et al., 1978; Blandina *et al.*, 1980), we investigated CHR expression in mast cells just in vicinity of eccrine glands. Serial sections were alternatively stained with toluidine blue and with anti-CHRM3 antibody. Mast cells were identified by positive toluidine blue staining (Figure 2c, upper panel), and CHRM3 expression by mast cells was examined in the antibody-stained adjacent sections (Figure 2c, lower panel). In a normal control, mast cells expressed CHRM3 at high levels. Mast cells in conjunction with the secretory portion expressed CHRM3 in the hypohidrotic but not anhidrotic areas. There was no significant difference in the number of mast cells between the anhidrotic and hypohidrotic areas.

Our study revealed that the skin of patients with CUAH is divided into the wheal-non-occuring anhidrotic and wheal-occuring hypohidrotic areas. Even in the hypohidrotic areas, the intradermal injection of autologous sweat did not yield wheal, suggesting the absence of sweat allergy (Tsuchiya *et al.*, 2004). We found the lack of CHRM3 expression in the anhidrotic skin, which may lead to the lack of sensitivity to acetylcholine. Mast cells are responsible for wheal formation and present just in the vicinity of eccrine glands. Neither eccrine gland cells nor mast cells expressed CHRM3 in the anhidrotic area, and it is thus reasonable that sweating and wheal formation were absent in this area. CHR mediates wheal development (Tong *et al.*, 1997), and acetylcholine can induce degranulation of mast cells (Fantozzi *et al.*, 1978; Blandina *et al.*, 1980). In the hypohidrotic area, we are tempting to speculate that acetylcholine released from nerves upon exercise cannot be completely trapped by CHR of eccrine glands and overflows to the adjacent mast cells. In this scenario, mast cells may be capable of producing histamine and resultant wheal in response to acetylcholine because of the expression of some degree of CHRM3.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Squamous Cell Carcinoma of the Skin Shows a Distinct MicroRNA Profile Modulated by UV Radiation

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TO THE EDITOR

Cutaneous squamous cell carcinoma (SCC) is the second most common skin malignancy in the general population. There are many risk factors for SCC, the most important one being solar radiation. The incidence of SCC is increased by 60–100-fold in organ transplant recipients (OTRs), making it the most common malignancy in these patients (Berg and Otley, 2002; Euvrard *et al.*, 2003). SCC in OTRs is characterized by a higher risk of metastasis in up to 20% of the patients and shows a more aggressive course than SCC in the general population (Euvrard *et al.*, 2003).

Recent work has delineated a class of small non-coding RNA species known as microRNAs. Owing to their association with the 3′-untranslated region region of target mRNAs, microRNAs have important regulatory roles in diverse cellular pathways, including

Abbreviations: OTR, organ transplant recepient; SCC, squamous cell carcinoma; UTR, untranslated region

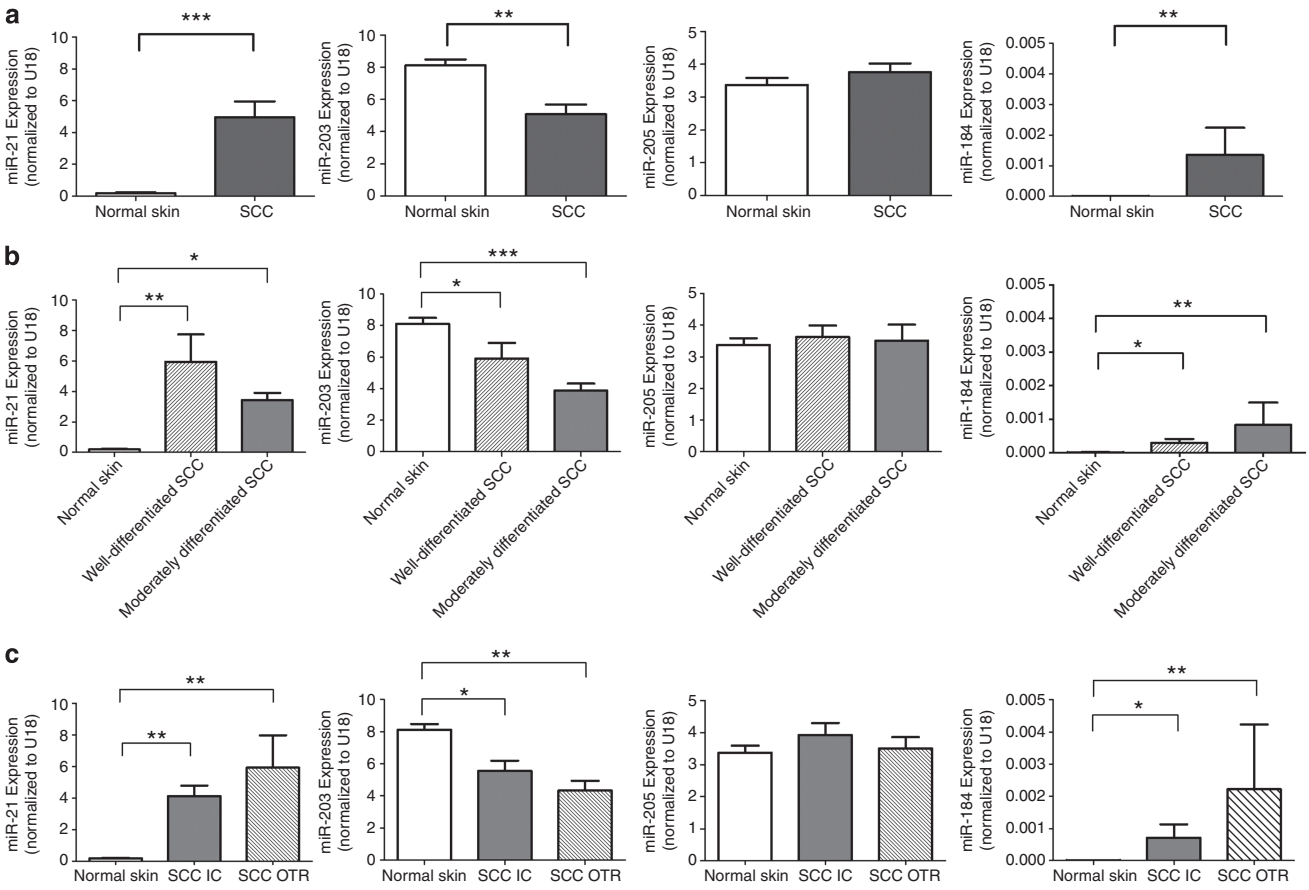

Figure 1. Altered microRNA expression in skin squamous cell carcinoma. (**a**) Real-time PCR showed increased expression of miR-21 and miR-184 and decreased expression of miR-203 in cutaneous SCC as compared with normal skin. There was no detectable difference in the expression of miR-205. Further analysis of SCC group according to histological differentiation (**b**) and drug-induced immunosuppression in organ transplant recepients (OTRs) (**c**) did not reveal any significant changes in the expression of these selected microRNAs. (*$P<0.05$, **$P<0.01$, ***$P<0.001$.)

development, differentiation, organogenesis, stem-cell and germ-line proliferation, and apoptosis (Chen *et al.*, 2004; Poy *et al.*, 2004; He *et al.*, 2005; O'Donnell *et al.*, 2005; Zhao *et al.*, 2005). Importantly, the successful use of antagomirs to silence microRNAs in mice and nonhuman primates suggests a possible therapeutic use of microRNAs (Krutzfeldt *et al.*, 2005; Elmen *et al.*, 2008).

Although SCC is one of the most common cancers, no studies so far have focused on microRNA expression and function in this particular tumor. We analyzed the expression of candidate microRNAs with at least partially described functions in keratinocytes: miR-21 and miR-184, for both of which oncogenic properties have been reported, miR-203 as a keratinocyte-specific microRNA, and miR-205 as an antagonist of miR-184, in the immunocompetent population and in OTRs. As UV radiation is the most important risk factor for SCC, we also investigated the influence of UV radiation on the expression of these miRs in normal human keratinocytes.

Analyses were performed on SCC tissue material from 13 OTRs, 17 immunocompetent patients and 7 normal skin samples obtained from healthy individuals. Treatment of OTRs consisted of cyclosporine A in combination with other immunosuppressive drugs. All SCC samples were diagnosed as well or moderately differentiated SCCs by a board-certified dermatohistopathologist (Supplementary Table S1 online). The *post-hoc* power analysis for statistically significant differences reached 60–98%, respectively.

Real-time reverse transcriptse-PCR analysis showed significantly increased expression of miR-21. This finding extends the list of tumors with upregulated miR-21, and suggests that miR-21 may have an essential role in the development or maintenance of SCC of the skin. Similarly, SCC showed increased expression of miR-184. Interestingly, miR-184 was barely detectable in the normal epidermis, but was much increased in SCC. Similar to our findings for miR-184 expression in the epidermis, we could not detect this microRNA in normal human keratinocytes. Interestingly, miR-184 was recently shown to be upregulated in tongue SCC, with strong data supporting the hypothesis that this microRNA may induce cell transformation and carcinogenesis (Wong *et al.*, 2008). In our samples, miR-205 did not seem to be important for SCC, judging by its expression, whereas its function may be important and may well be modulated by miR-184, which we found to be

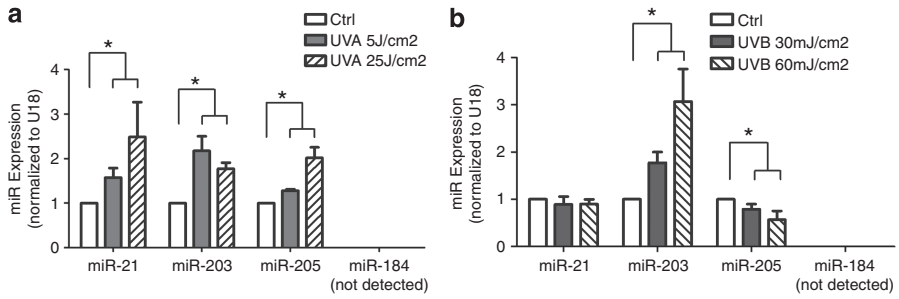

Figure 2. UVA and UVB irradiation differentially influence miR expression in normal keratinocytes. (**a**) UVA irradiation significantly increases expression of miR-21, miR-203, and miR-205, whereas (**b**) UVB has no effect on miR-21, upregulates miR-203, and downregulates miR-205 expression. miR-184 was not detected in normal keratinocytes before and after irradiation. $*P<0.05$.

elevated in SCC. Expression of miR-205 may thus be considered as a control for other measurements.

Although miR-203 was described in the context of cell senescence, which could suggest an increased expression in the older population, we found its expression to be decreased in the SCC group (i.e., the older group), suggesting differences to represent tumor-associated features, such as an increased stem-cell population and higher proliferative capacity rather than mere aging. It is known that SCC contains a higher percentage of highly proliferative, undifferentiated cells than normal skin. miR-203 antagonizes the expression of p63, which is a transcription factor having an essential role in the maintenance of ''stemness'' in the skin and which is known to be upregulated in SCC (Dotto and Glusac, 2006; Lena *et al.*, 2008). Our data suggest that decreased miR-203 may unleash p63 expression, leading to decreased cell senescence and supporting SCC formation.

Expression levels of these selected microRNAs were similar between well and moderately differentiated SCC (Figure 1b).

As long-term drug-induced immunosuppression is closely linked to greatly increased SCC formation (Caforio *et al.*, 2000), we compared the expression of the four microRNAs under investigation in SCC in immunocompetent patients and OTRs (Figure 1c), but did not find any significant differences. *In vitro* analysis of miR-21, miR-203, miR-205, and miR-184 expression in primary human keratinocytes exposed to different concentrations of cyclosporine A supported these findings (Supplementary Figure S1 online). We did not observe any influence of cyclosporine A on these microRNA expression levels. The greatly increased incidence of SCC in OTR seems, thus, unlikely to be mediated by these miRs directly. It is tempting to speculate that any SCC-promoting effects of miRs will have a greater impact in the context of a reduced tumor defense in OTR, thus potentially indirectly contributing to the greatly increased cutaneous carcinogenesis in OTR.

We found that UV irradiation alters miR expression. We observed that UVA radiation increased the expression of miR-21, miR-203, and miR-205 (Figure 2a). UVB radiation, however, increased the expression of miR-203 slightly, but significantly decreased the expression of miR-205 and had no effect on miR-21. Interestingly, UVA, but not UVB, increased the expression of miR-21, which is the best-described microRNA to date with potent carcinogenic properties. This finding is particularly interesting, as UVA radiation is responsible for SCC formation to a much higher extent than UVB. The mechanism by which UVA radiation upregulates miR-21 expression may further explain the differential influence of these two wavelengths on SCC formation. Both UVA and UVB induce miR-203 expression, which is possibly in line with the differentiation and aging of keratinocytes after solar irradiation. The diverging effects of UVA and UVB radiation on miR-205 expression, however, underline different mechanisms of cell damage mediated by these wavelengths. The consequence of miR-205 induction or down-modulation in skin keratinocytes remains to be investigated.

Topical non-steroidal anti-inflammatory drugs such as diclofenac are effective against early SCC and are the subject of attempts to prevent UV-mediated SCC formation (Ortonne *et al.*, 2006). In our study, however, diclofenac did not impact microRNA expression (Supplementary Figure S2 online). This finding argues against the cyclo-oxygenase pathway as a major modulator for selected miR expression induced by UV.

In summary, we show that the expression of miR-21, miR-184, and miR-205 is changed in SCC compared with that in the normal skin. UV, in particular UVA as a major risk factor for SCC, impacts miR expression, suggesting an early role for these miRs in SCC formation, possibly affecting such important processes as differentiation and apoptosis, whereas the calcineurin and cyclo-oxygenase pathways seem to be uninvolved.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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