ROBERT KOCH INSTITUT

Originally published as:

Janine Reiche, Georg Pauli and Heinz Ellerbrok.
Differential expression of human endogenous retrovirus K transcripts in primary human melanocytes and melanoma cell lines after UV irradiation.
(2010) Melanoma Research, 20, pp. 435-440.

DOI: 10.1097/CMR.0b013e32833c1b5d

This is an author manuscript.
The definitive version is available at: http://pt.wkhealth.com

Differential expression of human endogenous retrovirus K transcripts in primary human melanocytes and melanoma cell lines after UV irradiation

Janine Reiche, Georg Pauli and Heinz Ellerbrok

Abstract

Human endogenous retroviruses (HERVs) are discussed as causative agents of various diseases including cancers. Expression of endogenous retroviral sequences can be induced by ultraviolet (UV) light, which is also considered as a cofactor in the development of cutaneous melanoma.
Therefore, we investigated whether ultraviolet C (UVC) induces HERV-K rec and np9 expression in normal human epidermal melanocytes (NHEM) and in melanoma cell lines. NHEM and four different melanoma cell lines were irradiated with 10 and 30 mJ/cm2 UVC, respectively.
Expression of the HERV-K transcripts rec and np9 was measured 0, 6, 12, and 24 h after UV exposure by quantitative real-time PCR. In NHEM, HERV-K rec expression was significantly induced 24 h after UV exposure with 10 mJ/cm2 UVC, whereas np9 expression transiently increased 6 and 12 h after irradiation with both UV doses. In contrast, in melanoma cell lines.

Introduction

Cutaneous malignant melanoma is the leading type of skin cancer with increasing incidence worldwide [1]. It is caused by uncontrolled proliferation of epidermal melanocytes.
Exposure to sunlight is a significant risk factor for melanoma development [2]. In C57BL/6 mouse melanomas, the melanoma-associated retrovirus MelARV was expressed, and infection of normal melanocytes with MelARV induced malignant transformation [3]. Moreover, in human melanomas and metastases, but not in normal melanocytes, expression of the endogenous retrovirus K (HERV-K) protein Rec was detected [4,5].
HERV-K Rec and Np9 proteins are associated with tumorigenesis [6]. However, little is known about HERVK expression in irradiated melanocytes and melanoma cells.
To investigate whether HERV-K expression might be associated with transformation of primary melanocytes, we focussed our attention on ultraviolet C (UVC) as a tumor inducer [7] and the HERV-K rec and np9 transcription as markers for oncogenesis.
Methods Normal human epidermal melanocytes (NHEM; Promo-Cell, Germany) from two donors (referred to as NHEM-1and NHEM-2) and cells of the melanoma cell lines G-361, MEWO, MEL JUSO and GR-M were seeded into 50mm dishes (Nunc, Germany) (0.5–1_106/dish). NHEM were grown in supplemented melanocyte growth medium (PromoCell). The human melanoma cell lines were grown in RPMI-1640 medium (GR-M, MEL JUSO), in EMEM medium (MEWO), and in McCoy’s 5A medium (G-361), each supplemented with 10% FCS and 2mmol/l L-glutamine. Cells were maintained in a humidified air–5% CO2 atmosphere at 371C.
The next day, each cell culture was washed with 2 ml PBS. All liquid was taken off and cells were irradiated with B4 mW/cm2 for both 2.5 s (equal to 10 mJ/cm2) and 7.5 s (equal to 30 mJ/cm2) UVC (254 nm) in an UV Stratalinker 2400 (Stratagene, The Netherlands). Cell cultures were replenished with fresh culture medium and incubated for 0, 6, 12, and 24 h. At the timepoints given all cell cultures were washed with 2 ml PBS and lysed in 600 ml RLT lysis buffer (Qiagen, Germany). Thus samples for t0 were lysed approximately 5 min after the onset of UV irradiation. Unirradiated cultures served as controls and were treated identically except that UV treatment was omitted. For melanoma cell lines, all assays were performed in triplicate and in two independent experiments.
Analyses for melanocytes were also performed in triplicate but only once per donor.
RNA was extracted using the RNeasy Mini kit (Qiagen).

Residual DNA was removed by DNAse I digestion (Ambion, United Kingdom). cDNA was synthesized with the Superscript II RT kit according to the manufaturer's protocol (Invitrogen, Germany). Oligo(dT)18 primers and 1 mg RNA were incubated for 5 min at 651C, then snapcooled to 41C. Buffer, DTT, dNTPs, and 1 ml RTenzyme were added and the reaction mix was incubated for 50 min at 371C and for 15 min at 701C. HERV-K rec and np9 expression was determined by specific quantitative real-time PCR assays [25 ml reaction mix using 2.5 ml of cDNA product, 6.25 ml universal master mix (Applied Biosystems, Germany), 300 nmol/l of each of the primers and 100nmol/l probe (Table 1)] in the Applied Biosystems 7700 sequence detection system. Amplification
was carried out for 2 min at 501C, 5 min at 951C, followed by 45 cycles for 15 s at 951C and 1 min at 601C.
The number of rec-mRNA and np9-mRNA copies was normalized to 106 copies of L13-mRNA. L13 primers and probe were used as published earlier [8]. Data were analyzed with the one-way analysis of variance (post-hoc Tukey B, P<0.05).

Results

In untreated NHEM from both donors, no rec mRNA was detected (Fig. 1a and b). Irradiation with 10 mJ/cm2 UVC significantly induced rec expression 24 h after UV exposure, reaching levels of 300 and 400 copies in NHEM-1 and NHEM-2. UV irradiation with 30 mJ/cm2 had no influence on rec expression. Basal expression of np9 was between 40 and 110 copies in NHEM from both donors (Fig. 2a and b). After UV irradiation with both 10 and 30 mJ/cm2 np9 expression transiently increased to levels between 190 and 300 copies 6 and 12 h after UVexposure.
Twenty-four hours after irradiation np9 expression had returned to the initial level seen at t0.
In melanoma cell lines, MEWO and G-361, basal rec expression was highest with 10 100–30 800 and 8400–15 800 copies, respectively (Fig. 1d and e). Although rec expression was lower but clearly detectable in MELJUSO (<80 copies) (Fig. 1c) it was undetectable in GRM (not shown). After UV treatment rec expression was downregulated in MEWO and G-361, remained undetectable
in GR-M but was significantly increased after irradiation with both 10 and 30 mJ/cm2 UVC in MEL JUSO. In all four melanoma cell lines, basal np9 expression was between 50 and 1500 copies, however, in contrast to rec expression with some variations during the course of the experiment (Fig. 2c–f). Stimulation of np9 expression was seen in MEL JUSO in which the expression was significantly enhanced 6 and 12 h after UV exposure with 10 mJ/cm2 while np9 expression was reduced in MEWO and G-361 cells for both UVC conditions from t0 to 24 h (Fig. 2d and e).

Discussion

HERV-K expression is linked to a variety of cancer types including melanomas [4,5,9–12]. A recent study suggesting that endogenous retroviruses are associated with melanoma showed that HERV-K-MEL antigens are targeted by cytolytic T lymphocytes in melanoma patients [13]. The antigen was expressed in the majority of nevi, cutaneous, and ocular melanomas but not in normal
tissues such as keratinocytes and melanocytes. HERV-K protein expression was highly specific in patients with primary melanomas and metastases [5]. Increased malignancy of melanoma cell lines has been correlated with HERV-K gene expression as a response to stress [14]. So far for two HERV-K genes, np9 and rec, expressed from two HERV-K elements, type 1 and type 2, respectively [15,16], some evidence has been collected for their involvement in tumor induction [6]. We therefore investigated the effect of UVC irradiation on HERV-K rec and np9 expression in NHEM and compared it with different melanoma cell lines.
Investigation of basal rec and np9 expression showed that np9 encoded by HERV-K type 1 provirus was detected in NHEM and in all melanoma cell lines. In contrast, rec expressed from HERV-K type 2 was not detected in NHEM and only in three out of four melanoma cells.
Remarkable differences between the presence of rec and np9 have also been shown for primary tumor samples such as germ-cell tumors [16], mammary carcinomas [16], and melanoma tissues [17]. Possibly, the expression of HERVK elements is regulated differentially and is cell-type
specific [18]. Indeed, there are sequence differences in LTRs being linked to tissue-specific expression of HERVK elements [19], although they could not be associated with a particular type of provirus. As

expression levels for HERV elements are developmentally regulated in both somatic and germline tissue [20], this may also explain the observed differences of rec and np9 levels between NHEM and melanoma cell lines.

After UVC irradiation of NHEM expression of HERV-K, rec was significantly induced 24 h after UV exposure, whereas np9 expression transiently increased 6 and 12 h after irradiation and returned to control levels after 24 h.

On the contrary, rec and np9 expression was downregulated or remained unchanged after UV treatment in most of the melanoma cell lines. Only MEL JUSO cells showed an expression pattern comparable with NHEM post-irradiation treatment and lysis of the cells at t0. This suggests that the observed UV-dependent regulation of np9 and rec in NHEM and melanoma cell lines might be because of distinct biological mechanisms reflecting the actual state of the cells.

In NHEM, expression of rec was only induced after UV irradiation with 10 mJ/cm2, whereas np9 expression was increased after irradiation with both 10 and 30 mJ/cm2 UVC.
Earlier, expression of human endogenous retroviral pol sequences was described for primary epidermal keratinocytes and for a spontaneously immortalized keratinocyte cell line (HaCaT) after UV treatment [21]. Both keratinocytes and HaCaT irradiation with low doses of UVB (10 mJ/cm2) or even high doses of UVA (up to 20 J/cm2) had little or no effect on HERV expression. In contrast, irradiation with 30 mJ/cm2 UVB led to transcriptional activation of some retroviral pol sequences 6 and 24 h after UV irradiation in both cell types and inhibition of certain pol sequences in keratinocytes 6 h after UV exposure.
Similarly, our experiments point to a UV dose-dependent expression of HERV-K sequences. Both, the published activation of the endogenous retroviral pol sequences and the observed increase of rec and np9 transcripts in our study, are time-dependent. The late response to UV irradiation in keratinocytes may be mediated through an extracellular loop involving the action of growth factors and interleukins [21].
Nonetheless, the reason for the late or transient expression of rec or np9 transcripts in NHEM cannot be explained so far. It was shown recently that human melanoma cell lines undergo a transition from an adherent to a nonadherent more malignant phenotype [14]. These changes, initially observed as a spontaneous event in cell cultures, can also be induced experimentally by exposing melanoma cells to starvation. Moreover, these changes are associated both with the production of HERV-K-related virus-like particles and the expression of HERV-K env. It is assumed that the activation of HERV-K expression is a key element during melanoma progression contributing to morphological and functional cell changes.
Therefore, on the basis of our data an involvement of the HERV-K Rec and/or Np9 protein in melanoma development is conceivable.
For the first time, we have shown an UV-induced expression of HERV-K rec and np9 transcripts in NHEM. Both transcripts were activated upon UVC irradiation but at different timepoints after irradiation suggesting that they could have different biological functions during the development of melanoma. We might have shown that regulation of HERV-K rec and np9 expression in melanoma cell lines was a much faster process than in NHEM. Furthermore, although the basal expression level of rec in untreated melanoma cells was almost unchanged in the course of the experiment we observed substantial variation for np9. As rec and np9 are expressed from different HERV-K elements, this might be explained through differences in the transcriptional regulation between these elements in the cell lines that were analyzed for this study. So far, a potential role of these proteins in the process of transformation remains unclear and further studies are required to determine whether and how the expression of rec and np9 contributes to neoplastic transformation of human melanocytes.

Acknowledgement

The authors thank A. Nitsche and M. Reiche for helpful discussions.

References

1 Dennis LK. Analysis of the melanoma epidemic, both apparent and real: data from the 1973 through 1994 surveillance, epidemiology, and end results program registry. Arch Dermatol 1999; 135:275–280.
2 Gilchrest BA, Eller MS, Geller AC, Yaar M. The pathogenesis of melanoma induced by ultraviolet radiation. N Engl J Med 1999; 340:1341–1348.
3 Li M, Huang X, Zhu Z, Gorelik E. Sequence and insertion sites of murine melanoma-associated retrovirus. J Virol 1999; 73:9178–9186.
4 Buscher K, Trefzer U, Hofmann M, SterryW, Kurth R, Denner J. Expression of human endogenous retrovirus K in melanomas and melanoma cell lines. Cancer Res 2005; 65:4172–4180.
5 Muster T,Waltenberger A, Grassauer A, Hirschl S, Caucig P, Romirer I, et al. An endogenous retrovirus derived from human melanoma cells. Cancer Res 2003; 63:8735–8741.
6 Denne M, Sauter M, Armbruester V, Licht JD, Roemer K, Mueller-Lantzsch N. Physical and functional interactions of human endogenous retrovirus proteins Np9 and rec with the promyelocytic leukemia zinc finger protein. J Virol 2007; 81:5607–5616.
7 Devary Y, Gottlieb RA, Smeal T, Karin M. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell 1992; 71:1081–1091.
8 Radonic A, Thulke S, Mackay IM, Landt O, Siegert W, Nitsche A. Guideline to reference gene selection for quantitative real-time PCR. Biochem Biophys Res Commun 2004; 313:856–862.
9 Herbst H, Sauter M, Mueller-Lantzsch N. Expression of human endogenous retrovirus K elements in germ cell and trophoblastic tumors. Am J Pathol 1996; 149:1727–1735.
10 Wang-Johanning F, Frost AR, Jian B, Epp L, Lu DW, Johanning GL. Quantitation of HERV-K env gene expression and splicing in human breast cancer. Oncogene 2003; 22:1528–1535.
11 Boyd MT, Foley B, Brodsky I. Evidence for copurification of ERVK-related transcripts and a reverse transcriptase activity in human platelets from patients with essential thrombocythemia. Blood 1997; 90:4022–4030.
12 Depil S, Roche C, Dussart P, Prin L. Expression of a human endogenous retrovirus, HERV-K, in the blood cells of leukemia patients. Leukemia 2002; 16:254–259.
13 Schiavetti F, Thonnard J, Colau D, Boon T, Coulie PG. A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. Cancer Res 2002; 62:5510–5516.
14 Serafino A, Balestrieri E, Pierimarchi P, Matteucci C, Moroni G, Oricchio E, et al. The activation of human endogenous retrovirus K (HERV-K) is implicated in melanoma cell malignant transformation. Exp Cell Res 2009; 315:849–862.
15 Lower R, Boller K, Hasenmaier B, Korbmacher C, Muller-Lantzsch N, Lower J, et al. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc Natl Acad Sci USA 1993; 90:4480–4484.
16 Armbruester V, Sauter M, Krautkraemer E, Meese E, Kleiman A, Best B, et al. A novel gene from the human endogenous retrovirus K expressed in transformed cells. Clin Cancer Res 2002; 8:1800–1807.
17 Buscher K, Hahn S, Hofmann M, Trefzer U, Ozel M, Sterry W, et al. Expression of the human endogenous retrovirus-K transmembrane envelope, Rec and Np9 proteins in melanomas and melanoma cell lines. Melanoma
Res 2006; 16:223–234.
18 Casau AE, Vaughan JE, Lozano G, Levine AJ. Germ cell expression of an isolated human endogenous retroviral long terminal repeat of the HERV-K/ HTDV family in transgenic mice. J Virol 1999; 73:9976–9983.
19 Sugimoto J, Matsuura N, Kinjo Y, Takasu N, Oda T, Jinno Y. Transcriptionally active HERV-K genes: identification, isolation, and chromosomal mapping. Genomics 2001; 72:137–144.
20 Okahara G, Matsubara S, Oda T, Sugimoto J, Jinno Y, Kanaya F. Expression
analyses of human endogenous retroviruses (HERVs): tissue-specific and developmental stage-dependent expression of HERVs. Genomics 2004; 84:982–990.
21 Hohenadl C, Germaier H, Walchner M, Hagenhofer M, Herrmann M, Sturzl M, et al. Transcriptional activation of endogenous retroviral sequences in human epidermal keratinocytes by UVB irradiation. J Invest Dermatol 1999; 113:587–594.

Tables and Figures

Table 1 Characteristics of oligonucleotide primers and probes used for quantitative real-time PCR

F, 60-carboxy-fluorescein label; p, 30 phosphate; X, tetramethyl-rhodamin carrying thymidine.
aAll oligonucleotides were purchased from TIB MOLBIOL GmbH (Berlin, Germany).
bThese primers are designed to specifically bind to the rec and np9 mRNA, respectively, by spanning the specific splice junctions. Splice junctions are indicated
with a slash.

Gene	Name	Oligonucleotide sequence (5′–3′)[a]	Position	Accession number	Polarity
np9	np9 s[b]	CTCCACGGAGATG/TCTGCA	6481–6493/8118–8123	AF164509	+
	np9 as	CCCACATTTCCCCCTTTTC	8199–8217	AF164509	–
	np9 TM	F-CAGCTCCGAAGAGACAGCGACCATCX-p	8135–8159	AF164509	+
rec	rec s[b]	ACATCTACAGTCAGCCTTACG/ACATT	1482–1502/6860–6864	Y17832	+
	rec as[b]	TTGG/GTACACCTGCAGACACC	7134–7137/8837–8853	Y17832	–
	rec TM	F-TGCTCGATTGCGATGTCTCCGTCX-p	6916–6939	Y17832	–

Figure 1. Ultraviolet (UV)-dependent human endogenous retroviruses-K rec expression. Mean˘ SD (n=3) of rec mRNA was determined 0, 6, 12, and 24 h after UV irradiation with 10 and 30 mJ/cm2 ultraviolet C versus nonirradiated control cells in normal human epidermal melanocytes (NHEM) (a and b) and melanoma cell lines (c–e). Note the different scaling between NHEM and melanoma cell lines. The capital letters above each bar (A, B, C) indicate the mRNA cultures at each timepoint that were statistically significantly different from each other (analysis of variance post-hoc Tukey B, P<0.05). That means that averages followed by the same capital letters do not differ statistically and vice versa.

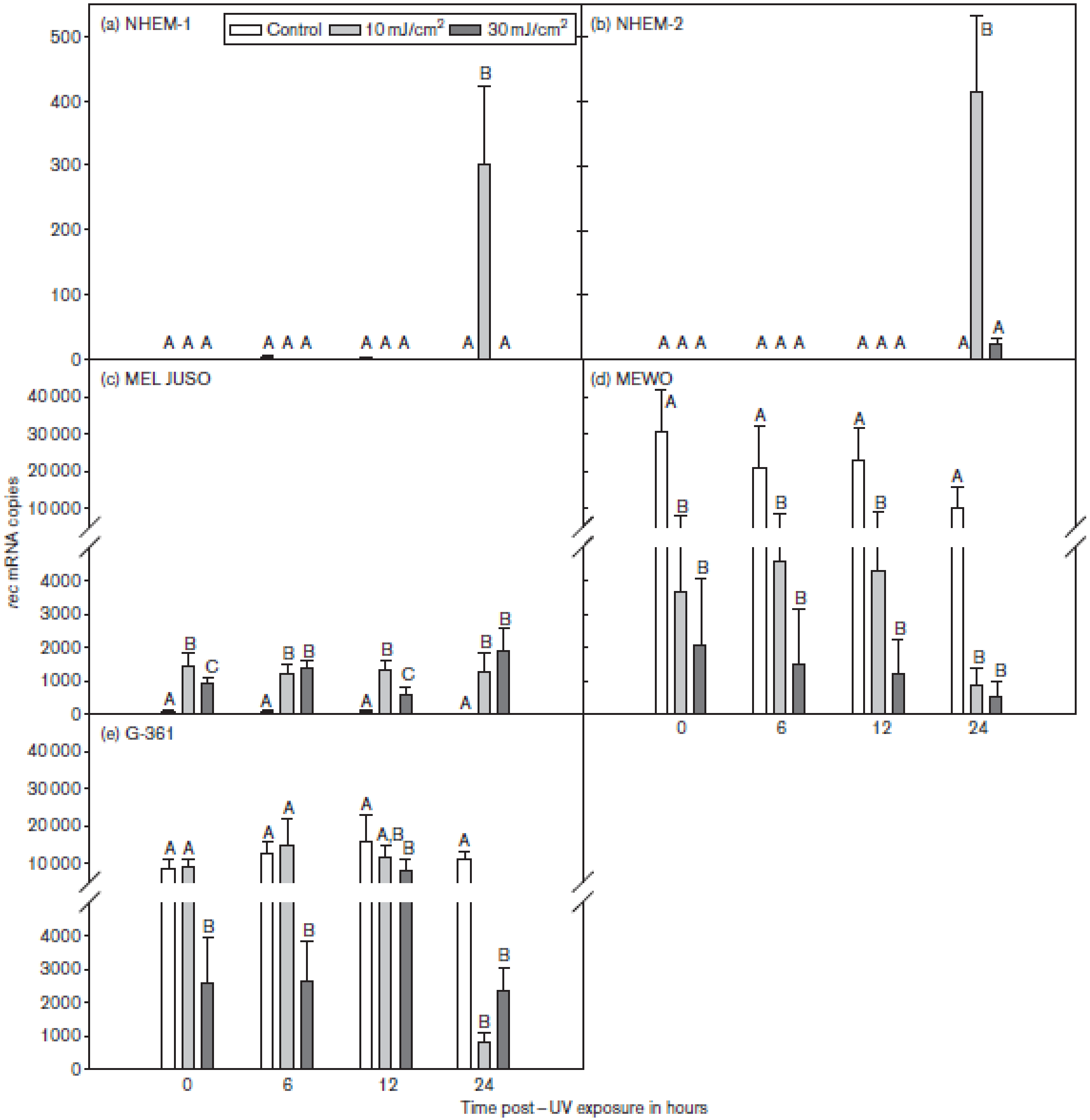

Figure 2. Ultraviolet (UV)-dependent human endogenous retrovirus-K np9 expression. Mean˘ SD (n=3) of np9 mRNA was determined 0, 6, 12, and 24 h after UV irradiation with 10 and 30 mJ/cm2 Ultraviolet C versus nonirradiated control cells in normal human epidermal melanocytes (NHEM) (a and b) and melanoma cell lines (c–f). Note the different scaling for melanoma cell line GR-M (f). The capital letters above each bar (A, B, C) indicate the mRNA cultures at each timepoint that were statistically significantly different from each other (analysis of variance post-hoc Tukey B, P<0.05). That means that averages followed by the same capital letters do not differ statistically and vice versa.