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T1799A BRAF Mutation is Common in PUVA Lentigines

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TO THE EDITOR

Psoralen plus UVA (PUVA) lentigines have been shown to develop in 40–50% of patients after long-term PUVA treatment (Rhodes *et al.*, 1983; Abel *et al.*, 1985; Gupta *et al.*, 1988). In addition to individual susceptibility factors of race, age, as well as tanning and burning response to sunlight, PUVA lentigines are related to the number of PUVA treatments and total UVA dose and are often found in patients who have received an overdose, as indicated by the development of sunburn-like phototoxic erythema (Hönigsmann *et al.*, 1999). Clinically, PUVA lentigines present as small brown unevenly pigmented lesions with irregular margins occurring on PUVA-exposed body sites (Basarab *et al.*, 2000). Histologically, they represent mainly a lentiginous proliferation of functionally active melanocytes, similar to solar lentigines. In contrast to solar lentigines, melanocytes in PUVA lentigines often display an increased size of melanosomes, clustering and binucleation with nuclear hyperchromatism, and cellular pleomorphism (Gupta *et al.*, 1988). Abel *et al.* (1985) found melanocytic atypia consisting of large or angular hyperchromatic nuclei in 57% of PUVA lentigines. Concern that PUVA lentigines could be the precursors of melanocyte dysplasia or malignancy has been increased by the fact that PUVA is known to be an effective proliferative stimulus for melanocytes. For instance, PUVA application enhances the incorporation of H^3-thymidine in benign melanocytic nevi (MN) and is associated with intraepidermal dysplastic melanocytic changes (Lagerholm and Frithz, 1982). *In vivo*, PUVA stimulates the growth of melanoma cells (Aubin *et al.*, 1991) and can induce melanocytic tumors in the mouse (Alcalay *et al.*, 1990). Indeed, several clinical studies have suggested that chronic PUVA exposure may lead to the formation of cutaneous malignant melanoma (CMM) (Marx *et al.*, 1983; Binet *et al.*, 1985; Gupta *et al.*, 1988; Stern *et al.*, 1997; Wolf *et al.*, 1998; Stern, 2001). Most importantly, a recent follow-up of the prospective US PUVA study showed an increased incidence of CMM in patients who had received more than 250 PUVA exposures (Stern *et al.*, 1997). PUVA may lead to CMM formation by its tumor initiating, promoting, and/or immunosuppressive effects (Wolf *et al.*, 2004 and references cited therein), possibly via PUVA lentigines as early precursor lesions. However, a definite pathogenic link between CMM and PUVA lentigines has not been demonstrated despite reports of PUVA lentigines with more or less severe atypical histologic features, persisting for years after therapy has been discontinued (Rhodes *et al.*, 1983; Abel *et al.*, 1985). Thus, the long-term course of atypical PUVA lentigines is still unknown as well as the alterations on the molecular level associated with these lesions.

B-type Raf (BRAF) mutations have been detected in a high proportion of CMM and MN (Brose *et al.*, 2002; Davies *et al.*, 2002; Gorden *et al.*, 2003; Pollock *et al.*, 2003; Uribe *et al.*, 2003; Lang and MacKie, 2005). The serine/threonine protein kinase BRAF is involved in the mitogen-activated protein kinase pathway that represents a membrane-to-nucleus signaling cascade regulating cell proliferation in response to extracellular mitogenic signals. A hotspot single mutation localized in exon 15 (T1799A) at codon 600 (V600E) (former designation V599E [T1796A]) of BRAF accounts for more than 80% of mutations in melanoma samples (Davies *et al.*, 2002). As demonstrated by *in vitro* and *in vivo* assays, abrogation of $BRAF^{V600E}$ leads to growth arrest and apoptosis indicating dependency of transformed phenotype on this mutation in melanocytes (Hingorani *et al.*, 2003). In this study, we analyzed PUVA lentigines, dysplastic MN, and CMM from PUVA-treated patients for BRAF mutations in exon 15 by an allele-specific (AS) PCR and direct sequencing of PCR products in order to gain insight into the molecular events of PUVA-associated tumorigenesis.

Formalin-fixed and paraffin-embedded material (surgical excisional and/or punch biopsy specimens) of 33 PUVA-associated pigmented and/or melanocytic lesions from 22 patients was retrieved from the Division of Dermatopathology, Department of Dermatology, Medical University Graz. Data concerning the PUVA treatment of these patients were obtained from the Research Unit for Photodermatology, Department of Dermatology, Medical University Graz. The lesions included 15 PUVA lentigines, 12 dysplastic MN, and six CMM (two of them *in situ*). The patient data and PUVA characteristics are given in Table 1. All lentigines had to be excised owing to clinical suspicion of CMM (Figure 1a). CMM arose in the PUVA-treated patients after a mean time of 15 years (range 3–26 years) following the first PUVA treatment, a lag time consistent with a previous report by Stern *et al.* (1997). All lesions had undergone routine histology for diagnosis, which was confirmed in a re-evaluation by two experienced dermatopathologists and coauthors (S.K. and H.K.). Three of the lentigines from the PUVA-treated patients showed particular severe melanocytic atypia, characterized by a profound increase of solitary, occasionally large, atypical melanocytes at the dermo-epidermal junction and foci with solitary melanocytes in higher levels of the epidermis (Figure 1b). Mitotic figures were also noted in these

Abbreviations: AS, allele-specific; CMM, cutaneous malignant melanoma; MN, melanocytic nevus; PCR, polymerase chain reaction; PUVA, psoralen plus UVA

Table 1. Clinical and histologic findings, PUVA characteristics, and BRAF mutation results in patients with PUVA-associated melanocytic lesions

Patient	Sex	Lesion	Age at lesion appearance (years)	Tumor location	Histologic diagnosis	Psoralen used	Total number of PUVA exposures	Total cumulative UVA dose (J/cm^2)	Lesion appearance/ excision after first PUVA treatment (years)	BRAF mutation
A	F	A1	60	Chest	MN	8-MOP	247	1821	26	T1799A
B	F	B1	42	Thigh	Lentigo	8-MOP	206	1021	8	T1799A[1]
		B2	50	Shank	Lentigo	8-MOP	844	4373	16	T1799A
		B3	50	Leg	Lentigo	8-MOP	844	4373	16	
		B4	53	Knee	MN	8-MOP	996	5096	19	T1799A
C	M	C1	44	Back	MN	8-MOP	31	152	1	T1799A
D	M	D1	55	Back	MN	8-MOP	107	505	10	
		D2	55	Back	CMM	8-MOP	107	505	10	
E	M	E1	57	Shank	Lentigo	8-MOP	30	46	9	
F	M	F1	67	Back	CMM (*in situ*)	8-MOP	1,430	8,580	20	T1799A
		F2	67	Chest	Lentigo	8-MOP	1,430	8,580	20	
		F3	67	Chest	Lentigo with SA	8-MOP	1,430	8,580	20	
		F4	68	Shoulder	Lentigo with SA	8-MOP	1,430	8,580	21	
		F5	66	Abdomen	MN	8-MOP	1,430	8,580	19	
		F6	67	Abdomen	Lentigo	8-MOP	1,430	8,580	20	
G	M	G1	57	Upper arm	CMM	8-MOP	NA	NA	21	
H	M	H1	41	Shoulder	MN	8-MOP	85	528	2	T1799A
I	M	I1	59	Thigh	Lentigo	8-MOP	NA	NA	24	T1799A
J	F	J1	69	Thigh	Lentigo	8-MOP	52	184	1	T1799A
K	F	K1	72	Upper arm	Lentigo	8-MOP	511	3,156	17	
L	M	L1	39	Abdomen	Lentigo with SA	8/5-MOP	30	93	3	
M	M	M1	62	Chest	CMM	8-MOP	NA	NA	26	T1799A
N	M	N1	56	Buttocks	MN	8-MOP	46	121	<1	T1799A
O	F	O1	26	Back	MN	8-MOP	225	1,745	6	T1799A
P	M	P1	41	Abdomen	CMM	5-MOP	67	352	3	
Q	M	Q1	47	Buttocks	MN	8-MOP	86	484	2	T1799A
	M	Q2	47	Back	MN	8-MOP	86	484	2	T1799A
R	M	R1	73	Ear	Lentigo	8/5-MOP	523	2,375	25	
S	F	S1	68	Back	Lentigo	8-MOP	19	42	6	
T	F	T1	31	Back	MN	8-MOP	75	113	4	T1799A[1]
		T2	37	Chest	MN	8-MOP	75	113	10	T1799A
U	M	U1	56	Chest	Lentigo	8-MOP	418	1,847	18	T1799A
V	F	V1	46	Calf	CMM (*in situ*)	8-MOP	31	56	11	

CMM, cutaneous malignant melanoma (invasive, if not otherwise indicated); CN, congenital nevus; MN, melanocytic nevus; MOP, methoxypsoralen; SA, severe atypia.
[1]Mutation was detected by direct sequencing and AS-PCR.

PUVA lentigines. In one case, pagetoid spread of solitary melanocytes was accompanied by a pronounced epidermal hyperplasia. These lesions were classified as PUVA lentigines with severe atypia (Table 1). In addition, 15 randomly selected melanomas and 14 MN from non-PUVA-treated patients were used as control samples for the possible presence of the T1799A BRAF mutation. All clinical and histological data were collected after obtaining informed patient consent and the sampling was carried out in accordance with the Declaration of Helsinki Principles.

Sections (6 μm thick) of the paraffin-embedded specimens were microdissected to eliminate normal tissue and

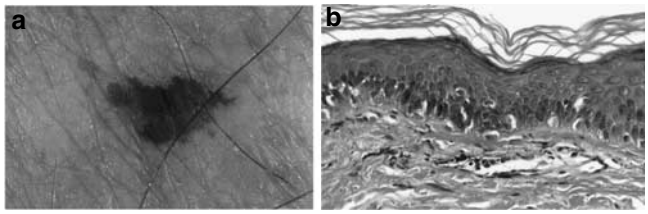

Figure 1. PUVA lentigo with severe atypia (patient F, lesion F3). (**a**) Clinical features showing an irregular, pigment lesion simulating CMM. (**b**) Histologic findings revealing hyperpigmentation of the basal layer and an increase of relatively large, solitary melanocytes at the dermo-epidermal junction as well as scattered melanocytes in higher layers of the epidermis.

obtain tissue with a content of more than at least 30–50% melanocytes or nevus cells. DNA extraction and direct sequencing of BRAF exon 15 was performed, as described previously (Worda *et al.*, 2005). AS-PCR for BRAF T1799A was carried out with mutant-specific primers and a pair of glyceraldehyde-3-phophate dehydrogenase primers as internal control, as described by Pollock *et al.* (2003) (primer sequences and PCR conditions available from authors upon request). The AS-PCR of DNA from melanocytic lesions of PUVA-treated patients revealed that T1799A BRAF mutation was present in two of six (33%) CMM, 10 of 12 (83%) MN, and five of 15 (33%) PUVA lentigines (Table 1). AS-PCR of DNA from melanocytic lesions of non-PUVA-treated control patients disclosed T1799A BRAF mutation in eight of 15 (53%) CMM and seven of 14 (50%) MN. Direct sequencing of DNA from all PUVA-associated melanocytic lesions showed T1799A BRAF mutated in only two lesions (B1 and T1), indicating that the AS-PCR is much more sensitive than direct sequencing for mutation detection (Miller *et al.*, 2004). Indeed, our own sensitivity testing of the AS-PCR by diluting DNA hetereozygous for the T1799A mutation with wild-type DNA revealed that our PCR setup was capable of detecting the mutant allele when diluted to 0.4% of the total DNA (data not shown). To ensure reliability of the mutation analysis, we always screened at least one melanoma with a known T1799A BRAF mutation obtained from a non-PUVA-treated patient in conjunction with the specimens of PUVA-treated patients. The chance of false-negative results in the group of PUVA-associated lesions is not very high as the randomly selected, simultaneously microdissected and analyzed CMM from non-PUVA-treated patients displayed a mutation rate of 53% in CMM and 50% in MN, which is well in line with published data showing a mutation rate ranging from ~20 to 90% in BRAF of CMM and MN from the non-PUVA-treated patient population (Brose *et al.*, 2002; Davies *et al.*, 2002; Gorden *et al.*, 2003; Pollock *et al.*, 2003; Uribe et al., 2003; Lang and MacKie, 2005). Taken together, this study demonstrates that T1799A BRAF mutation is a common occurrence in PUVA lentigines and perhaps in PUVA-associated CMM, indicating that PUVA lentigines might be precursors of CMM.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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