSP (which remains to be determined) then that is unlikely to result from an intrinsic genomic mechanism. Our data further highlight the fact that MMSP is genetically different from conventional melanoma.

Acknowledgments

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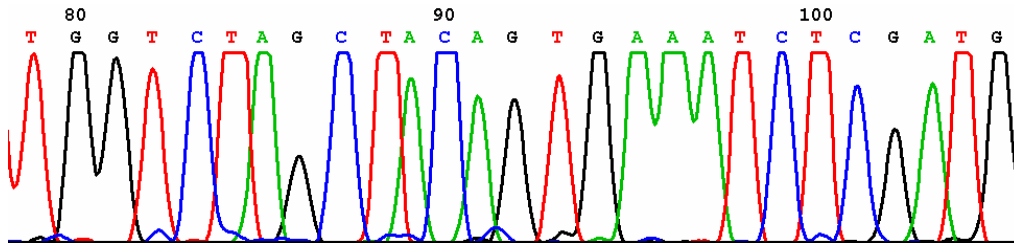
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Figure 1

Partial sequence chromatogram showing the *BRAF* exon 15 T1796A (V599E) mutation in the positive control and wild type sequence in an MMPS tumor biopsy.

Normal



T1796A mutation

