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SHORT COMMUNICATION

Mutation Scanning of D1705 and D1709 in the RNAse IIIb Domain of MicroRNA Processing Enzyme Dicer in Cutaneous Melanoma

Michael Sand[1,2] · Falk G. Bechara[1] · Marina Skrygan[1] · Daniel Sand[3] · Thilo Gambichler[1] · Michael Bromba[2] · Eggert Stockfleth[1] · Schapoor Hessam[1]

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Abstract Since the discovery of microRNAs (miRNAs) there have been performed several studies showing perturbations in the expression of miRNAs and the miRNA expression machinery in cutaneous melanoma. Dicer, a pivotal cytosolic enzyme of miRNA maturation has shown to be affected by both somatic and germline mutations in a variety of cancers. Recent studies have shown that recurrent somatic mutations of Dicer frequently affect the metal-ion-binding sites D1709 and D1705 of its RNase IIIb domain, therefore called hot spot mutations. The present study investigates metal-ion-binding sites D1709 and D1705 of the Dicer RNase IIIb domain in cutaneous melanomas and melanoma metastasis by Sanger sequencing. All investigated samples showed wildtype sequence and no single mutation was detected. The miRNA processing enzyme Dicer of melanoma and melanoma metastasis does not appear to be affected by mutation in the metal-ion-binding sites D1709 and D1705 of its RNase IIIb domain.

Keywords Melanoma · Dicer · Mutation · MicroRNA

✉ Michael Sand
michael.sand@ruhr-uni-bochum.de

[1] Dermatologic Surgery Unit, Department of Dermatology, Venereology and Allergology, Ruhr-University Bochum, St. Josef Hospital, Gudrunstr. 56, 44791 Bochum, Germany

[2] Department of Plastic Surgery, St. Josef Hospital, Catholic Clinics of the Ruhr Peninsula, Heidbergweg 22-24, 45257 Essen, Germany

[3] University of Michigan Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105, USA

Introduction

Dicer is a pivotal cytosolic enzyme in the maturation of microRNAs and therefore mRNA expression. Although several authors have investigated Dicer expression levels in primary cutaneous malignant melanoma (PCMM) there has been no screening of the mutational status of Dicer in PCMM or cutaneous malignant melanoma metastasis (CMMM). The RNase III enzyme Dicer together with its cofactors protein activator of PKR (PACT) and double-stranded RNA-binding domain (dsRBD) protein Tar RNA binding protein (TRBP) cleaves the precursor-miRNA (pre-miRNA) into a ~ 22 nt miRNA-3p/miRNA-5p duplex with a 2 nt overhang at each of its 3′ end by removing a pre-miRNA hairpin loop [1]. Both, germline and somatic mutation in Dicer have been identified and linked with a variety of different types of cancer [2]. Recurrent somatic mutations of Dicer and inactivation of its RNase IIIb domain by mutation of metal-ion-binding sites D1709 and D1705, which results in complete loss of 5p-derived mature miRNAs and partial reduction in 3p-derived mature miRNAs, have recently been reported in non-epithelial ovarian cancer and for D1709 in thyroid carcinoma [3]. There is no available data on D1705 and D1709 mutation status of Dicer in PCMM or CMMM. Therefore the present pilot study was performed to examine a heterogeneous group of PCMM and CMMM specimens regarding their mutational status of D1709 and D1705 in the RNAse IIIb domain of Dicer.

Report

The present study was approved by the Ethical Review Board of the Ruhr-University Bochum, Germany (registration number: 3265-08) and was performed at an academic university hospital within the declaration of Helsinki.

Tumor Samples

A total of 40 specimen consisted of 30 PCMM and 10 CMMM paraffin embedded tissue samples. The PCMM group consisted of 12 nodular melanomas, 10 superficial spreading melanomas, 5 acral lentiginous melanomas, and 3 amelanotic melanomas. CMMM specimens were included in the analyses as it has been shown that they have an increased mutation rate compared to PCMM [4].

DNA Isolation and Amplification

DNA was extracted from paraffin embedded specimens using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden/Germany) according to the manufacturer's protocol. DNA quality was quantified photometrically using a spectrophotometer at 260 nm and 280 nm. DNA amplification was performed by means of polymerase chain reaction (PCR) in 50 μl reactions using the HotStarTaq® *Plus* DNA Polymerase (Qiagen, Hilden/Germany) and TRIO-Thermoblock (Biometra, Göttingen/Germany). The following primer pairs were used for Dicer1 Exon 26 as 1st forward and reverse primer: 5′-ACGGTTCCACTTCGGATCCCCT-3′ and 5′-AGCCAGCGATGCAAAGATGGTGT-3′. The cycle conditions were 95° for 5″ (initial denaturation), 94° for 30 s then 45 cycles of 94° for 30 s (denaturation), 60° for 30 s (annealing), 72° for 1″ (extension), followed by 10″ at 72°(end-extension).

DNA Sequencing and Sequence Data Analysis

Sequence analysis was performed by Seqlab (Göttingen, Germany) according to the method of Sanger et al. [5]. The final reaction volume of 20 μl consisted of 2 μl of BigDye Terminator v. 3.1 mix (Applied Biosystems), 3 μl of dilution buffer (Applied Biosystems), 30 pmol of primer, and 0.2 μg of template DNA. The primer pairs were the same as described above. The cycle conditions were 96° for 2″ (initial denaturation), then 35 cycles of 96° for 10 s (denaturation), 60° for 15 s (annealing), 60° for 3″ (extension), followed by 1″ at 72°(end-extension). After removal of excess dye terminators with CleanSeq magnetic bead sequencing reaction clean up kit, samples were resuspended off the beads in 50 μl double distilled water (ddH2O). A 96 well PCR plate with 10 μl of each sample with an additional 10 μl of ddH2O in each well was used. The PCR plate was loaded onto an Applied Biosystems 3730xl automated DNA sequencing instrument according to the manufacturer's instructions. After samples were electrophoresed using 50 cm capillary arrays and POP-7 polymer, data were analyzed by using PE-Biosystems (version 3.7) of Sequencing Analysis. All PCMM and CMMM specimen showed wildtype status for D1705 and D1709. No mutation was detected.

Discussion

Expression levels of the microRNA machinery on mRNA level have been investigated showing both up- and down-regulation for Dicer in a variety of malignancies including PCMM. Reduced expression of Dicer has shown to be an independent predictor of poor survival and to be present in advanced metastatic melanoma [6]. Dicer expression in melanoma has shown to be inversely correlated with melanoma progression and to be up-regulated on protein level [7]. Besides Sox4, adenosine deaminase acting on RNA enzyme 1 (ADAR1) has shown to regulate Dicer via the tumor suppressor let-7 [8]. Interestingly Dicer expression on mRNA level showed no differences in melanoma compared to benign melanocytic naevi or melanoma metastases. An upregulation of Dicer at protein level together with no change of mRNA expression could be counted as a first hint for a possible somatic mutation of the Dicer gene. Mione and Bosserhoff analyzed the COSMIC (Catalogue Of Somatic Mutations In Cancer at www.cancer.sanger.ac.uk) database regarding mutations in miRNA processing enzymes and rarely found mutations with no obvious recurrence or hot spots [9]. When searching for Dicer mutation in the latter database we were able to find 26 Dicer mutations in 694 analyzed melanoma samples (3.8 %). Zhang et al. were the first who identified high frequency copy number abnormalities of Dicer in melanoma [10]. Previously described somatic mutations in Dicer are heavily concentrated in the gene segment coding for metal-ion-binding sites D1705 and D1709 with the absence of tissue specificity, which leads to relatively lower 5p miRNA abundance compared to wild-type [2, 3]. We did not observe a single mutation in our heterogeneous group of PCMM and CMMM. We assume that oncogenic miRNA profiles observed in PCMM cannot be attributed to hotspot mutations as previously described for D1705 and D1709 in other malignant tumors such as Sertoli-Leydig cell or Yolk sac tumor. Nevertheless, further studies are needed to investigate the mutational status of other members of the miRNA machinery in general and in other functional domains of Dicer specifically since previous studies strongly indicate that alterations of miRNA maturation and expression can be critical in melanoma.

Compliance with Ethical Standards

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Ethics This study conforms to the applicable local requirements regarding ethical and investigational committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in medical research (Ethical

Review Board of the Ruhr-University Bochum, Germany, registration number: 3265-08).

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