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Localised multiple spiradenomas are caused by somatic mosaicism in *ALPK1* hotspot mutation

Dear Editor,

Malignant eccrine spiradenoma (MES) is a rare skin adnexal tumour, mostly arising from a pre-existing benign eccrine spiradenoma (ES). MES typically presents sporadically as a solitary nodule or in association with Brooke-Spiegler syndrome[1]. Localised multiple ESs are exceptionally rare and have been reported to be arranged in a linear, zosteriform, nevoid, or Blaschkoid pattern[2,3]. Notably, one of these ESs may undergo malignant transformation into MES[4]. Genomic profiling identified pervasive *ALPK1* p.V1092A hotspot mutation in MES, ES, and adjacent morphologically normal skin[5]. A previous report suggested that multiple ESs result from somatic mosaicism because of their Blaschkoid hemicorporal distribution[4]. In line with this, Sanger sequencing detected the *ALPK1* hotspot mutation in spiradenomas organised linearly[2]. However, definite evidence showing the role of somatic mosaicism is lacking. To clarify the evolutionary trajectories and mutational profile of MES and ES, we analysed MES arising from localised multiple ESs by exome-, targeted- and amplicon-sequencing.

A 39-year-old man presented with an MES accompanied by localised multiple ESs and an axillary lymph node (LN) metastases donated the tissues (Fig a). An LN section was sent for FoundationOne CDx clinical sequence (Foundation Medicine). MES, adjacent ESs, and adjacent normal tissue were microdissected from formalin-fixed, paraffin-embedded tissue, followed by DNA extraction using QIAamp DNA Micro Kit (QIAGEN). Exome sequencing libraries were generated using xGen cfDNA & FFPE DNA Library Prep Kit, and xGen Exome Hyb Panel v2 (Integrated DNA Technologies) and sequenced on DNBSEQ-G400 (MGI). *ALPK1* amplicons were amplified using QuickTaq HS DyeMix (Toyobo) with the following primers: (forward: 5'-TGGGTCAGCTAGAATGAGTGT-3', reverse: 5'-ACAGGGACAACACACAAGGA-3'). Amplicon libraries were generated using xGen DNA library Prep Kit EZ UNI (Integrated DNA Technologies) and sequenced on NovaSeq 6000 (Illumina). Genomon2 pipeline was used to identify somatic mutations and cnacs software was used to infer copy number alterations as described previously[6].

Exome sequencing identified 42, 11, 17, 17, and 10 mutations/exome in one MES and four adjacent ESs, while the FoundationOne analysis of the LN estimated the mutation burden to be 3 mutations/Mb (approximately 165 mutations/exome) (Fig b). *ALPK1* p.V1092A mutation was detected in all MES and ES samples. Encouraged by these findings,

morphologically normal dermis or epidermis adjacent to the tumour were analysed by amplicon-sequencing targeting the *ALPK1* hotspot. However, no *ALPK1* hotspot mutation was detected in the surrounding normal dermis or epidermis at the variant allele frequency threshold 0.01. *TP53* p.264_265del mutation, accompanied by loss-of-heterozygosity, was exclusively detected in the primary MES sample, while the *TP53* p.Q331* mutation was identified in the LN sample. *CDKN2A/B* loss was detected in the primary MES and LN samples (Fig c). No copy number alteration was found in the ES samples. The FoundationOne analysis also revealed *PIK3R1* p.V661fs*3, *CTNNA1* p.A685fs*19, and *RAD51B* loss.

Somatic mosaicism can develop anytime after the first zygotic division, leading to various manifestations. In nevus sebaceous (NS), *HRAS*/*KRAS* mutations have been found exclusively in NS keratinocytes, not in adjacent non-lesional skin, regional fibroblasts, or peripheral blood-derived genomic DNA, confirming postzygotic mosaicism[7]. Provided that only the *ALPK1* mutation was shared across tumours and that the *ALPK1* mutation was not detected in normal dermis or epidermis, the mutation was likely acquired early in life, post-gastrulation. Moreover, our case suggested that loss of function of *TP53* and *CDKN2A/B* are the likely drivers of malignant transformation to MES, as seen in other malignancies. In summary, our data clarify that somatic mosaicism in the *ALPK1* mutation hotspot leads to the parallel evolution of localised multiple ESs.

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Conflicts of interest: none to declare.

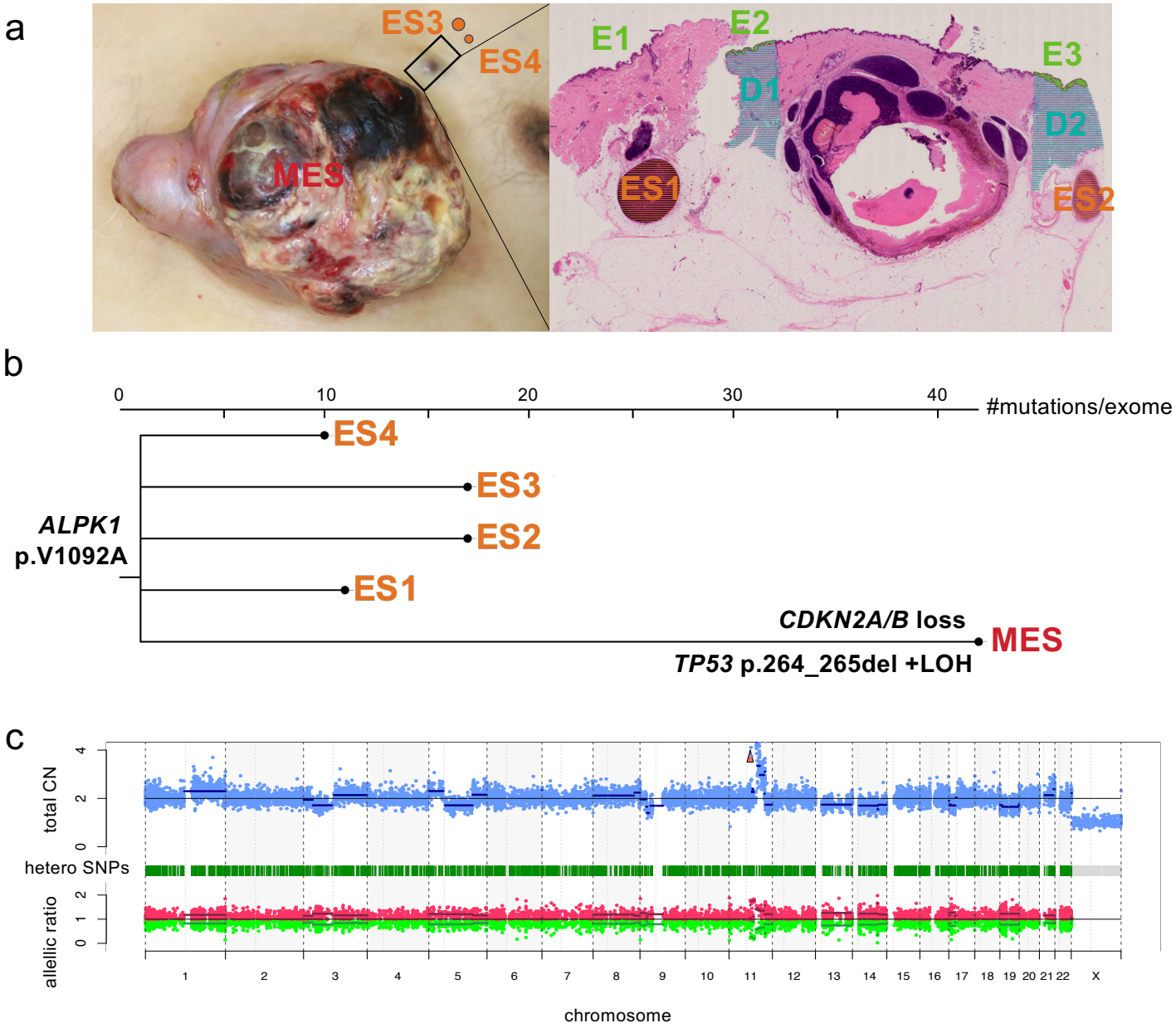
Figure legends

a) A clinical photograph of the primary lesion of malignant eccrine spiradenoma (MES) and surrounding eccrine spiradenomas (ES) on his chest (left panel). A histological overview of the surrounding ESs (ES1, 2) (right panel). The scale bar indicates 10 mm (×10). E: epidermis, D: dermis.

b) The phylogenic tree of the primary lesion of MES and surrounding ESs. LOH: loss of heterogeneity.

c) Copy number alterations of the malignant eccrine spiradenoma. *Upper panel:* total copy number; *lower panel:* B allele frequency.

Figure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ES4
ES3
ALPK1
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ES1
CDKN2A/B loss
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TP53 p.264_265del +LOH
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