TITLE:

A case of Langerhans cell sarcoma on the scalp: Whole - exome sequencing reveals a role of ultraviolet in the pathogenesis

AUTHOR(S):

Katsuragawa, Hiroyuki; Yamada, Yosuke; Ishida, Yoshihiro; Kaku, Yo; Fujimoto, Masakazu; Kataoka, Tatsuki R.; Haga, Hironori

CITATION:

Katsuragawa, Hiroyuki ...[et al]. A case of Langerhans cell sarcoma on the scalp: Whole - exome sequencing reveals a role of ultraviolet in the pathogenesis. Pathology International 2020, 70(11): 881-887

ISSUE DATE:

2020-11

URL:

<http://hdl.handle.net/2433/259188>

RIGHT:

This is the peer reviewed version of the following article: ['Pathology International', 70(11), 881-887], which has been published in final form at <https://doi.org/10.1111/pin.13007>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.; The full-text file will be made open to the public on 16 August 2021 in accordance with publisher's 'Terms and Conditions for Self-Archiving'; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。; This is not the published version. Please cite only the published version.

**A Case of Langerhans Cell Sarcoma on the Scalp:
Whole-Exome Sequencing Reveals a Role of Ultraviolet in the Pathogenesis**

Hiroyuki Katsuragawa, MD¹, Yosuke Yamada, MD, PhD¹, Yoshihiro Ishida, MD, PhD²,
Yo Kaku, MD, PhD², Masakazu Fujimoto, MD, PhD¹, Tatsuki R. Kataoka, MD, PhD¹, and
Hironori Haga, MD, PhD¹

¹ Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan

² Department of Dermatology, Kyoto University Hospital, Kyoto, Japan

Running head

UV Meets Langerhans Cell Sarcoma

Author Note

Corresponding author: Yosuke Yamada

Address: 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

Fax number: +81-75-751-3499

Phone number: +81-75-751-4946

Email: yyamada@kuhp.kyoto-u.ac.jp

There are no Abbreviated words in this submission

21 **Abstract (200 words)**

22 Langerhans cell sarcoma (LCS) is a high-grade neoplasm with overtly malignant cytological
23 features and a Langerhans cell phenotype. The underlying genetic features are poorly
24 understood, and only a few alterations, such as those of the MARK pathway-related genes,
25 *CDKN2A* and *TP53* have been reported. Here we present a 70-year-old male with LCS on the
26 scalp and pulmonary metastasis. The multinodular tumor, 3.0 cm in diameter, consisted of
27 diffusely proliferated pleomorphic cells with numerous mitoses (53/10 HPFs).
28 Immunohistochemically, the tumor cells were positive for CD1a, Langerin, and PD-L1, and
29 the Ki-67 labeling index was 50%. These pathological features were consistent with LCS,
30 and were also observed in the metastatic tumor. Whole-exome sequencing revealed that both
31 the primary and metastatic tumors harbored a large number of mutations (>20
32 mutations/megabase), with deletion of *CDKN2A* and *TP53* mutation, and highlighted that the
33 mutational signature was predominantly characteristic of ultraviolet (UV) exposure
34 (W=0.828). Our results suggest, for the first time, that DNA damage by UV could
35 accumulate in Langerhans cells and play a role in the pathogenesis of LCS. The high
36 mutational burden and PD-L1 expression in the tumor would provide a rationale for the use
37 of immune checkpoint inhibitors for treatment of unresectable LCS.

38

39 **Keywords:** *CDKN2A*, Langerhans Cell Histiocytosis, Langerhans Cell Sarcoma, MAPK
40 pathway, PD-L1, Whole Exome Sequencing, *TP53*, Ultraviolet

41 Introduction

42 Langerhans cell sarcoma (LCS) is an aggressive neoplasm with apparent malignant
43 cytological features and a Langerhans cell phenotype. It is extremely rare, with an incidence
44 of 0.2 per 10,000,000 population (1), and often develops in skin and underlying soft tissues,
45 mostly in adults. Microscopically, LCS consists of atypical pleomorphic cells, sometimes
46 with the complex grooves reminiscent of Langerhans cells. The tumor shows high
47 proliferative activity, and mitotic figures often exceed >50/10 high-power fields.
48 Demonstration of a Langerhans cell phenotype by immunohistochemistry or by examination
49 of the ultrastructure is required for diagnosis. The prognosis is poor, with a median overall
50 survival period of only 19 months (1, 2).

51 These clinical and pathological features are considerably different from those of
52 Langerhans cell histiocytosis (LCH), the most common form of Langerhans cell neoplasm.
53 LCH often develops in children, especially as a bone tumor. It does not show obvious
54 cytological atypia, although the phenotypic features resemble LCS, and it generally exhibits
55 indolent behavior, sometimes even showing spontaneous regression (2).

56 However, the underlying molecular features of LCS that could explain its aggressive
57 behavior or pathogenesis, and lead to the development of more effective treatment strategies,
58 are poorly understood, except for the reported presence of a few genetic alterations, such as
59 mutations of MAPK-related genes, *CDKN2A*, or *TP53* (3-8).

60 Here, we report a case of LCS on the scalp of an elderly male with pulmonary
61 metastasis. As well as histological and immunohistochemical analyses, we performed whole-
62 exome sequencing for both the primary and metastatic sites and attempted to clarify the
63 molecular features.

64

65 **Clinical summary**

66 The patient was a 70-year-old male who had been treated for prostate cancer ten years
67 previously. He had no history of any hematopoietic malignancy. Three months before
68 presentation, he had undergone surgery for cervical spondylotic myelopathy. During the
69 postoperative rehabilitation period, a 3.0-cm nodule was found on the scalp, corresponding to
70 a site the patient reported to have been struck hard against a showerhead two years previously.
71 A biopsy and histologic examination of the scalp tumor suggested LCS. Computed
72 tomography (CT) for systemic screening revealed a nodule 1.0 cm in diameter in the upper
73 lobe of the left lung. This was suspected to be a metastasis of the LCS, as it had not been
74 detected in a CT examination performed six months previously. 18F-fluorodeoxyglucose
75 (18F-FDG) positron emission tomography (FDG-PET) demonstrated no other possible
76 metastatic tumors other than that in the lung. The patient underwent resection of the scalp
77 tumor and received two courses of CHOP (cyclophosphamide, hydroxydaunorubicin,
78 oncovin, and prednisone) therapy. However, as the lung nodule gradually enlarged, it was
79 resected for pathological examination.

80

81 **Pathological findings**

82 Macroscopically, the scalp tumor formed an irregular nodule 3.0 cm in diameter. The
83 formalin-fixed specimen showed that a whitish-gray tumor had invaded the periosteum from
84 the epidermis (Figure S1A-B). Microscopically, the tumor consisted of a diffuse proliferation
85 of atypical cells, which had pleomorphic nuclei with complex grooves, conspicuous nucleoli,
86 and moderate amounts of cytoplasm (Figure 1A-B). Mitotic figures were plentiful (53/10
87 HPFs), and variable numbers of eosinophils and small lymphocytes were present within the
88 tumor (Figure 1C-D). Immunohistochemistry (IHC) showed that the tumor cells were

positive for CD1a, CD4, CD68, Langerin, and S-100. The Ki-67 labeling index was about 50% (Figure 1E-H). BRAF V600E, CD21, CD23, CD123, CD163, MCPyV, and SOX10 were negative (data not shown). These pathological features were consistent with Langerhans cell sarcoma (LCS). There were no areas containing proliferations of bland histiocytic cells, which is a feature reminiscent of Langerhans cell histiocytosis (LCH). The resected lung tumor also consisted of atypical cells with a morphology and immunophenotype similar to the skin tumor, although they are more pleomorphic than tumor cells in the primary site (Figure 2A-D). Thus, this was diagnosed as a metastasis from the LCS on the scalp.

We next performed whole-exome sequencing (WES) for both the primary and metastatic tumors to clarify the genetic profile. Both tumors harbored a large number of mutations (663 SNVs/indels in the primary site and 634 SNVs/indels in the metastasis), most of which (569 genes) were shared between the two lesions (Figure 3A), including *TP53*/Y88C, *KMT2D*/R3703X, and *STK19*/G265A mutations (Table 1). Copy number analysis showed that both tumors harbored homozygous deletions in 6q and 9p, the latter being consistent with the locus of *CDKN2A* (Figure 3B and Figure S2A-B). The *TP53* mutation and *CDKN2A* deletion were supported by the findings of IHC because the tumor cells were strongly positive for p53 and negative for p16, the protein encoded by *CDKN2A* (Figure 4A-B). Because the number of mutations was large, we were able to decompose the mutational signature into known COSMIC signatures; surprisingly, it was dominated by COSMIC signature 7, i.e., an ultraviolet (UV)-mutational signature (W=0.828) (Figure 3C and Figure S2C). The mutation spectra further supported the role of UV in the mutagenesis: 77.3% (primary site) and 77.1% (metastatic site) of the SNVs were C to T mutations at dipyrimidine sites, and 5.4% (primary) and 4.5% (metastatic) of them were CC to TT substitutions (9).

Considering its possible therapeutic relevance, we also performed immunohistochemistry for PD-L1 and confirmed that both the primary and metastatic tumors partially expressed PD-L1, as well as accompanying macrophages (Figure 4C).

Discussion

A literature searched revealed ten English-language papers reporting genetic alterations in LCS (3-8, 10-13). Six case reports (3-8), three of which included WES data (4-6), indicated causative alterations such as mutations related to activation of the MAPK pathway (*BRAF*, *KRAS*, *MAPK2A1*, or *NRAS*), homozygous deletions of *CDKN2A/(2B)*, and/or a loss-of-function mutation of *TP53* (Table 1). The present case also exhibited *CDKN2A* deletion and a pathogenic missense mutation in *TP53* (Figure 3B and Table 1), which was supported by IHC (Figure 4A-B). Xerri et al. reported that deletion of *CDKN2A/(2B)* was not found among their 22 LCS samples (3), and Badalian detected *TP53* mutation in only one of 61 LCH cases (14), while mutations related to the MAPK pathway overlapped in LCS and LCH (14, 15). Thus, the dysfunction of these common tumor suppressor genes would underlie the aggressiveness of LCS and differentiate it from LCH. The present case also harbored truncating mutations of *KMT2D* and *STK19*, which are also known to be tumor suppressor genes in some cancers including those of the skin (16).

Our most important discovery in the present case was that the mutational signature was highly correlated with that of UV mutagenesis (Figure 3C). Murine experiments have shown that Langerhans cells have a rather long lifespan (at least 18 months) (17), and UV can change the phenotype of human Langerhans cells through alteration of the surface molecules (18). Through our analysis of this Langerhans cell neoplasm, we demonstrated for the first time that UV could affect not only the phenotype but also the genome of the Langerhans cells.

LCS can arise through at least three mechanisms: *de novo*, progression from LCH, and transdifferentiation from a B-cell neoplasm (4, 10, 12, 19). Our results suggest that *de novo* LCS, corresponding to our case, can occur as a UV-related cancer, as is the case for skin cancers of various lineages (e.g., basal cell carcinoma, squamous cell carcinoma, and melanoma, etc.). The association of UV with LCS could also explain the tendency of LCS to arise more frequently in adult skin, including sun-exposed areas, in comparison with LCH. Taken together, we speculate that if human Langerhans cells are able to remain in the epidermis for a sufficient period of time as murine Langerhans cells (17), ultraviolet (i.e., sun) exposure could cause genetic alterations (some of the genes might be related to their migration capacity), ultimately leading to their malignant transformation (i.e., [*de novo*] LCS) (Figure 4D).

Unfortunately, we were unable to detect any possible driver mutations. As well as the typical histological features, because IHC showed the tumor to be strongly positive for EZH2 (Figure S1C), whose expression is known to be highly correlated with the phosphorylation of ERK in histiocytic and dendritic cell neoplasms (20), we speculate that the present case might also have been driven by the MAPK pathway through unknown mechanisms. However, other pathways may play a key role in the pathogenesis, as has been reported for some LCH cases (15).

Lastly, we attempted to consider some therapeutic options for LCS, as few established treatments are currently available. Because the tumor showed a high tumor mutation burden (>20/Mb), we speculated that immune checkpoint inhibitors might be an option, especially as the tumor cells expressed PD-L1 (Figure 4C). Although no previous reports have documented the use of this type of drug for Langerhans cell neoplasms, in view of the generally dismal

160 prognosis of LCS, our present findings suggest that immune checkpoint inhibitors would be
161 worth considering for unresectable cases, under careful monitoring.

162 In summary, we have reported a patient with LCS on the scalp associated with
163 pulmonary metastasis. As well as the typical pathological features, exome sequencing
164 revealed that the tumor harbored a large number of mutations, including *CDKN1A* deletion
165 and *TP53* mutation, and strongly suggested that the mutations had arisen through UV
166 exposure. These findings advance our understanding of the biology of Langerhans cells and
167 their neoplasms, and might contribute to the development of more effective treatment
168 strategies.

169

170 **Disclosure Statement**

171 The authors have no conflicts of interest to declare.

172

173 **Author Contributions**

174 Drafting the manuscript and figures; HK and YY. Acquisition and analysis of data; YI.
175 Correction and approval of manuscript; All authors.

176 **Figure Legends**

177 Figure 1. Microscopic features of the primary tumor (skin) (A-D, H&E sections; E-H,
178 immunohistochemistry).

179 A multinodular tumor is evident on the scalp (A). The tumor cells have pleomorphic
180 nuclei with complex grooves, conspicuous nucleoli, and moderate amounts of cytoplasm (B),
181 with numerous mitoses (arrows) (C). Focal eosinophilic infiltration is evident (D). The tumor
182 cells are positive for CD1a (E), Langerin (F) and S-100 (G), and the Ki-67 labeling index was
183 50% (H).

184

185 Figure 2. Microscopic features of the metastatic tumor (lung) (A-B, H&E sections; C-D,
186 immunohistochemistry).

187 A nodule 10 mm in diameter is evident in the lung (A). The tumor consists of highly
188 atypical cells morphologically similar to those at the primary site, with necrosis (upper right)
189 (B). The tumor cells are positive for CD1a (C) and Langerin (D).

190

191 Figure 3. Whole-exome sequencing of the primary and metastatic tumors.

192 Variant allele frequency (VAF) in the primary and metastatic tumors (A). Both
193 tumors harbor a large number of genomic mutations, which are mostly shared between the
194 two lesions, including mutations in *TP53*, *KMT2D*, and *STK11* (A). Copy number analysis of
195 the primary tumor (B-C). The tumor exhibits a homozygous deletion of 9q (B), which
196 corresponds to the locus of *CDKN2A* (C). The mutational signature of the tumor is highly
197 correlated with COSMIC signature 7, i.e., an ultraviolet mutational signature (D).

198

199 Figure 4. Immunohistochemistry for p16, p53, and PD-L1 in the primary tumor, and a
200 possible role of ultraviolet in the pathogenesis of Langerhans cell sarcoma: a hypothesis.

201 The tumor is completely negative for p16 (A) and strongly/diffusely positive for p53
202 (B). Some of the tumor cells, including one with mitosis (arrow), exhibit membranous PD-L1
203 expression (C). A possible role of ultraviolet in the pathogenesis of Langerhans cell sarcoma:
204 a hypothesis (D). If human Langerhans cells remain in the epidermis for a sufficient period of
205 time, ultraviolet (i.e., sun) exposure could cause genetic alterations, ultimately leading to
206 their malignant transformation (i.e., [*de novo*] LCS).

207

208 **Table**

209 Table 1. Reported genetic alterations in Langerhans cell sarcoma.

210

211 **Supplementary information**

212 Figure S1. Macroscopic and immunohistochemical features of the skin tumor.

213 The multinodular whitish tumor with a diameter of 3.0 cm is seen on the skin (A). The
214 cut section of the tumor shows expansion from the epidermis to the periosteum (B). The
215 tumor is diffusely positive for EZH2 (C).

216

217 Figure S2. Whole-exome sequencing of the primary and metastatic tumors.

218 The primary tumor shows a homozygous deletion of 6p (A). The copy number
219 validation of the metastatic tumor is common to that of the primary tumor (B). The

220 mutational signature of the metastatic tumor is highly correlated with COSMIC signature 7,
221 i.e., an ultraviolet mutational signature (C).

222 References

- 223 1. Tella SH, Kommalapati A, Rech KL, Go RS, Goyal G. Incidence, Clinical Features,
224 and Outcomes of Langerhans Cell Sarcoma in the United States. *Clin Lymphoma Myeloma*
225 *Leuk.* 2019;**19**:441-6.
- 226 2. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of*
227 *Haematopoietic and Lymphoid Tissues.* Lyon: IARC; 2017.
- 228 3. Xerri L, Adélaïde J, Popovici C, et al. CDKN2A/B Deletion and Double-hit Mutations
229 of the MAPK Pathway Underlie the Aggressive Behavior of Langerhans Cell Tumors. *Am J*
230 *Surg Pathol.* 2018;**42**:150-9.
- 231 4. Choi SM, Andea AA, Wang M, et al. KRAS mutation in secondary malignant
232 histiocytosis arising from low grade follicular lymphoma. *Diagn Pathol.* 2018;**13**:78.
- 233 5. Kim SW, Choi MK, Han HS, et al. A Case of Pulmonary Langerhans Cell Sarcoma
234 Simultaneously Diagnosed with Cutaneous Langerhans Cell Histiocytosis Studied by Whole-
235 Exome Sequencing. *Acta Haematol.* 2017;**138**:24-30.
- 236 6. Karai LJ, Sanik E, Ricotti CA, Susa J, Sinkre P, Aleodor AA. Langerhans cell sarcoma
237 with lineage infidelity/plasticity: a diagnostic challenge and insight into the pathobiology of
238 the disease. *Am J Dermatopathol.* 2015;**37**:854-61.
- 239 7. Mourah S, Lorillon G, Meignin V, et al. Dramatic transient improvement of
240 metastatic BRAF(V600E)-mutated Langerhans cell sarcoma under treatment with dabrafenib.
241 *Blood.* 2015;**126**:2649-52.
- 242 8. Zwerdling T, Won E, Shane L, Javahara R, Jaffe R. Langerhans cell sarcoma: case
243 report and review of world literature. *J Pediatr Hematol Oncol.* 2014;**36**:419-25.
- 244 9. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in
245 human cancer. *Nature.* 2013;**500**:415-21.
- 246 10. Ambrosio MR, De Falco G, Rocca BJ, et al. Langerhans cell sarcoma following
247 marginal zone lymphoma: expanding the knowledge on mature B cell plasticity. *Virchows*
248 *Arch.* 2015;**467**:471-80.
- 249 11. Chang NY, Wang J, Wen MC, Lee FY. Langerhans Cell Sarcoma in a Chronic
250 Myelogenous Leukemia Patient Undergoing Imatinib Mesylate Therapy: A Case Study and
251 Review of the Literature. *Int J Surg Pathol.* 2014;**22**:456-63.
- 252 12. Muslimani A, Chisti MM, Blenc AM, Boxwala I, Micale MA, Jaiyesimi I.
253 Langerhans/dendritic cell sarcoma arising from hairy cell leukemia: a rare phenomenon. *Ann*
254 *Hematol.* 2012;**91**:1485-7.
- 255 13. Shao H, Xi L, Raffeld M, et al. Clonally related histiocytic/dendritic cell sarcoma and
256 chronic lymphocytic leukemia/small lymphocytic lymphoma: a study of seven cases. *Mod*
257 *Pathol.* 2011;**24**:1421-32.

- 258 14. Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in
259 Langerhans cell histiocytosis. *Blood*. 2010;**116**:1919-23.
- 260 15. Diamond EL, Durham BH, Haroche J, et al. Diverse and Targetable Kinase
261 Alterations Drive Histiocytic Neoplasms. *Cancer Discov*. 2016;**6**:154-65.
- 262 16. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma.
263 *Cell*. 2012;**150**:251-63.
- 264 17. Merad M, Manz MG, Karsunky H, et al. Langerhans cells renew in the skin
265 throughout life under steady-state conditions. *Nat Immunol*. 2002;**3**:1135-41.
- 266 18. Aberer W, Schuler G, Stingl G, Hönigsmann H, Wolff K. Ultraviolet light depletes
267 surface markers of Langerhans cells. *J Invest Dermatol*. 1981;**76**:202-10.
- 268 19. Nakamine H, Yamakawa M, Yoshino T, Fukumoto T, Enomoto Y, Matsumura I.
269 Langerhans Cell Histiocytosis and Langerhans Cell Sarcoma: Current Understanding and
270 Differential Diagnosis. *J Clin Exp Hematop*. 2016;**56**:109-18.
- 271 20. Tian X, Xu J, Fletcher C, Hornick JL, Dorfman DM. Expression of enhancer of zeste
272 homolog 2 (EZH2) protein in histiocytic and dendritic cell neoplasms with evidence for p-
273 ERK1/2-related, but not MYC- or p-STAT3-related cell signaling. *Mod Pathol*. 2018;**31**:553-
274 61.
- 275

A Shared and private mutations

B Copy number (The primary tumor)

C Mutational signature (The primary tumor)

Signature.7 : 0.827 & Signature.8 : 0.094 & Signature.11 : 0.069

Table 1. Reported genetic alterations in Langerhans cell sarcoma

Authors	Age	Gender	Organ	Pathogenic genetic alterations	The primary tumor
The present case	70	Male	Skin	<i>KMT2D</i> (R3703X) <i>STK19</i> (G265A) <i>TP53</i> (Y88C)	<i>De novo</i>
Choi et al., 2018 (4)	NA	NA	Lymph node	<i>IgH/BCL2</i> (rearrangement) <i>KRAS</i> (G13D)	Follicular lymphoma
Xerri et al., 2018 (3)	55	Male	Lymph node	<i>CDKN2A/B</i> (homozygous deletion) <i>KMT2D</i> (E2989X) <i>MAP2K1</i> (C121S) <i>NOTCH1</i> (Q2403X) <i>NRAS</i> (Q61K)	<i>De novo</i>
Kim et al., 2017 (5)	73	Male	Lung	<i>TP53</i> (R196*)	<i>De novo</i>
Karai et al., 2015 (6)	62	Female	Skin	<i>CDKN2A</i> (homozygous deletion) <i>KRAS</i> (Q61H) <i>TP53</i> (R282W)	<i>De novo</i>
Mourah et al., 2015 (7)	58	Male	Lymph node	<i>BRAF</i> (V600E)	<i>De novo</i>
Zwerdling et al., 2014 (8)	7	Female	Soft tissue	<i>BRAF</i> (V600E)	<i>De novo</i>

NA, not available

A Chromosome 6 (The primary tumor)

B Copy number (The metastatic tumor)

C Mutational signature (The metastatic tumor)

Signature.7 : 0.874 & Signature.8 : 0.114