Nanotomography of lesional skin using electron microscopy reveals cytosolic release of nuclear DNA in psoriasis

Eric Lindberg, PhD,[a] Yvonne Baumer, PhD,[a,b] Erin S. Stempinski, MS,[a] Justin A. Rodante, PA,[b] Tiffany M. Powell-Wiley, MD, MPH,[c] Amit K. Dey, MD,[b] Saeko Nakajima, MD, PhD,[d] Martin P. Playford, PhD,[b] Christopher K. E. Bleck, PhD,[a] and Nehal N. Mehta, MD, MSCE, FAHA[b]
Bethesda, Maryland and Kyoto, Japan

Key words: DNA release; FIB-SEM; inflammation; keratinocytes; psoriasis.

INTRODUCTION

Psoriasis is a common, chronic inflammatory skin disease associated with thick, scaly erythematous plaques on the extensor and flexor surfaces of the skin. Psoriasis is linked to cardiometabolic comorbidities leading to decreased life expectancy,[1] and although the underlying mechanisms are not yet fully understood, inflammation is a key driver of these associations. Normally, DNA is confined to the nucleus and the mitochondria. In disease, DNA is released to the cytosol[2,3] and is an important danger-associated molecular pattern in keratinocytes, which is accelerated in the presence of psoriasis-associated proinflammatory cytokines.[4] Circulating cell-free DNA and mitochondrial DNA are higher in psoriasis patients than in healthy controls, and both types of DNA are believed to be released into the circulation by apoptotic cells and activated immune cells.[5,6] Circulating free DNA may also originate from low-density granulocytes that undergo spontaneous NETosis,[7] whereby DNA is released into the extracellular space. However, these processes have not been studied in human skin lesions from patients with psoriasis using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM).

Abbreviations used:

FIB-SEM:	Focused Ion Beam Scanning Electron Microscopy
NHLBI:	National Heart, Lung and Blood Institute
NIH:	National Institutes of Health
SEM:	scanning electron microscope

CASE REPORT

We obtained a biopsy specimen from nonlesional and lesional skin of a 65-year-old woman with psoriasis, with a Psoriasis Area and Severity Index score of 21.6 (Fig 1), who was not on systemic or biological treatment for psoriasis, and from a healthy volunteer without psoriasis (Fig 1).

Hematoxylin and eosin staining (Fig 2, top) showed an increase in the thickness of the epidermal layer in the lesion consistent with psoriasis. The same features were observed by scanning electron microscopy (Fig 2, middle). We further investigated the ultrastructure by FIB-SEM 3-dimensional volume tomography (Fig 2, bottom). Image segmentations of 3-dimensional datasets allowed for quantification of keratinocyte nuclear membrane volumes (Fig 2),

From the Electron Microscopy Core Facility,[a] Section of Inflammation and Cardiometabolic Diseases,[b] and Social Determinants of Obesity and Cardiovascular Risk Laboratory,[c] National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda; and Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto.[d]

Authors Lindberg, Baumer, Bleck, and Mehta contributed equally to the experiments and design of the study.

Funding sources: Supported by the Intramural Research Program of the National Institutes of Health HL006193-07 to Nehal N. Mehta. Dr Baumer was financially supported by the National Psoriasis Foundation Robertson Translational Research Fellowship. Dr Nakajima was financially supported by the Japan Agency for Medical Research and Development (AMED)-PRIME (19gm6010014h0003).

IRB approval status: Reviewed and approved by the Institutional Review Board of the National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), in accordance with the principles of the Declaration of Helsinki.

Data can be provided by the corresponding author upon reasonable request.

Correspondence to: Nehal N. Mehta, MD, MSCE, FAHA, Chief, Section of Inflammation and Cardiometabolic Diseases, National Heart, Lung and Blood Institute, National Institutes of Health, Building 10, 10 Center DriveBethesda, MD 20814. E-mail: nehal.mehta@nih.gov.

JAAD Case Reports 2021;9:9-14.
2352-5126

https://doi.org/10.1016/j.jdcr.2020.12.024

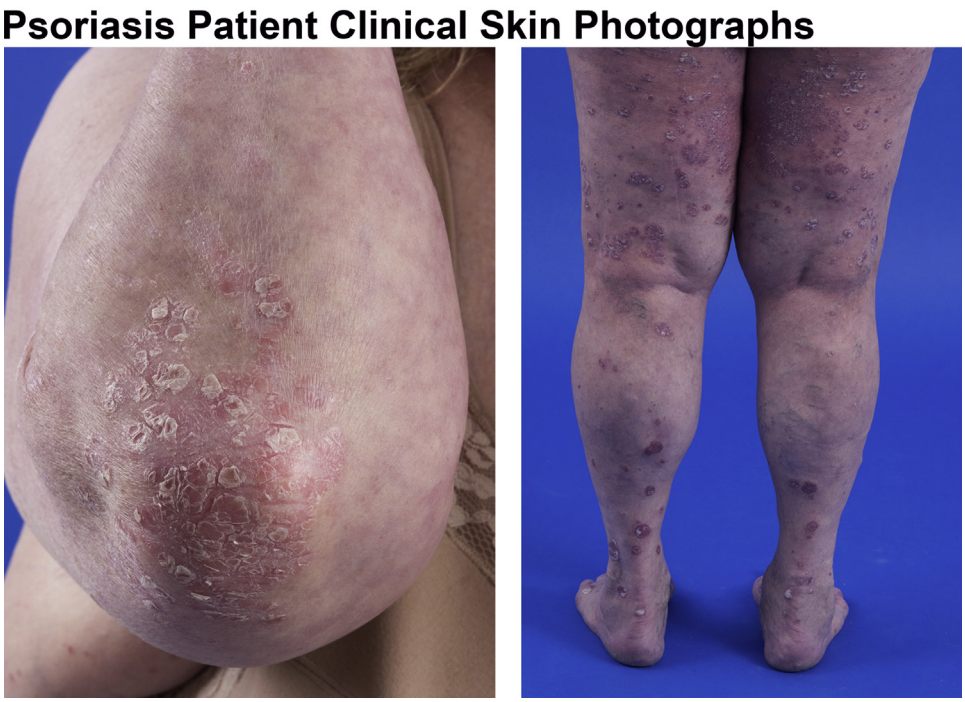

Fig 1. Photographic images of psoriasis study participants' skin. Images of the study participants' skin were taken at the day of the clinical visit.

where the nuclei in lesional psoriasis skin appeared to be larger than the nuclei in nonlesional psoriasis skin and healthy volunteer skin (Fig 2).

Upon further analysis, we found evidence of nuclear DNA release into the cytosol of keratinocytes in lesional psoriasis skin. Four different regions were characterized by FIB-SEM (Fig 3), two in the stratum spinosum (Fig 3, regions 1 and 3) and two in the stratum granulosum layer (Fig 3, regions 2-4), with DNA release observed only in the stratum spinosum layer of psoriasis skin.

As shown in Fig 4, nuclear release of DNA in keratinocytes in the stratum spinosum was accompanied by the nuclear membrane partially opening and releasing the DNA into the cytosol, with the rest of the nuclear membrane remaining fully intact. There was no rupture of the keratinocyte cell membrane itself. DNA release from mitochondria was not observed.

3D surface renderings of image segmentations of keratinocyte nuclei showed the DNA being released in a common direction (Fig 5 and Supplementary Videos 1 and 2). These findings were not observed in nonlesional skin or in healthy skin.

DISCUSSION

A possible involvement of intracellular DNA in the pathology of psoriasis has previously been suggested,[8] but it was also stated that the origin of self-DNA in psoriatic skin remained to be determined.[3] Given our findings in lesional psoriatic skin, further investigation is required to pinpoint the exact underlying factors that induce DNA release into the cytosol of keratinocytes and whether this process is similar to vital NETosis. We also observed apparent differences in the size of nuclei between healthy skin, nonlesional psoriatic skin, and lesional psoriatic skin, a phenomenon that might be explained by changes in lamin subtype expression.[9,10] Lamins have been reported to be crucial for controlling the size and flexibility of the nucleus, with reduced expression levels resulting in fragile and rupture-prone nuclei,[9] as seen in the context of neutrophil NETosis. Lamin expression varies widely between different cell types and is crucial for keratinocyte differentiation but therefore potentially presenting a plausible cellular mechanism regulating size and rupture of keratinocyte nuclei as seen in psoriasis lesional skin.[11] Furthermore, differences in lamin expression throughout the epidermal layer might explain why we detected cytosolic DNA release in the stratum spinosum only. Interestingly, keratinocytes in the stratum granulosum of psoriatic skin retained their nuclei, a finding supported by the literature,[12] which might also be explained by differences in lamin subtype expression in psoriasis. Our findings of DNA release by keratinocyte nuclei in lesional psoriatic skin suggest that DNA extrusion from keratinocytes and subsequent release may contribute to the increased levels of circulating

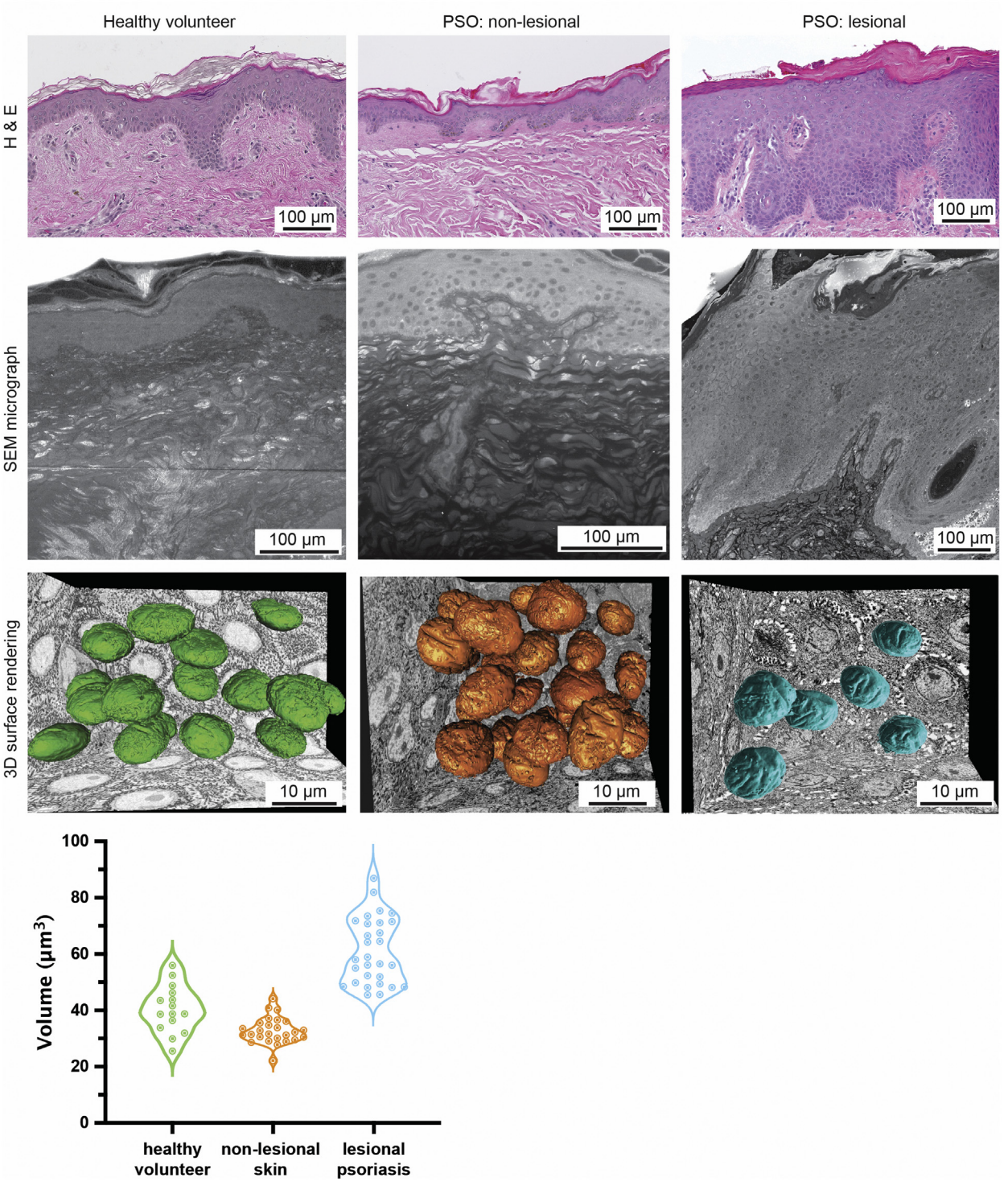

Fig 2. Histopathology and overview images of analyzed skin samples. Images of hematoxylin and eosin staining as well as scanning electron microscope (SEM) overview images, and whole nuclei volume renderings of image segmentations of healthy volunteer skin (left), psoriasis (PSO) nonlesional skin (middle), and psoriasis lesional skin (right). Graph shows volume of whole nuclei image segmentations.

cell-free DNA driving innate immunity in psoriasis. Our data warrant further investigation into the role of nuclear DNA release in psoriasis and demonstrate how FIB-SEM holds promise to further define cellular ultrastructure to better understand psoriasis cutaneous pathology.

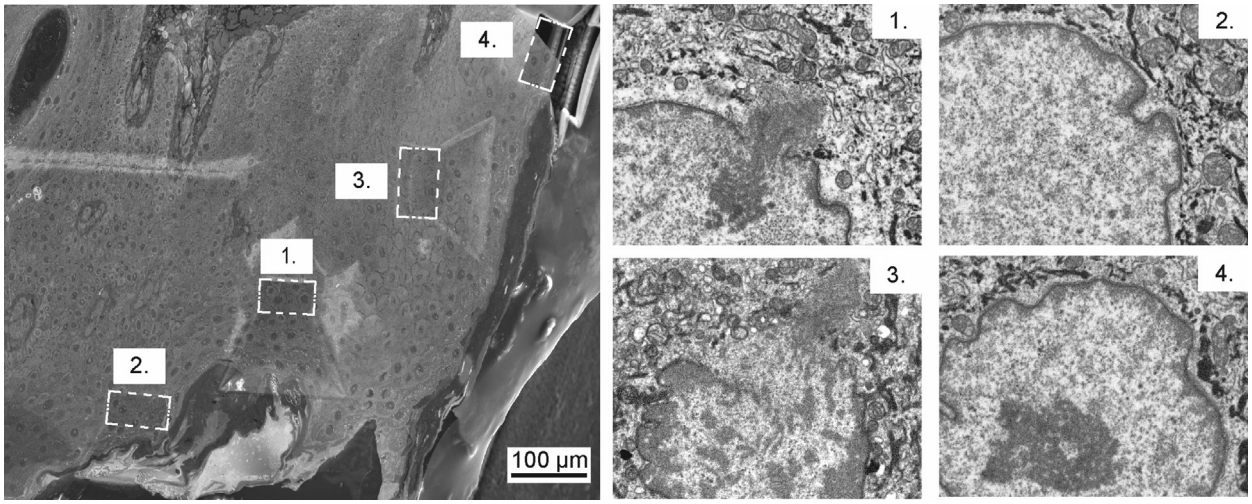

Fig 3. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) overview image of lesional psoriasis skin. Overviews showing regions of interest selected for FIB-SEM in psoriasis lesional skin sample (left). Representative FIB-SEM images of each region of interest shown on the right.

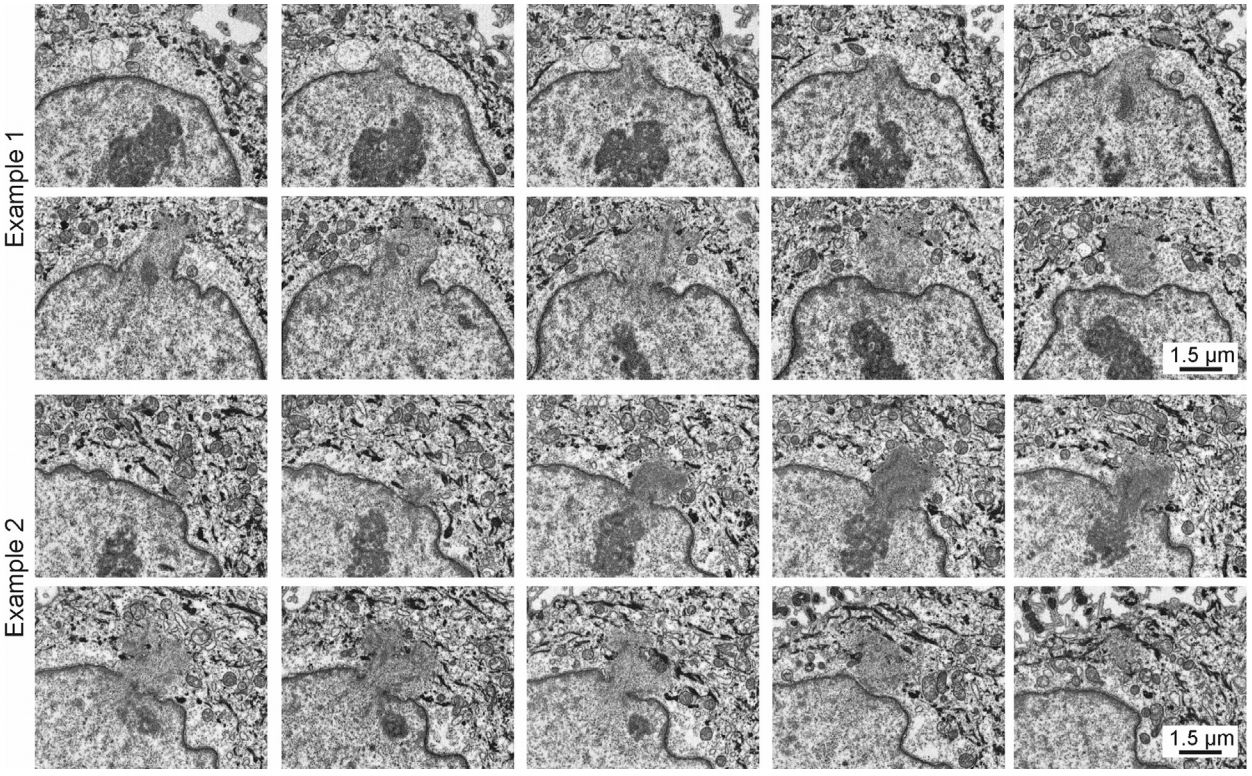

Fig 4. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) serial images of lesional psoriasis skin. Serial images from two keratinocytes in the stratum spinosum layer of lesional psoriasis skin are displayed. The nuclear membrane opens, followed by nuclear material being released into the cytosol. At a later point in the z-stack, the nuclear membrane is fully closed again. The secreted nuclear material is clearly visible in the cytosol.

MATERIALS AND METHODS

Human subjects

Study approval was obtained from the Institutional Review Board of the National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), in accordance with the principles of the Declaration of Helsinki. All guidelines for good clinical practice and those set forth by the NIH and in

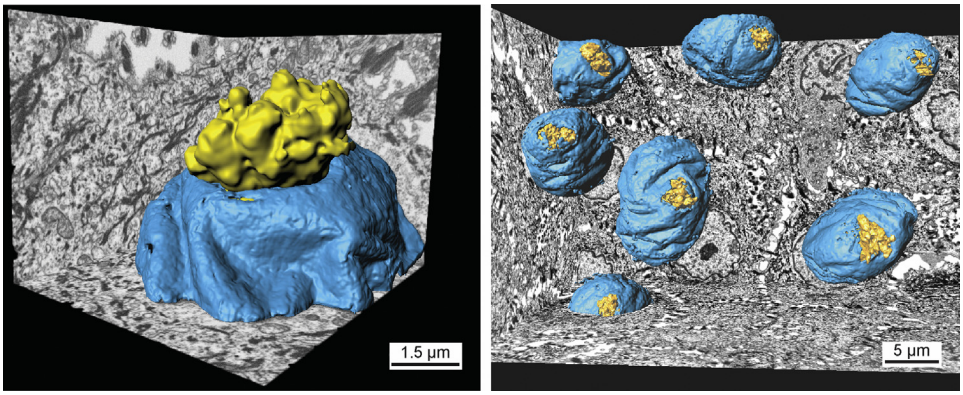

Fig 5. 3D reconstruction of Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) serial images. 3D reconstruction of keratinocyte nuclear membranes of DNA excreting nuclei (blue) and secreted DNA (yellow).

the Belmont Report (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research) were followed. All study participants in the cohort provided written informed consent. All the participants were adequately compensated. Data for all psoriasis patients under the cohort study at NHLBI/NIH were obtained from the Psoriasis, Atherosclerosis, and Cardiometabolic Disease Initiative protocol (NCT01778569), while healthy subjects were accrued under a separate Inflammatory Characterization of Known or Possible Cardiovascular Diseases protocol (NCT0193 4660) at NIH/NHLBI.

Human skin biopsies

First, the procedure was explained to the study participants and informed consent was obtained. An appropriate area of skin in the healthy and the psoriatic volunteers was identified. Care was taken to avoid higher-risk areas such as the distal extremities, face, and areas directly over tendons or ligaments. The area was then prepared with chlorhexidine, draped in a sterile manner, and infiltrated with 3 to 5 cc of lidocaine 2% with epinephrine solution. After a few minutes and with analgesia ensured, a round 3-mm punch biopsy was performed with a 3-mm punch biopsy tool. The sample was then placed in a sterile cup and transferred to the laboratory for immediate preparation by a laboratory member. Local homeostasis at the wound was achieved with pressure. Gauze and tape bandage and, if needed, steri-strips were applied. The study participant was provided with post-care instructions.

Focused Ion Beam Scanning Electron Microscopy

By performing consecutive sectioning and imaging, FIB-SEM is a new and innovative technique capable of achieving the ultrastructural resolution of transmission electron microscopy in a 3-dimensional system, which to our knowledge has not been performed on human psoriatic skin samples. Tissue samples were prepared for FIB-SEM as previously. The samples were imaged inside a Zeiss Crossbeam 540 FIB-SEM microscope. Platinum and carbon were deposited over the region of interest, and the run was set up and controlled by Atlas software (Fibics). The SEM settings were 1.5 kV, 2.5 nA, milling probes 700 or 300 pA. The slice thickness and the imaging pixel size were set to 10 nm. The total volume acquired per tissue sample is listed below (XYZ): HV = 49.80 × 24.90 × 25.62 μm; Pso-Les = 39.93 × 34.84 × 25.63 μm (region 1); 44.99 × 29.94 × 26.16 μm (region 2); 39.94 × 34.94 × 12.09 μm (region 3); 49.87 × 24.66 × 23.19 μm (region 4); NL = 40.15 × 28.02 × 27.77 μm.

Advanced imaging and analysis

The FIB-SEM datasets were aligned using Atlas 5 software (Fibics). The data were then imported into Fiji software and binned 4 times, to 40 × 40 × 40 nm isotropic voxels. The contrast was then normalized using Enhance Local Contrast (CLAHE3Dtool in ImageJ). Segmentation of membranes and nuclear material was performed using the Pixel Classification module in the Ilastik software package (ilastik.org). The probability maps were then imported into Imaris (Bitplane.com), and surfaces were generated around fully segmented nuclei (partially segmented nuclei in which no DNA excretion could be observed were excluded). Images and videos were rendered using Imaris software.

We are grateful for the contributions of our clinical team at the NIH Clinical Center and our study participants.

Conflicts of interest

Dr Mehta is a full-time US government employee and has served as a consultant for Amgen, Eli Lilly, and Leo Pharma receiving grants and other payments; as a principal investigator and/or investigator for AbbVie, Celgene, Janssen Pharmaceuticals, and Novartis receiving grants and/or research funding; and as a principal investigator for the National Institutes of Health receiving grants and/or research funding. Authors Lindberg, Baumer, Stempinski, Rodante, Powell-Wiley, Dey, Nakajima, Playford, and Bleck have no conflicts of interest to declare.

REFERENCES

1. Egeberg A, Skov L, Joshi AA, et al. The relationship between duration of psoriasis, vascular inflammation, and cardiovascular events. *J Am Acad Dermatol*. 2017;77:650-656.e3.
2. Paludan SR, Reinert LS, Hornung V. DNA-stimulated cell death: implications for host defence, inflammatory diseases and cancer. *Nat Rev Immunol*. 2019;19:141-153.
3. Schauber J, Dombrowski Y, Besch R. Pathogenic DNA: cytosolic DNA promotes inflammation in psoriasis. *Cell Cycle*. 2011;10:3038-3039.
4. Chiliveru S, Rahbek SH, Jensen SK, et al. Inflammatory cytokines break down intrinsic immunological tolerance of human primary keratinocytes to cytosolic DNA. *J Immunol*. 2014;192:2395-2404.
5. Beranek M, Fiala Z, Kremlacek J, et al. Changes in circulating cell-free DNA and nucleosomes in patients with exacerbated psoriasis. *Arch Dermatol Res*. 2017;309:815-821.
6. Therianou A, Vasiadi M, Delivanis DA, et al. Mitochondrial dysfunction in affected skin and increased mitochondrial DNA in serum from patients with psoriasis. *Exp Dermatol*. 2019;28:72-75.
7. Teague HL, Varghese NJ, Tsoi LC, et al. Neutrophil subsets, platelets, and vascular disease in psoriasis. *JACC Basic Transl Sci*. 2019;4:1-14.
8. Dombrowski Y, Peric M, Koglin S, et al. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci Transl Med*. 2011;3:82ra38.
9. Manley HR, Keightley MC, Lieschke GJ. The neutrophil nucleus: an important influence on neutrophil migration and function. *Front Immunol*. 2018;9:2867.
10. Melero JL, Andrades S, Arola L, Romeu A. Deciphering psoriasis. A bioinformatic approach. *J Dermatol Sci*. 2018;89:120-126.
11. Naeem AS, Zhu Y, Di WL, Marmiroli S, O'Shaughnessy RF. AKT1-mediated Lamin A/C degradation is required for nuclear degradation and normal epidermal terminal differentiation. *Cell Death Differ*. 2015;22:2123-2132.
12. Roberson EDO, Bowcock AM. Psoriasis genetics: breaking the barrier. *Trends Genet*. 2010;26:415-423.