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Genetic Diversity in Melanoma Metastases from a Patient with Xeroderma Pigmentosum

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TO THE EDITOR

Melanoma is among the most severe and lethal forms of human skin cancer (Mackie, 2006; Miller and Mihm Jr, 2006; Fecher *et al*., 2007; Gray-Schopfer *et al*., 2007). For 2008, more than 110,000 new cases of melanoma (about 50,000 melanoma *in situ* (MIS) and 60,000 invasive melanomas) were estimated with more than 8,000 deaths (American Academy of Dermatology, 2008; Ries *et al*., 2008). To examine genetic diversity, we studied metastatic melanoma lesions from a patient with xeroderma pigmentosum (XP). XP is a rare genetic disease with defective DNA repair and a more than 1000-fold increase in melanoma frequency (Kraemer *et al*., 1987, 1994; Ruenger *et al*., 2008).

The XP patient, (XP4BE), had no blistering reaction to sunlight despite living on a farm with unrestricted exposure to sunlight. By 8 years of age he had extensive freckling on his face (Figure 1a). At the age of 9 years a large warty tumor was excised from his nose. He subsequently developed more than 100 cancers in sun-exposed skin, primarily basal cell carcinomas and squamous cell carcinomas, which were treated with electrodessication and curettage or surgical excision followed by skin grafting (Figure 1b). At the age of 23 years a primary malignant melanoma of the right post-auricular area had been widely excised, and evidence of spread was found in two of the 35 posterior cervical nodes.

XP4BE was admitted to the NIH Clinical Center in 1969 at the age of 25 years (Robbins *et al*., 1974) and studied in accordance with the NIH human research guidelines then in effect. He was the first XP “variant” patient recognized, a form of XP with normal nucleotide excision repair (Robbins *et al*., 1974). His cells were subsequently found to have a defect in the error-prone polymerase, polymerase eta (Johnson *et al*., 1999; Masutani *et al*., 1999). At admission, his neurological examination was normal. In 1971, a melanoma nodule was present on his scalp (Figure 1c). He had no beneficial response to two courses of bis-

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The authors state no conflict of interest.

choroethyl-nitrosourea (BCNU), steroids, or radiation to the brain and lumbar areas for treatment of the metastatic melanoma. He died at the age of 27 years in 1971. Autopsy performed at NIH revealed thromboemboli with pulmonary infarctions, aspergillus pneumonia and cerebral abscess, and metastatic melanoma involving his brain, spinal cord, stomach, small bowel, liver, gall bladder, adrenal glands, kidneys, lymph nodes, left testis, right lung, pancreas, thyroid gland, and soft tissue of the right thigh. The paraffin blocks from this autopsy were recently retrieved, sectioned, and stained. We do not have tissue from the primary lesion, which was removed before coming to NIH. We analyzed seven metastatic lesions from this patient, the metastatic scalp nodule obtained before BCNU treatment (Figure 1c–e), and six lesions obtained at autopsy (central nervous system (CNS) × 2, muscle, liver, kidney, and pancreas) (Table 1) as well as normal tissue. Using laser capture microdissection, we isolated about 500 tumor or normal cells for DNA analysis (Figure 1d and e) as described previously (Wang *et al.*, 2009). To minimize formalin sequencing artifacts (Williams *et al.*, 1999), we used more than 500 cells for analysis and repeated the sequencing on independent PCR products obtained from adjacent regions of the tumor for several tumors.

We studied the tumor suppressor gene *PTEN* (phosphatase and tensin homolog), which is one of the most frequently mutated genes in human cancer, including melanoma (Goel *et al.*, 2006; Baker, 2007) as well as the *NRAS* and *BRAF* oncogenes that have been reported to be mutated in melanomas (Curtin *et al.*, 2005). We used sequencing techniques previously described (Curtin *et al.*, 2005; Wang *et al.*, 2009 and references therein). Previously, we screened 59 primary cutaneous melanomas from 8 other XP patients and 56% had PTEN base substitution mutations. There were 1–4 mutations in individual melanomas, including MIS, the earliest stage of melanoma (Wang *et al.*, 2009).

Table 1 summarizes the pathological and mutational features of all seven metastatic melanoma lesions tested. Samples from six (86%) of the melanomas showed base substitution mutations in the PTEN tumor suppressor gene. No insertions or deletions were observed. Fifteen base substitution mutations were detected and 14 of these were different from each other. There was 1 nonsense mutation (Gln245X), 12 missense mutations, and 2 synonymous mutations that did not alter the amino-acid sequence. Individual metastatic lesions showed marked genetic diversity with 1–4 different PTEN base substitution mutations: the liver lesion had one mutation; a CNS and a skeletal muscle metastasis each had two mutations; kidney and pancreas metastases each had three mutations; and the metastatic melanoma lesion on the scalp had four different mutations. Thirteen (87%) of the mutations were present at dipyrimidine sites, a feature of UV-induced mutations (Wang *et al.*, 2009). Loss of heterozygosity was found in two of the metastatic melanomas that also had *PTEN* missense mutations. A Thr167Ala missense mutation was reported previously in an astrocytoma (Raffel *et al.*, 1999). The missense mutations were located in the dual-specificity protein phosphatase domain (amino acids 25–179) as well as in the calcium/lipid binding region (amino acids 190–347).

Two of four (50%) of the metastatic lesions tested had *NRAS* base substitution mutations. One mutation in *NRAS* was found in the kidney metastasis and two *NRAS* mutations were found in the pancreas metastasis. However, these three *NRAS* mutations were all synonymous. None of the five metastatic lesions tested had *BRAF* base substitution mutations. The low frequency of mutations in *NRAS* and *BRAF* contrasts with the higher frequency of mutations in the *PTEN* gene obtained from the same samples and suggests that *PTEN* mutations were not the result of formalin artifacts that would be expected to similarly affect all genes (Williams *et al.*, 1999). Thus, mutations in the *NRAS* and *BRAF* oncogenes did not appear to have a major role in metastasis of melanomas in this XP patient.

Patient XP4BE presented with metastatic lesions. In our study of early melanomas in other XP patients (Wang *et al.*, 2009), we found the UV type mutations, gGc131gAc and aGg164aAg, in MIS lesions. These same mutations were also present in three of the metastatic lesions (liver, CNS, and kidney) (Table 1) and might have been present in the primary melanoma(s) in XP4BE. These mutations result in amino-acid alterations (Gly44Asp and Arg55Lys) in the protein phosphatase domain of PTEN and may represent persistent UV-induced genetic alterations associated with metastasis.

Surprisingly, there was no single mutation present in all of the metastatic lesions, as would be expected if all of the metastases arose from a single primary melanoma (Wang *et al.*, 2006; Sabatino *et al.*, 2008). Multiple tumor samples from the same patient had different mutations, indicating the presence of different clonal populations of tumor cells in different metastatic lesions. Independent PCR amplification of laser capture microdissected tumor tissue from adjacent areas of tumors 1, 2, and 4 revealed the identical mutations in *PTEN* exon 2 as shown in Table 1, with no new mutations found, which is evidence that these mutations were not formalin artifacts (Williams *et al.*, 1999). In addition, normal *PTEN* sequence was obtained for exons 4, 6, 7, and 8 for tumors 1–6, suggesting that there were local sequence variations in these metastatic melanomas. These metastatic lesions with different *PTEN* mutations might have arisen from different primary melanomas. The high frequency of UV type mutations in these metastatic lesions (87 vs 54% expected (Wang *et al.*, 2009)) is consistent with their origin from sunlight-induced primary melanomas. Alternatively, alterations in other gene(s) might have induced the metastases (Wang *et al.*, 2006; Sabatino *et al.*, 2008). As in the primary melanomas (Wang *et al.*, 2009), the finding of multiple *PTEN* mutations in metastatic lesions indicates that there is a marked genetic diversity in these tumors, perhaps reflecting the hypermutability of XP variant cells (Waters *et al.*, 1993; Stary *et al.*, 2003; Wang *et al.*, 2007). A similar genetic heterogeneity of metastatic melanomas from non-XP patients was reported based on the studies of loss of heterozygosity and X-chromosome inactivation (Katona *et al.*, 2007). This genetic diversity may affect melanoma development, progression toward metastasis, and response to therapy.

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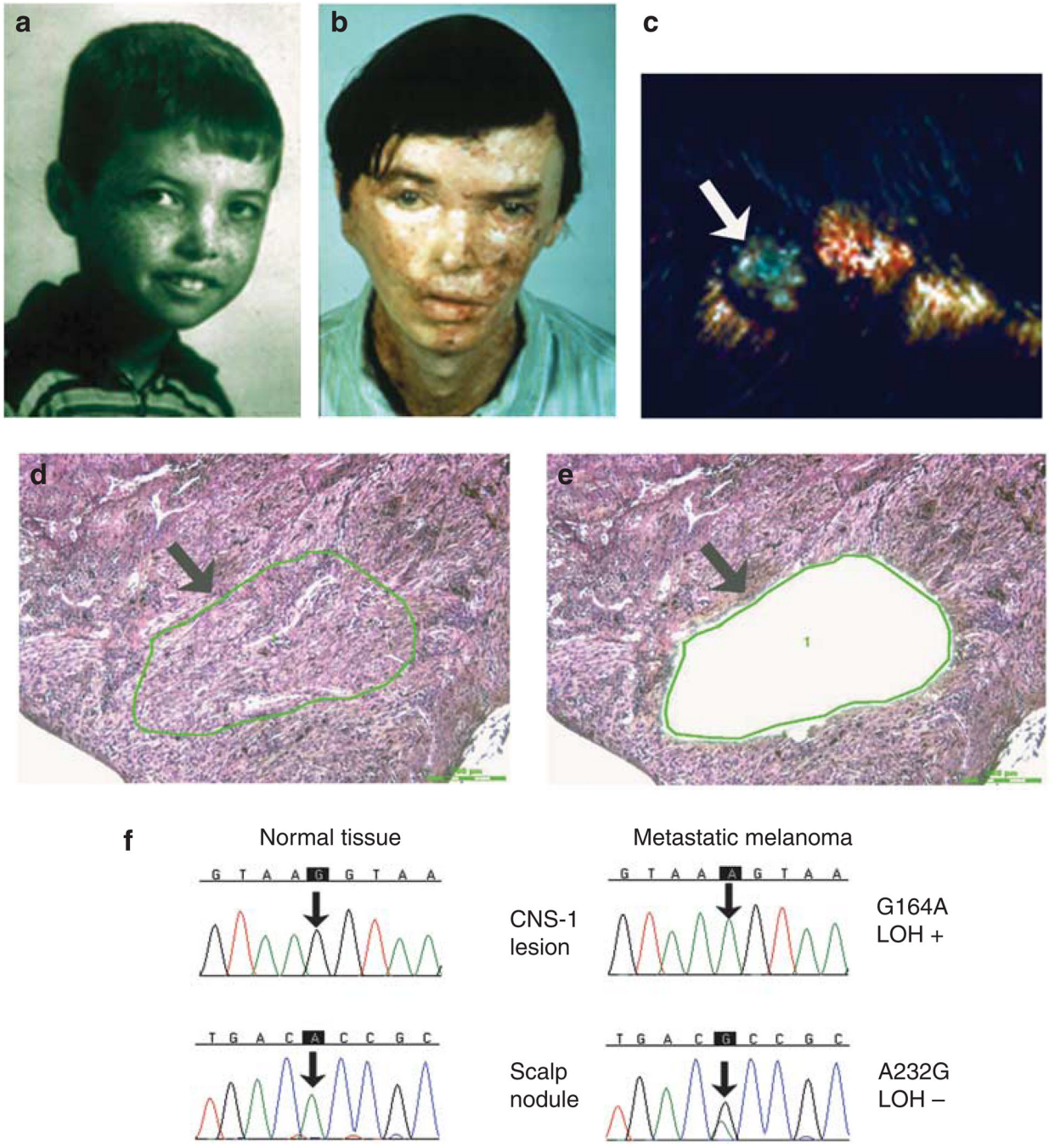

Figure 1. Clinical appearance, histology, and DNA sequencing of metastatic melanoma lesions in patient XP4BE

(**a**, **b**) Comparison of the face of patient XP4BE at the ages of 8 and 27 years. By the age of 27, he had had surgery with grafting for many skin cancers (reproduced from Robbins *et al.* (1974)). (**c**) Metastatic melanoma of the scalp before treatment with bis-choroethyl-nitrosourea (arrow). (**d**) Histology of metastatic melanoma lesion in scalp. Atypical melanocytes are arranged in sheets and nests (arrow) (hematoxylin and eosin (H&E) staining, bar = 200 μm). (**e**) After capture of the melanoma cells, the remaining tissue was inspected (arrow), and the transfer efficiency of about 500 captured cells was evaluated (H&E staining, bar = 200 μm). (**f**) Sequencing chromatograms for determination of PTEN

mutations. The metastatic melanoma DNA shows a mutation compared with the normal tissue (arrow). LOH, loss of heterozygosity. (Details of method are described in Wang *et al.* (2009)).

Table 1

Mutations in *PTEN*, *NRAS,* and *BRAF* in metastatic melanoma lesions from patient XP4BE

		PTEN				*NRAS*		*BRAF*
Metastatic lesion no.	**Metastatic melanoma site**	**Mutation**	**UV type mutation?[1]**	**LOH?[2]**	**Amino-acid change**	**Mutation**	**Amino-acid change**	**Mutation**
1	Liver	gGc131gAc[3]	+	−	Gly44Asp[3]	Normal	Normal	Normal
2	CNS-1	aGg164aAg[3]	+	+	Arg55Lys[3]	NT[4]	NT	Normal
2	CNS-1	tAt521tGt	−	−	Tyr174Cys			
3	Skeletal muscle	tCa733tTa	+	−	Gln245X	NT	NT	NT
3	Skeletal muscle	gTc882gCc	+	−	Ser294Ser[5]			
4	Kidney	aGg164aAg[3]	+	−	Arg55Lys[3]	aTc99aCc	Asp33Asp[5]	NT
4	Kidney	aAc499aGc	+	−	Thr167Ala[6]			
4	Kidney	aGg663aTg	+	+	Lys221Asn			
5	Pancreas	tAa162tGa	+	−	Val54Val[5]	aAt93aGt	Glu31Glu[5]	Normal
5	Pancreas	gGa494gTa	+	−	Gly165Val	cCa105cTa	Thr35Thr[5]	
5	Pancreas	tTa562tCa	+	−	Tyr188His			
6	Scalp	gAt152gGt	+	−	Asp51Gly	NT	NT	Normal
6	Scalp	cAc232cGc	−	−	Thr78Ala			
6	Scalp	aAg548aGg	+	−	Lys183Arg			
6	Scalp	gTt583gCt	+	−	Phe195Leu			
7	CNS-2	Normal			Normal	Normal	Normal	Normal

[1] +, Dipyrimidine; −, not dypyrimidine.

[2] +, Loss of heterozygosity; −, no loss of heterozygosity.

[3] Mutation reported in *in situ* melanoma in XP patients (Wang *et al.*, 2009).

[4] NT, not tested.

[5] Synonymous mutation (no amino-acid change).

[6] Mutation reported in an astrocytoma (Raffel *et al.*, 1999).