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Expression and Activity of Alcohol and Aldehyde Dehydrogenases in Melanoma Cells and in Melanocytes

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ABSTRACT

Disturbances in vitamin A metabolism are an important attribute of some cancer cells. Most evidence point that these disturbances lead to decreasing of the retinoic acid concentration in tumor cells. Up to now, in benign and malignant skin cells the features of vitamin A metabolism with its participating enzymes are not entirely understood. Alcohol and aldehyde dehydrogenases (ALDH) are involved in the retinol metabolism, oxidizing retinol, and retinal in retinoic acid or reducing retinal in retinol. In this work we investigated the expression and enzymatic activity of alcohol and ALDH in melanoma cells compared to their benign counterparts. We demonstrated that melanoma cell lines and melanocytes despite similar pattern of the enzyme expression, show different general ALDH activity. Retinal, the substrate of ALDH, could stimulate the ALDH activity through up-regulation of retinaldehyde dehydrogenase 1 and aldehyde dehydrogenase 6. Furthermore, we found that retinoids regulate alcohol dehydrogenase activity, probably via effects on alcohol dehydrogenase expression at the post-transcriptional level. We suggest that melanoma cells in contrast to melanocytes should favor the retinal reduction over its oxidation. The decreasing cellular amount of the precursor molecules of retinoic acid could result in a changed gene regulation in melanoma cells. J. Cell. Biochem. 113: 792–799, 2012.

KEY WORDS: MELANOMA; MELANOCYTES; ALCOHOL DEHYDROGENASES; ALDEHYDE DEHYDROGENASES

Two important physiological roles are assigned to vitamin A: participation in the visual cascade [Wald, 1968] and regulation of growth and development [De Luca, 1991]. For a long time it has been known, especially in skin, that retinoids, particularly retinoic acid, have critical functions to regulate gene expression, and appear to modulate tumor development and progression [Jarrett and Spearman, 1970]. All-*trans* retinoic acid (ATRA) is the major physiological active form of vitamin A, regulating the expression of different genes both in the embryo and in the adult organism [Ross et al., 2000].

In order to regulate gene expression, all-*trans* retinol (ATRol) needs to be oxidized to ATRA [Duester, 2000]. All-*trans* retinal (ATRal), in turn, can be produced through oxidation of ATRol or as an intermediate metabolite [Napoli and Race, 1988] in an ATRol-independent metabolic pathway through cleavage of β-carotene. In liver, the storage form of vitamin A is retinyl ester (RE), which can be hydrolyzed to ATRol and subsequently oxidized to ATRal [Napoli, 1996]. Enzymes which are involved in the ATRol oxidation belong to two families of retinol dehydrogenases: cytosolic alcohol dehydrogenases (ADH1, ADH2, ADH4, and ADH7) and microsomal retinol dehydrogenases—short-chain dehydrogenase/reductase (RoDH1 and RoDH4) [Duester, 2000; Duester et al., 2003]. Both enzyme families have wide substrate specificity taking part in the metabolism of ethanols, sterols, and retinoids [Duester, 2000]. Cytosolic aldehyde dehydrogenases (ALDH), also known as retinaldehyde dehydrogenases (RALDH), oxidize ATRal to ATRA in an irreversible reaction [Labrecque et al., 1995; Molotkov and Duester, 2003]. Alternatively, ATRal can be also reduced in ATRol catalyzed by short-chain aldehyde dehydrogenases/reductases (AD/R, RDH12, and retSDR1) and by ADH8 [Duester, 2000]. ALDH show

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Additional Supporting Information may be found in the online version of this article.

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also wide substrate specificity oxidizing both retinal and other important aldehydes [Duester, 2000].

Disturbances in vitamin A metabolism are the attribute of some cancer cells. The most evidence point that such disturbances lead to decreasing of the ATRA concentration in tumor cells [Mongan and Gudas, 2007]. It was shown that the ATRA production in breast cancer and in prostate cancer cells is reduced comparing to normal epithelial cells [Mira et al., 2000; Kim et al., 2005]. Thus, ADH and ALDH could have an impact on the vitamin A metabolism in cancer [Jelski and Szmitkowski, 2008].

ATRal is reduced in ATRol in a first step within the esterification pathway of the vitamin A metabolism. The key enzyme of the esterification is lecithin/retinol acyltransferase (LRAT) which metabolizes ATRol in all-*trans* RE in several tissues, including liver, lung, pancreas, intestine, testis, and the retinal pigment epithelium (RPE) [Ruiz et al., 1999]. Isomerization of RE in 11-*cis* retinol (11cisRol) in the RPE is catalyzed by the RPE65 protein [Xue et al., 2004; Moiseyev et al., 2005]. RPE65 has been also found to be expressed in human keratinocytes functioning probably as a receptor for the plasma retinol-binding protein [Hinterhuber et al., 2004]. Our recent data about vitamin A metabolism in benign and malignant melanocytic skin cells showed that ATRal could be metabolized in 11cisRol by melanoma cell lines [Amann et al., 2011], making the vitamin A metabolism in melanoma cells similar to it in the RPE. Besides, melanoma cells favor ATRal reduction over its oxidation into ATRA. We proposed that esterification of ATRol by LRAT with the subsequent isomerization of RE by RPE65 could be important for removal of ATRol as a substrate for ATRA production in melanoma cells [Amann et al., 2011].

However, another possibility could be that melanoma cells express low level of ADH and/or ALDH to metabolize ATRol and ATRal into ATRA, or the activity of these enzymes is disturbed. In this work we analyzed expression of ADH and ALDH and their enzymatic activity to get a hint why melanoma cells favor the reduction of ATRal over its oxidation into ATRA.

MATERIALS AND METHODS

MATERIALS

Actinomycin D, ATRA, ATRol, ATRal, and NAD^+ (monosodium salt) were purchased from Sigma–Aldrich (Germany). A polyclonal (monospecific) goat antibody against RALDH1 was purchased by Everest Biotech Ltd (UK). $NADP^+$ (monosodium salt) was purchased by Calbiochem (Germany). Protease inhibitor cocktail tablets were purchased from Roche Diagnostics GmbH (Germany). Normal human mRNA comprised pools of 1–12 donors and were from a commercial source (Clontech, Palo Alto).

CELL CULTURES

Established melanoma cell lines (Ma-Mel-01, -04, -05, -11, -12, -17, -19, -37b, 42b, -47, -52, UKRV-Mel-02, -06a, -21a, -27, -31, MeWo, and SK-Mel-023) from the Skin Cancer Unit at the German Cancer Research Center, Heidelberg, Germany were cultivated in RPMI-1640 medium. The keratinocyte cell line HaCaT [Boukamp et al., 1988] was cultivated in DMEM High Glucose (4.5 g/L) medium. Melanocyte cells (Melanocyte01, Melanocyte02, and Melanocyte03) are from a commercial source (Promocell, Heidelberg, Germany) or they were derived from foreskin in our lab (Melanocyte04 and Melanocyte05) as described [Amann et al., 2011]. Melanocytes were cultivated in MEM Alpha Medium with ribo- and deoxyribonucleotides (Promocell). All media were supplemented with 10% fetal calf serum, L-glutamine (2 mM), and penicillin/streptomycin solution (5 U/ml), and were purchased from PAA Laboratories (Coelbe, Germany). Cell lines were cultivated at 37°C and 5% CO_2.

RNA isolation and analysis, RT-PCR analysis, and real-time RT-PCR were performed as described in Bazhin et al. [2008].

CELL FRACTIONATION

Lysated cells ($\sim 1.5 \times 10^7$) were homogenized in 10 mM HEPES buffer (pH 7.9), containing 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, protease inhibitors cocktail, and 0.1% Triton X-100. After 10 min centrifugation at 800*g*, the pellet containing nuclei was resuspended in 20 mM HEPES buffer (pH 7.9), containing 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, and a protease inhibitor cocktail, and was extracted for 30 min at 4°C on a rocking platform. After 30 min centrifugation at 4,000*g*, the supernatant was used.

For the microsomal fraction obtaining, cells ($\sim 1.5 \times 10^7$) were homogenized in 60 mM Na_2HPO_4 buffer (pH 8.0) containing 570 mM sucrose, 1 mM DTT, and protease inhibitor cocktail and then were disrupted thermally by freezing in fluid nitrogen and thawing at 30°C three times. After centrifugation at 10,000*g* and 4°C for 30 min supernatant with protein fraction was separated in cytosolic fraction and microsomal fraction by 1 h centrifugation at 100,000*g* and 4°C. The supernatant containing the cytosolic protein fraction was removed. The pellet was resuspended in Na_2HPO_4 buffer mentioned above and centrifuged at 100,000*g* and 4°C for 15 min. After removal of the supernatant this washing step was repeated two times. Subsequently, the microsomal fraction was resuspended in sucrose-free 60 mM Na_2HPO_4 buffer and disrupted by UP50H ultrasonic treatment (Hielscher Ultrasonics GmbH, Teltow, Germany) for 1 min on ice.

MEASUREMENT OF THE GENERAL ALDH, ADH, AND AD/R ACTIVITY

Measurement of the general ALDH activity was performed as described previously [Moreb et al., 2005] with minor modifications. Briefly, cells were lysed in 50 mM Tris buffer (pH 8.0) containing 25 mM EDTA, 5 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 0.1% sarcosyl, and proteinase inhibitor cocktail. After centrifugation at 10,000 rpm for 15 min at 4°C, the supernatant was collected and used for determination of ALDH activity. Aliquots of 100 μl incubated at 37°C with the addition of protein extract, 5 mM NAD^+, and 5 mM propionaldehyde (as a substrate) were used to measure spectrophotometrically the change in absorbance at 340 nm over 5 min. A probe without substrate was used as a control for the endogenous rate of NAD^+ reduction.

The cytosolic and microsomal protein fractions obtained from melanoma cells and melanocytes were used as source of ADH. Measurement of the general ADH activity was conducted as described previously [Koivusalo et al., 1989] with modifications. Briefly, aliquots of 100 μl in 60 mM Na_2HPO_4 buffer (pH 8.0)

incubated at 37°C with the addition of protein extract, 1.5 mM NAD^+ (or 1.5 mM $NADP^+$ for microsomal fraction), and 10 mM ethanol (as a substrate) were used to measure the change in absorbance at 340 nm over 5 min spectrophotometrically. A probe without substrate was used as a control for the endogenous rate of $NAD^+/NADP^+$ reduction.

The reduction of propionaldehyde by AD/R was monitored spectrophotometrically at 340 nm as previously described [Tabakoff and von Wartburg, 1975] with modifications in 100 μl of 60 mM Na_2HPO_4 (pH 8.0) reaction mixture containing NADH or NADPH (0.15 mM), cytosolic fraction of cells, and 1 mM propionaldehyde (as a substrate) at 37°C. A probe without substrate was used as a control. All ALDH, ADH, and AD/R data are expressed in catal (cat) per 1 g protein.

Western blot analysis was performed as described elsewhere [Bazhin et al., 2007].

IMMUNOCYTOLOGY

For immunocytology 1×10^5 cells were spun per slide and fixed in 3.7% formaldehyde. Cells and sections were preabsorbed using the Avidin/Biotin Blocking solution (Vector Laboratories, USA) and subsequently 5% normal swine serum. Then the slides were incubated overnight with antibodies against RALDH1 (4 μg/ml). After incubation with a swine anti-goat antibody (1:800) coupled to biotin for 1 h and subsequent incubation with a streptavidin–biotin–phosphatase complex, specific staining was visualized by alkaline phosphatase substrate (all components from the Alkaline Phosphatase Rabbit IgG ABC Kit and AP substrate Kit; Vector Laboratories) as described by the manufacturer. Hemalaun solution was used for counterstaining.

STATISTICAL ANALYSIS

Statistical analysis was performed by Student's *t*-test with significant differences determined as $P < 0.05$.

RESULTS

ADH AND ALDH ARE PRESENT ON mRNA LEVEL IN MELANOMA CELLS AND IN NORMAL MELANOCYTES

As described previously [Amann et al., 2011], reduction of ATRal in melanoma cell lines is higher than in melanocytes. Thus, we first wanted to know whether the difference in ATRal reduction would be a cause of different expression of enzymes involved in ATRal metabolism. We performed a gene expression analysis with conventional RT-PCR for ADH involved in ATRol and 11cisRol oxidation (ADH1, -2, -4, -7, RoDH1 and RoDH4, RDH5 and RDH11), for AD/R involved in ATRal reduction (RDH12 and retSDR1) and for aldehyde dehydrogenases (RALDH1 and -2, and ALDH6) oxidizing ATRal to ATRA. Results of the analysis are summarized in Table I. Hence, both benign and malignant melanocytic skin cell counterparts possess ADH and ALDH on mRNA level.

MELANOMA CELL LINES AND MELANOCYTES SHOW DIFFERENT GENERAL ALDH AND AD/R ACTIVITIES WHICH CAN BE MODULATED BY ATRal

First, we measured the general ALDH and AD/R activities in five melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27) and in melanocytes obtained from three individuals. This analysis (Fig. 1A) revealed that melanoma cell lines possess a lower general ALDH activity ($6.5 \times 10^{-4} \pm 2.57 \times 10^{-4}$ cat/g) than

TABLE I. mRNA Expression of the Enzymes Involved in Retinol Oxidation (RDH, ADH, and RoDH), in ATRal Reduction (RDH12 and retSDR1), and in ATRal Oxidation (RALDH and ALDH6) in Normal and Malignant Skin Cells

	RDH		ADH				RoDH				RALDH		
Tumor cell lines	−5	−11	−1	−2	−4	−7	−1	−4	RDH12	retSDR1	−1	−2	ALDH6
MeWo	−	−	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel01	−	+	−	−	−	−	+	−	−	+	+	+	+
Ma-Mel04	−	+	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel05	−	+	−	−	−	−	+	−	−	+	+	+	−
Ma-Mel11	−	+	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel12	−	+	−	−	−	−	+	−	+	+	+	−	−
Ma-Mel17	−	+	−	−	−	−	+	−	−	+	+	+	+
Ma-Mel19	−	+	−	−	−	−	+	−	+	+	+	−	+
Ma-Mel37b	−	+	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel42b	−	+	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel47	−	+	−	−	−	−	+	−	+	+	+	+	+
Ma-Mel52	−	+	−	−	−	−	+	−	+	+	+	−	+
Sk-Mel023	−	+	−	−	−	−	+	−	+	+	+	+	+
UKRV-Mel02	−	+	−	−	−	−	+	−	+	+	+	−	+
UKRV-Mel06a	−	+	−	−	−	−	+	−	−	+	+	+	+
UKRV-Mel21a	−	+	−	−	−	−	+	−	−	+	+	+	+
UKRV-Mel31	−	+	−	−	−	−	+	−	+	+	+	−	+
UKRV-Mel27	−	+	−	−	−	−	+	−	−	+	+	+	+
Normal skin cells													
Melanocyte01	−	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	+	−	+
Melanocyte02	−	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	+	−	+
Melanocyte03	−	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	−	+
Melanocyte04	−	−	−	−	−	+	+	−	n.d.	n.d.	n.d.	n.d.	n.d.
Melanocyte05	−	−	−	−	−	+	+	−	n.d.	n.d.	n.d.	n.d.	n.d.
HaCaT	−	−	−	−	−	+	+	−	+	+	+	−	+
Normal skin tissues													
Skin1	−	+	−	−	−	+	+	−	+	+	+	−	+
Skin2	+	+	−	−	−	+	+	−	+	+	+	−	+

n.d., no data.

melanocytes ($3.17 \times 10^{-3} \pm 3.3 \times 10^{-4}$ cat/g). At the same time the general AD/R activity (Fig. 1B) was higher in melanoma cells ($1.05 \times 10^{-2} \pm 3.45 \times 10^{-3}$ cat/g) than in melanocytes ($1.23 \times 10^{-3} \pm 6.7 \times 10^{-4}$ cat/g). Based on the data obtained, we suggest that melanoma cells in contrast to melanocytes favor the ATRal reduction over its oxidation although the ALDH expression is not different in these cell types.

We assumed that ATRal as a substrate of ALDH and ATRA as a product of the reaction catalyzed by ALDH may have an effect on the activity of ALDH. After stimulation with ATRA, ATRol, and ATRal we measured the general ALDH activity in the melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27). We found no effect on the general ALDH activity after stimulation with ATRA and ATRol. However, ATRal was capable to stimulate this activity (Fig. 1C). Thus, although the baseline ALDH activity in melanoma cells is lower than in melanocytes, it can be stimulated by the ALDH substrate—ATRal.

We supposed that the increase of ALDH activity by ATRal stimulation is attributed to the up-regulation of ALDH gene expression. Indeed, a real-time PCR analysis revealed the ATRal-depended up-regulation of mRNA expression for RALDH1 and ALDH6 but not for RALDH2 (Fig. 1D). To exclude that this modulation of ALDH expression is only caused by changes in mRNA stability we applied a mRNA decay assay using actinomycin D, which prevents transcription. No increase in specific mRNA was observed (data not shown). Besides, such stimulation with the retinoids did not lead to de novo mRNA expression of RALDH2 and ALDH6 in MaMel12 (data not shown) which was virtually negative for mRNA expression of these genes (Table I). Using Western blot analysis with an antibody against RALDH1 we found protein expression of this gene in about 50% of all melanoma cell lines tested, in HaCaT cells, but not in melanocytes (Fig. 2A). To investigate the regulation of RALDH1 expression by ATRal we performed both a semi-quantitative ELISA and an immunocytology. These analyses revealed an up-regulation of RALDH1 protein expression by ATRal (Fig. 2B,C). Hence, the general ALDH activity may be modulated by their substrate ATRal through the up-regulation of RALDH1 protein expression.

ADH ACTIVITY IN MELANOMA CELL LINES AND MELANOCYTES

First we fractioned cell pellets from five melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27) and melanocytes obtained from three individuals to cytosolic and microsomal fractions. Cytosolic fraction was used for measurement of general ADH activity for ADH1, -2, -4, and -7. General ADH activity for RoDH4 (NAD^+-depended activity) and RoDH1 ($NADP^+$-depended activity) was measured in microsomal fraction. We found a background level of ADH activity in both benign and malignant melanocytic counterparts (Fig. 3A–C). Even there is a tendency that melanoma cells have higher ADH activity than melanocytes, we did

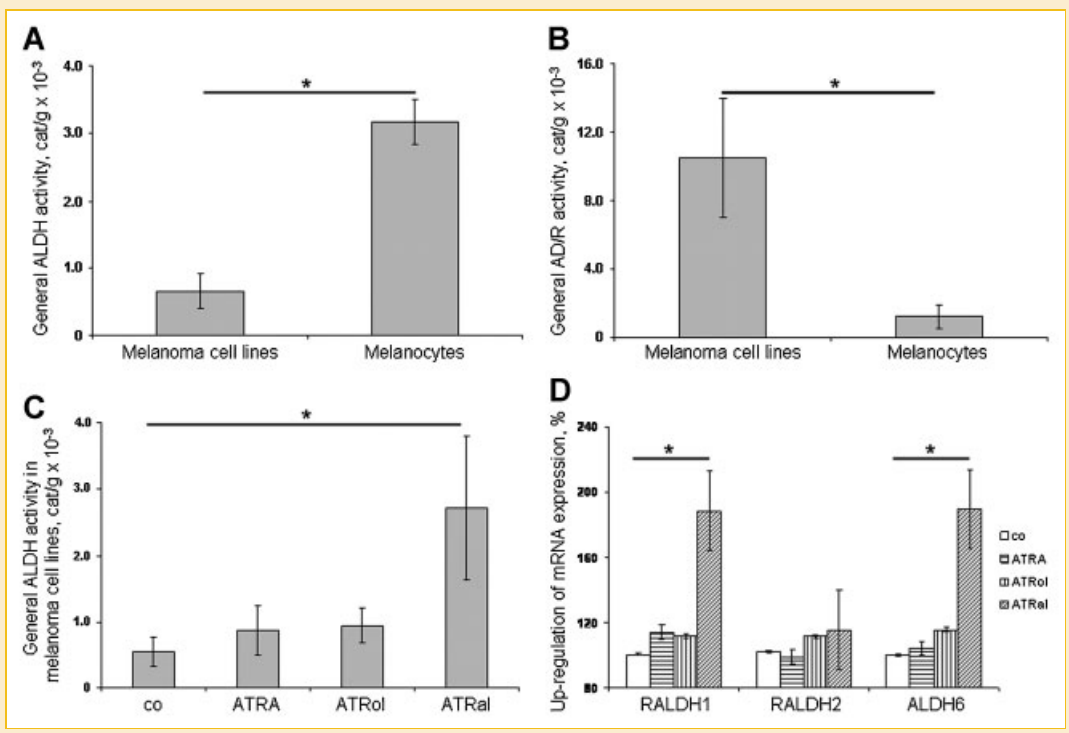

Fig. 1. Melanoma cell lines and melanocytes show different general ALDH and AD/R activities which can be modulated by ATRal. General ALDH (A) and AD/R (B) activities measured in five melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27) and in melanocytes obtained from three individuals. C: Measurements of general ALDH activity before and after 24 h treatment with the retinoids (10μM ATRA, 30 μM ATRol, and 30 μM ATRal) (D) a real-time PCR analysis of RALDH1, RALDH2, and ALDH6 in MaMel11 before and after treatment with the retinoids. Data are presented in means ± SEM. Three independent experiments performed in triplicates are depicted. *$P < 0.05$, significant differences between groups indicated with the lines.

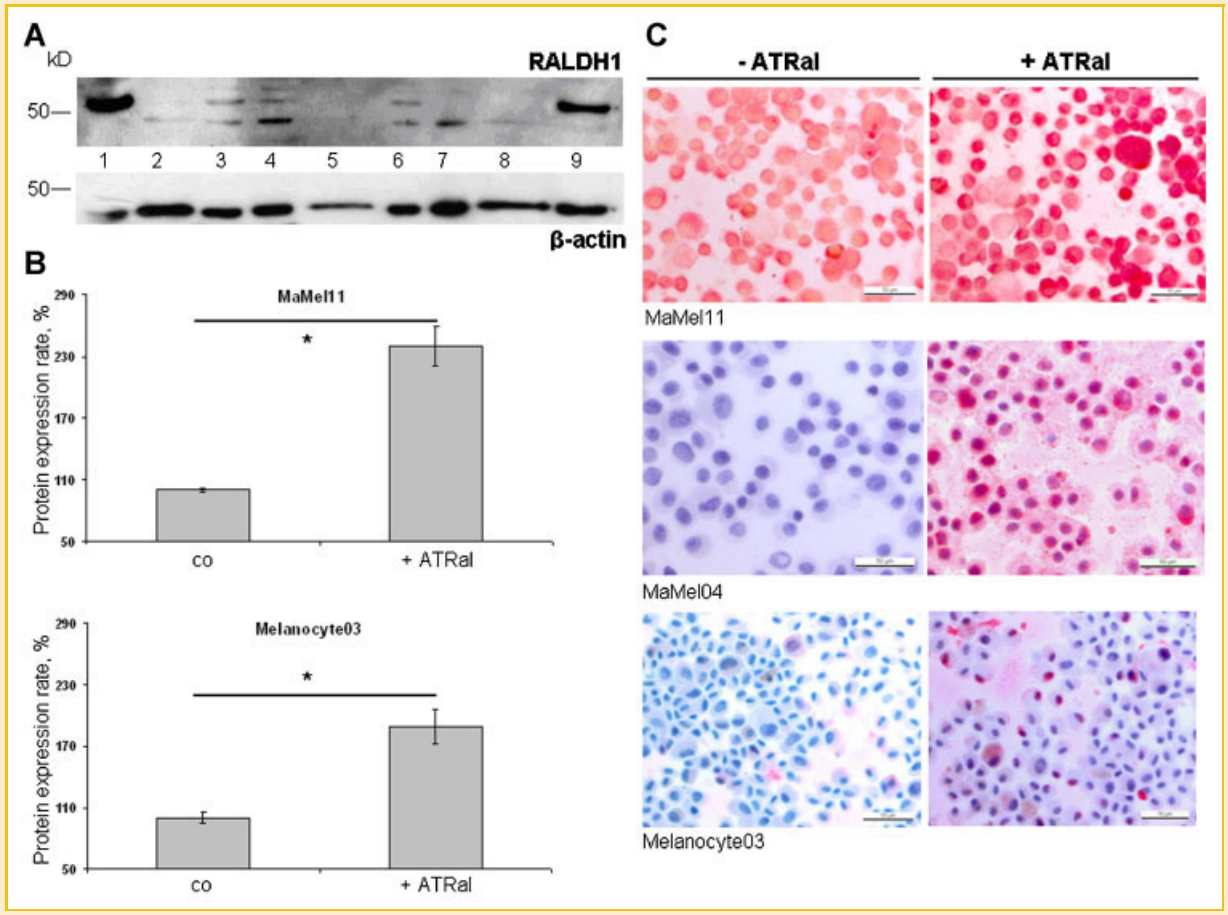

Fig. 2. RALDH1 can be up-regulated by ATRal. A: A representative Western blotting of 1—liver (as a positive control), 2—melanocyte02, 3—UKRV-Mel27, 4—MaMel11, 5—MaMel04, 6—SkMel023, 7—MaMel12, 8—MeWo, and 9—HaCaT. B: RALDH1 protein expression rate in % normalized to β-actin protein expression before and after 24-h stimulation with ATRal (30 μM) measured by semi-quantitative ELISA. Data are presented in means ± SEM. Three independent experiments performed in triplicates are depicted. $^{*}P < 0.05$, significant differences between groups indicated with the lines. C: A representative immunocytology of melanoma cell lines and melanocytes with a RALDH1 antibody (0.5 μg/ml). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

not find a significant difference in the general ADH activity in these cell types (Fig. 3A–C). The presence of background level of ADH activity in spite of the absence of ADH1, -2, -4, and -7 expression and presence of RoDH1 could be explained by the presence of an additional cytosolic ADH with substrate specificity for ethanol which has been used for the ADH activity measurement.

Next question was whether the ADH activity could be stimulated by retinoids like the ALDH activity. The same five melanoma cell lines were stimulated with ATRA, ATRal, and ATRol, and the general ADH activities were measured. We found that all general ADH activities could be stimulated by ATRal and ATRol, but not by ATRA (Fig. 4A–C). In case of RoDH1, microsomal $NADP^{+}$/ADH activity could be stimulated by ATRA in addition to ATRal and ATRol (Fig. 4C). However, a real-time PCR analysis did not reveal any stimulatory effect at RoDH1 expression after stimulation of MaMel04 with these retinoids (Fig. 4D). Hence, we can suppose that the activity of ADH might be regulated at the post-transcriptional level.

DISCUSSION

The main aim of this work was to investigate expression and activity of ADH, AD/R, and ALDH in normal and malignant melanocytic skin cells.

Our RT-PCR data showed that the important enzymes of ATRal oxidation—RALDH1, RALDH2, and ALDH6 are expressed in melanoma cells and in their benign counterparts (except RALDH2). These data are in agreement with whole genome expression profiling data of biopsies from melanoma tissues, NHEM and nevi [Smith et al., 2005; Talantov et al., 2005]. However, Hoek et al. [2004] did not find expression of RALDH2 in melanoma cells after short-time cultivation or expression of RALDH1 and ALDH6 in NHEM. This discrepancy could be explained by the low number of cell lines used by the authors. It should be noted, that the expression profiles do not give us any information about the enzymatic activity of these proteins.

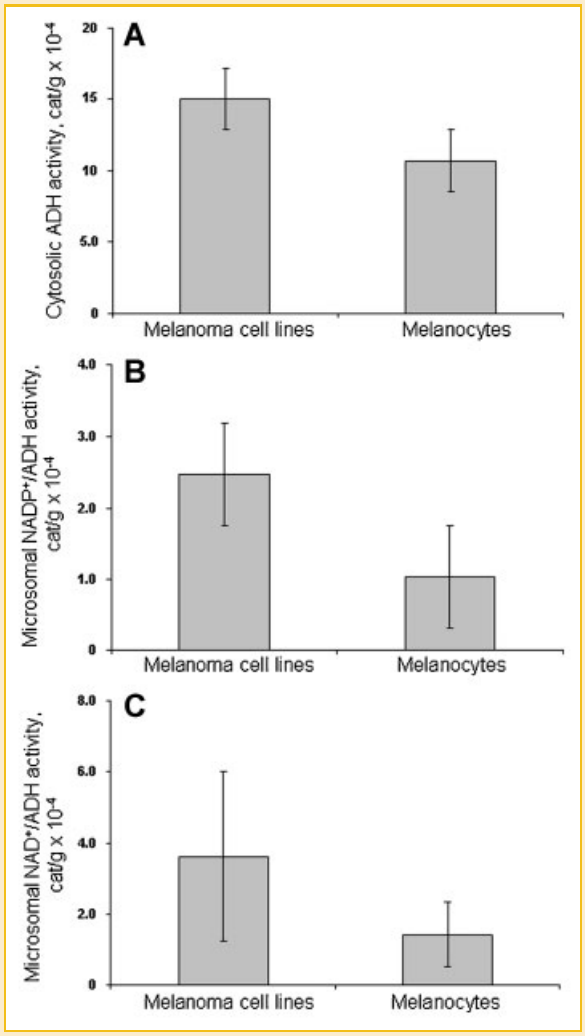

Fig. 3. ADH activity in melanoma cell lines and melanocytes. General ADH activity in cytosolic (A) fraction and in microsomal ($NADP^+$/ADH activity (B) and NAD^+/ADH activity (C)) fractions from five melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27) and melanocytes obtained from three individuals. Cytosolic fraction was used for measurement of the general ADH activity for ADH1, -2, -4, and -7 (A). General ADH activity for RoDH1 ($NADP^+$-depended activity (B)) and RoDH4 (NAD^+-depended activity (C)) was measured in the microsomal fraction. Data are presented in means ± SEM. Three independent experiments performed in triplicates are depicted.

Data of this work show that the general ALDH activity in melanoma cells is low compared to melanocytes. However, our recent data of a cell culture system showed that melanoma cell lines but not normal melanocytes are able to oxidize ATRal [Amann et al., 2011]. Since in this work we present data from an in vitro study using protein extracts, it could be that in a cell culture system ATRal (or its derivates) as substrates of the oxidative reactions would activate the general ALDH activity in melanoma cells through regulation of its gene expression. Indeed, we found that ATRal up-regulates RALDH1 and ALDH6 expression. This up-regulation in turn correlates with the increased general ALDH activity, providing evidence that the ALDH activity can be regulated by increasing amounts of enzymes due to up-regulation by retinoids. However, since we used propanal as a substrate for the measurement of general ALDH activity, we cannot exclude that such increase in the activity belongs only to propanal oxidation but not to ATRal. Low level of general ALDH activity in melanoma cells is in agreement with the work of Jelski et al. [2004] which revealed low general ALDH activity in colorectal cancer compared to normal epithelium. However, general ALDH activity cannot serve as a general discrimination factor between malignant and benign cells. In liver cancer and pancreatic carcinoma ALDH activity is higher than in normal liver or pancreas tissues, respectively [Jelski et al., 2007, 2008]. Moreover, RALDH2 is important for malignant epithelial cells [Touma et al., 2009; Williams et al., 2009], and it has a potential role to be a tumor suppressor in prostate cancer [Kim et al., 2005]. Interestingly to note is that ALDH1 has been identified as a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome in breast carcinoma [Ginestier et al., 2007]. In the context of melanoma-initiating cells, an enhanced tumorigenicity was also found recently in ALDH activity positive melanoma cells over ALDH activity negative melanoma cells [Boonyaratanakornkit et al., 2010]. However, Prasmickaite et al. [2010] showed that ALDH was not associated with more aggressive subpopulations in malignant melanoma and does not distinguish between tumor-initiating or therapy-resistant cells.

RT-PCR data from this work indicated no differences in mRNA expression of AD/R between malignant and benign melanocytic skin cells. These data are consistent with global gene expression micro array analysis of melanocytes and melanoma cells from skin biopsies, respectively [Smith et al., 2005; Talantov et al., 2005]. In contrast to ALDH the general AD/R activity was higher in melanoma cells as in melanocytes. This difference in AD/R activity can explain the phenomenon of the high ATRal reduction capacity of melanoma cells [till 117 times higher than by melanocytes; Amann et al., 2011]. However, we found expression of the AD/R enzymes retSDR1 and RDH12, which reduce ATRal to ATRol, in all types of skin cells. In the oncological context, it is interesting to note that retSDR1 can be involved in neuroblastoma development [Cerignoli et al., 2002].

From all ADH tested in this work, we found only RoDH1 to be expressed in both malignant and benign cellular counterparts and expression of ADH7 in NHEM, but not in melanoma cell lines. Presently, there are no micro array data available for RoDH1 and RoDH2 to compare these with the data from our work. Our data for ADH1 and ADH2 mRNA expression are in consistence with the expression profiling data from Hoek et al. [2004], who did also not find expression of these enzymes neither in melanoma cells nor in NHEM. In contrast to our and Hoek's data the works from Smith et al. [2005] and Talantov et al. [2005] revealed expression of ADH1 and ADH2 in some melanoma and nevus tissues and in NHEM. This could be explained by the fact that some mRNA got lost during the cell cultivation [Gazdar et al., 1980]. In the same time absence of ADH4 expression was also identified in the work of Smith et al. [2005]. Regarding ADH7, which expression was found in this work only in benign skin cells, expression of this gene in micro array data does not match among each other [Hoek et al., 2004; Smith et al., 2005; Talantov et al., 2005], indicating necessity for new investigations. At the functional level we did not find any

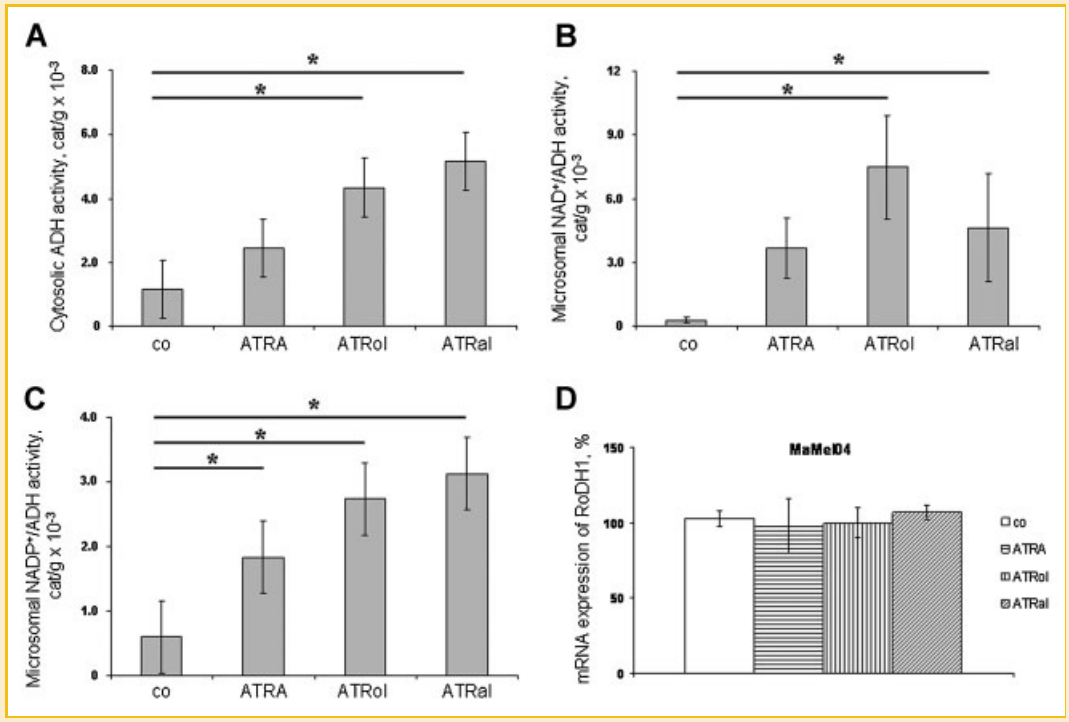

Fig. 4. ADH expression and their activity can be up-regulated by the retinoids. Five melanoma cell lines (MaMel04, MaMel11, MaMel12, Sk-Mel23, and UKRV-Mel27) were stimulated 24 h with ATRA (10 μM), ATRal (30 μM), and ATROl (30 μM), and the general ADH activities were measured. (A), (B), and (C) the same as (A), (B), and (C) in Figure 3. D: A real-time PCR analysis of RoDH1 in MaMel04 before and after treatment with the retinoids. Data are presented in means ± SEM. Three independent experiments performed in triplicates are depicted. $^{*}P < 0.05$, significant differences between groups indicated with the lines.

differences in ADH activity between melanoma cells and melanocytes. ADH activity seems to be elevated in the cancer cells from patients with gastrointestinal tumors compared with the corresponding normal cells [Jelski et al., 2004, 2007, 2008]. At the same time in breast cancer cells the ADH activity is as low as in healthy counterparts [Jelski et al., 2006]. This indicates the heterogeneity of ADH activity in respect of the origin of tumor cells.

In this work we also showed that RDH11 which is normally expressed in RPE [Parker and Crouch, 2010] could also be expressed both in melanoma cell lines and melanocytes. Our data are consistent with the global gene expression data [Hoek et al., 2004; Smith et al., 2005; Talantov et al., 2005] which also showed expression of RDH11.

Summarizing, these and our previous data [Amann et al., 2011] about vitamin A metabolism in melanocytes and malignant melanoma cells showed that all components of molecular machinery of the retinoid metabolism are present in malignant and benign melanocytic skin cells. Considering the presence and activity of these enzymes, this enzyme constellation underpins the hypothesis of ATRal and ATROl removal in malignant melanoma. The decreasing cellular amount of the precursor molecules of ATRA could result in a changed gene regulation.

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