Virchows Arch (2003) 443:741–744
DOI 10.1007/s00428-003-0883-2

ORIGINAL ARTICLE

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Sequence analysis and high-throughput immunhistochemical profiling of KIT (CD 117) expression in uveal melanoma using tissue microarrays

Received: 11 June 2003 / Accepted: 17 July 2003 / Published online: 26 September 2003

Abstract We aimed to immunohistochemically examine the expression of KIT (CD 117) in human posterior uveal melanoma and to analyze KIT-positive tumors for gene mutations. Brought into a tissue microarray (TMA) format were 101 formalin-fixed, paraffin-embedded posterior uveal melanomas. Immunhistochemistry was performed using the polyclonal anti-CD117 antibody from Dako (A4502). In ten selected KIT-positive tumors, exons 2, 8, 9, 11, 13 and 17 were sequenced. Of the 101 cases, 89 (88%) could be evaluated on the TMAs. Immunohistochemistry for CD 117 was weakly positive in 5 cases (6%), moderately positive in 10 cases (12%) and strongly positive in 57 cases (69%). No KIT mutations were detected in the analyzed exons. In conclusion, human posterior uveal melanoma frequently expresses CD117 at high levels. Although KIT mutations could not be found, it appears justified to investigate the utility of imatinib mesylate in the treatment of these patients.

Keywords Uveal melanoma · CD 117 · cKIT · Tissue microarray · Sequence analysis

Introduction

Although posterior uveal melanoma is the most common primary intraocular malignancy, the neoplasm is rare, with an overall incidence of 0.1–1.5. Its frequency increases with advanced age. The average age of detection of posterior uveal melanoma ranges between 55 years and 60 years [19]. The tumor has not only the capacity to destroy vision, but can also metastasize and cause death. In recent years, a marked progress in local tumor control was achieved using therapeutic options such as enucleation, radiation, photocoagulation, microsurgical resection or transpupillary thermotherapy [19]. However, none of these treatments can prevent metastases. Therefore, the prognosis of locally treated posterior uveal melanoma has not changed during recent years. The metastatic rate of the malignant uveal melanoma is about 35% at 10 years. Results of systemic treatment for metastatic uveal melanoma are still discouraging.

It is hoped that novel adjuvant cancer drugs targeting specific gene products in cancer cells, such as imatinib mesylate (STI 571; Glivec), will improve the success of systemic therapy in uveal melanoma and other tumor entities. Imatinib mesylate is a small molecule that specifically inhibits several kinases, including ABL, KIT (CD117) and platelet derived growth factor receptor. Imatinib mesylate is of special interest in melanomas because melanocytes are known to express at least one of its molecular targets (KIT). KIT is a transmembrane tyrosine kinase and acts as a type-III receptor for mast cell growth factor or stem cell factor. KIT expression has been described in various tumor entities [1, 7, 12, 14, 23]. Remarkably, metastatic and/or advanced gastrointestinal stroma tumors (GIST), the tumor entities with the highest KIT expression, responded well to imatinib mesylate therapy in initial studies [4, 24]. There is hope that imatinib mesylate might also be effective in other KIT-expressing tumor entities. Several clinical studies are currently investigating the effect of imatinib mesylate on KIT-positive tumors of various sites, such as gliomas, soft tissue sarcomas/bone sarcomas, ovarian cancers, primary peritoneal cancers, pediatric solid tumors and colorectal cancers [16].

As far as melanomas are concerned, only skin melanomas have been analyzed for KIT expression.

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These studies have indicated a frequency of KIT expression ranging between 20% and 90% [1, 7, 23]. Uveal melanoma has not been examined for KIT expression up to now. To investigate the importance of KIT as a potential therapeutic target in uveal melanomas, we analyzed KIT expression in a series of 101 uveal melanomas using the tissue microarray (TMA) technique [2, 3]. In addition, we sequenced exons 2, 8, 9, 11, 13 and 17 in a subset of KIT-expressing tumors to identify possible mechanisms of KIT activation in uveal melanoma.

Materials and methods

Tumors, TMA construction

Formalin-fixed, paraffin-embedded tissue material from 101 posterior uveal melanomas from the years 1985–2002 were collected from the archives of the Institute of Ophthalmopathology and Pathology, University of Basel. All cases were classified according to the pTNM classification [UJCC 5th edition, 1997]. The series included 44 males and 57 females. The mean age was 79 years (range 39–98 years). The pT stage was pT1a in 3, pT1b in 1, pT2a in 25, pT2b in 27, pT2c in 6, pT3 in 15 and pT4 in 20 cases. pT stage could not be determined in four cases. Histologically, a spindle cell type was present in 50 cases, an epitheloid type in 13 cases and a mixed type in 38 cases. The construction of TMAs was done exactly as described [2, 3]. In brief, tissue cylinders with a diameter of 0.6 mm were taken from representative tumor areas of each "donor" tissue block using a robotic precision instrument and brought into a recipient paraffin block. Sections 4-μm thick of the resulting TMA blocks were transferred to an adhesive coated slide system (Instrumedics Inc., Hackensack, New Jersey).

Immunohistochemistry

Immunhistochemistry was performed using the polyclonal anti-CD117 antibody A4502 (Dako, Glostrup, Denmark). In a comparison of different commercially available anti-CD117 antibodies, A4502 yielded the highest specificity and the least background (unpublished data). A4502 was applied at a dilution of 1:300 at 4°C for 18 h, after 3 min pressure cooking in 10 mM sodium citrate buffer (pH 6.0) for antigen retrieval. Endogenous peroxidase was blocked using 0.3% hydrogen peroxidase diluted in methanol for 30 min. A standard avidin-biotin complex (ABC) technique (Vector ABC kit) using diaminobenzidine for visualization was used. For specificity control, a preabsorption of the antibody was performed (CD117 peptide stock solution; Neomarkers PP1518). This antigen matches the sequence of the epitope recognized by the polyclonal antibody of Dako. A case of KIT-positive GIST was used for control. The percentage of positive cells was estimated and the staining intensity was semi-quantitatively recorded as 1+, 2+ or 3+. For statistical analyses, the staining results were categorized into four groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in less than 60% of cells and 2+ intensity in less than 30% of cells were considered weakly positive. Tumors with 1+ staining intensity in 60% of cells or more, 2+ intensity in 30% or more up to 80% (not inclusive) or 3+ intensity in less than 30% were considered moderately positive. Tumors with 2+ intensity in 80% or more or 3+ intensity in 30% or more of cells were considered strongly positive.

Sequence analysis

Ten melanomas showing intense CD117 staining were selected for KIT mutation analysis. Deparaffinizing of the formalin-fixed tissues and DNA extraction was performed using a commercial DNA extraction kit (Qia Amp DNA minkit, cat. #51304, Qiagen GmbH, Hilden, Germany). Exons 2, 8, 9, 11, 13 and 17 were amplified using a semi-nested polymerase chain reaction approach [10] and were sequenced using the Big Dye Terminators Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). Sequence products were analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems; Foster City, CA, USA).

Statistical analysis

Statistical analyses were performed using the statistical package StatView 5.0 (Abacus Concepts, Berkley, CA). The chi-square statistic was used for frequency comparisons of nominal categorized variables. In the case of 2×2 contingency tables, significances were calculated using Fisher's exact test.

Results

Immunohistochemistry

Immunohistochemistry was interpretable in 89 of 101 cases (88%). Analysis failures were due to either lack of tissue on the TMA section or a lack of vital tumor cells in the arrayed samples. A positive CD 117 staining was seen in 72 of 83 interpretable tumors (87%). The staining was membranous in all cases (Fig. 1). Positivity was weak in 5 cases (6%), moderate in 10 cases (12%) and strong in 57 cases (69%). Examples of positive and negative staining for CD 117 are shown in Fig. 2. There was no difference in frequency or intensity of KIT staining between males and females or between tumors of different stages or different tumor cell types.

Mutation analysis

Sequence alterations of exons 2, 8, 9, 11, 13 and 17 were not detected in any of the ten analyzed tumors.

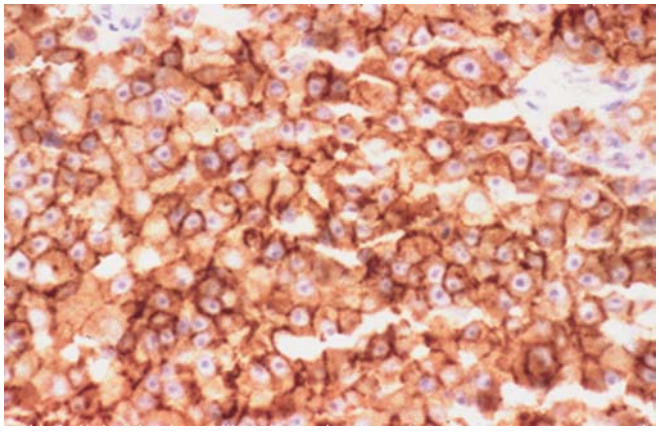

Fig. 1 Example of a CD 117-positive tumor (magnification ×100)

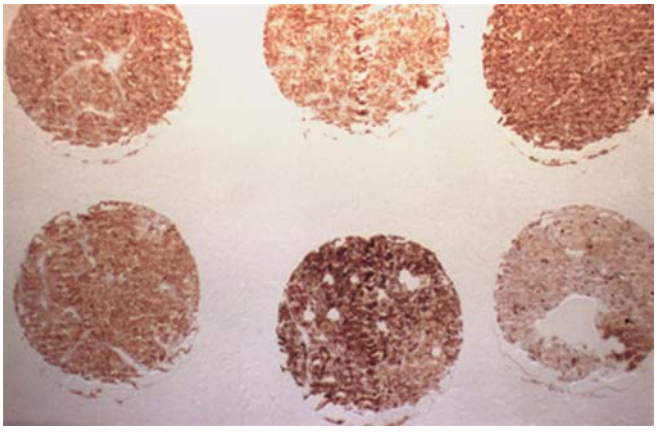

Fig. 2 Examples of positive and negative tumors. Single punches (0.6 mm diameter) of posterior uveal melanoma positive and negative for CD 117, respectively (magnification ×5)

Discussion

A TMA was used to evaluate the expression frequency of KIT in a series of clinically well-documented 101 uveal melanomas. Using this method, minute tissue samples (diameter 0.6 mm) are analyzed on one microscope glass slide. It is likely that focal alterations in gene expression are not always detected in a TMA setting. However, this perceived disadvantage seems to be compensated by a maximal standardization of the analysis (where all samples are processed under identical conditions) and the high number of tumors that can be included in a TMA study. Numerous studies have shown that representative information can be obtained in TMA analyses [9, 17, 20, 21, 22] (Went et al., personal communication). For example, the known frequencies of amplifications of various genes were confirmed in TMA studies in various tumor types [9, 18]. Other TMA projects confirmed the previously established prognostic impact of molecular markers such as the expression of estrogen receptor, progesterone receptor and p53 in breast cancer [22] or of the Ki67-labeling index in bladder cancer [15].

The results of this study show that KIT is frequently expressed in uveal melanoma. More than 81% of our tumors were KIT positive. Although it is difficult to compare immunohistochemical studies if different antibodies, staining conditions and scoring procedures are applied, these data suggest that KIT expression is more frequent in uveal than in skin melanoma. Such a high frequency of KIT expression in uveal melanoma raises the possibility that imatinib mesylate could be a therapeutic option in these tumors. Imatinib mesylate was initially designed to inhibit the bcr/abl fusion protein in chronic myeloid leukemia, for which it was FDA approved in May 2001 [5]. Later it was shown that imatinib mesylate is also effective in KIT-positive GIST [24]. Several studies are now ongoing to assess whether imatinib mesylate has a beneficial effect on other KIT-positive tumors [16].

Our data suggest that uveal melanoma is one of the tumors with the highest frequency of immunohistochemically detectable KIT expression. This could make uveal melanoma a prime candidate for imatinib mesylate therapy. However, c-KIT protein is also expressed in normal melanocytes, and there is evidence that its expression is downregulated or even lost in skin melanoma [13]. It is therefore possible that KIT expression in uveal melanoma does not reflect "overexpression" but rather represents preservation of normal protein expression. The lack of mutations in all ten tumors sequenced for exons 2, 8, 9, 11, 13 and 17 also does not provide evidence for presence of KIT alterations in uveal melanoma. It has recently been shown that the response rate to imatinib mesylate may depend on presence and type of KIT mutation in a tumor [8]. If this is true in general, the likelihood of uveal melanomas responding favorably to imatinib mesylate may be low. However, other authors have reported at least some therapeutic effect in KIT-positive tumors expressing wild-type protein [6, 11].

Taken together, our data show that posterior uveal melanoma is one of the most frequently KIT-positive tumor entities. Although this seems to reflect preservation of "normal level KIT expression" in melanocytes rather than KIT mutations, these data may justify investigating possible beneficial effects of imatinib mesylate in uveal melanoma.

Acknowledgement The authors thank Miriam Vonlanten and Andreas Meyer for their technical assistance.

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