

## Myeloid sarcoma arising in malignant phyllodes tumour: clonal relationships revealed by comparative genome-wide analyses

Myeloid sarcomas (MS) are rare neoplasms defined as tumour masses composed of myeloblasts with or without maturation occurring at anatomical sites other than the bone marrow. Although the breast can be involved, it is an uncommon site (Valbuena *et al.*, 2005). Myeloid sarcomas may occur independently of, prior to, or concurrently with acute myeloid leukaemia, myeloproliferative neoplasms, or myelodysplastic syndromes. Only a limited number of MS cases have been studied genetically, and a few genes, such as *FLT3* and *NPM1*, were reported to be recurrently mutated (Li *et al.*, 2015). Phyllodes tumours, rare fibroepithelial tumours of the breast, are histologically characterized by a double-layered epithelial component arranged in clefts surrounded by a stromal/mesenchymal component, and are classified as benign, borderline or malignant lesions. Recent studies reported that *MED12* mutations are very common in phyllodes tumours (Yoshida *et al.*, 2015). Here, we report the first case of a malignant phyllodes tumour (MPT) with an MS component.

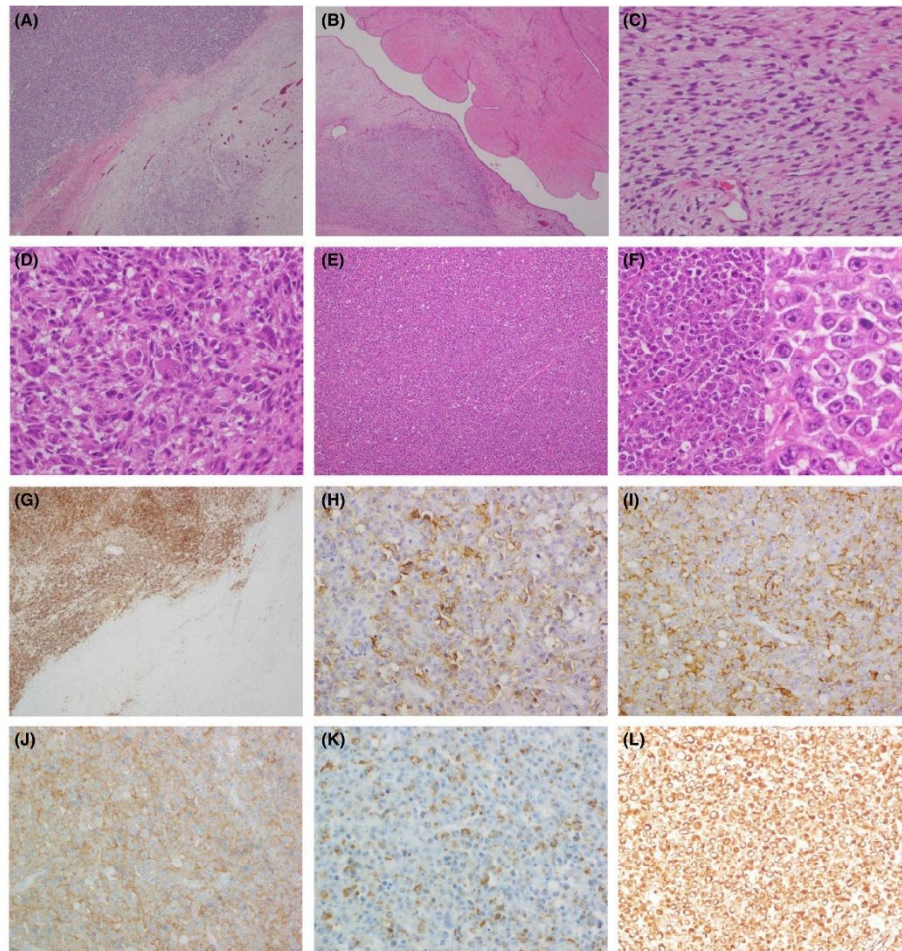
The patient was a 55-year-old woman who was admitted for a huge mass in her right breast, first noticed 4 months before admission. A contrast-enhanced computed tomography (CT) scan showed a 12 cm mass shadow with peripheral enhancement (Figure S1A) without any distant metastases. The preoperative histological diagnosis following tumour biopsy was MPT or stromal sarcoma, and total mastectomy was performed. Two months after the operation, CT scan revealed multiple lung metastases. She was treated with chemotherapy and repeated radiation; however, systemic metastases were detected in the brain, thyroid gland, liver, adrenal gland and skin. The patient died 10 months after the operation. During the course of disease, white blood cell counts, which ranged from  $2.8$  to  $8.0 \times 10^9/l$ , did not suggest leukaemia. An autopsy was not performed. This study was approved by the Gunma University Ethical Committee.

Methods are detailed in the Supplementary methods (Data S1). Grossly, a well-circumscribed grey to tan solid tumour with cystic components, clefts and haemorrhage were observed in the resected right breast (Figure S1B). Histologically, the tumour consisted of two distinctive MPT and MS components. Although the border was focally indistinct, these components were separated by narrow collagenous stroma for the most part (Fig 1A; Figure S1B and C). The MPT element had leaf-like architectures composed of stromal components partly showing variable cellularity and cellular atypia as well as benign epithelial components (Fig 1B). In areas with lower

cellularity, stromal cells were composed of monomorphic spindle cells with few mitoses. In areas with higher cellularity, stromal cells exhibited pleomorphism (Fig 1C and D) – spindle cells, multinucleated cells and polygonal cells – with a high mitotic rate and coagulative necrosis. The ductal and slit-like structures lined by benign epithelium with two cell layers were seen particularly in areas with lower cellularity. The MS component demonstrated markedly high cellularity and consisted of discohesive round cells with scant eosinophilic cytoplasm, and round to oval nuclei showing minimal variation in size and one or two distinct nucleoli (Fig 1E and F). Numerous mitoses, apoptosis, and coagulative necrosis were seen in the MS element. Immunohistochemically, the MS cells were positive for leucocyte common antigen (LCA), CD68 (KP-1 and PG-M1), CD13, CD14, CD4, and vimentin (Fig 1G–L), while they were negative for keratin, HMB-45, S-100 protein, CD1a, CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD33, CD34, CD56, CD79a, CD117, CD163, MAC387, MPO, lyzosome, and desmin. The MPT cells were negative for LCA (Fig 1G).

By array comparative genomic hybridization using DNA extracted from paraffin-embedded sections separately from MPT and MS components, five shared copy number alterations including a homozygous deletion encompassing *CDKN2A* were found in both components (Fig 2A and B; Table S1), while a gain of the entire chromosome 5 was observed only in the MS component, which was validated by fluorescence *in situ* hybridization analysis (Fig 2C). We next performed whole-exome sequencing (WES), with the mean coverage depths for the MPT and MS components being 484.7 and 503.7-fold, respectively, and found 139 shared variants in both components, ten of which are reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>) (Fig 2D; Table S2). Furthermore, two variants in *SNAP91* and *ACSM6* (previously termed *C10orf129*) were found only in the MS component (Fig 2D; Table S3); however, only the *SNAP91* mutation could be confirmed by Sanger sequencing (Fig 2E). The *SNAP91* mutation is not reported in the COSMIC database, and mutations in *SNAP91* have not been shown to be involved in tumorigenesis. In addition, Sanger sequencing revealed a *MED12* mutation (c.138\_164del27) in both components (Fig 2F), which could not be detected by WES and the filtering criteria, probably due to the relatively large size of the deletion, but was observed by manual inspection of aligned reads using Integrative Genomics

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**Fig 1.** Microscopic images. Histological features of the malignant phyllodes tumour (MPT) (A–D) and myeloid sarcoma (MS) components (A, E, F). A. The MS (top left) and MPT (bottom right) components are separated by narrow collagenous stroma. B. The MPT element has leaf-like architectures composed of stromal components partly showing variable cellularity and benign epithelial components. Stromal cells show pleomorphism: spindle cells (C), multinucleated cells and polygonal cells (D). The MS component demonstrates markedly high cellularity (E) and consists of discohesive round cells (F). Immunohistochemistry (G–L): G. The MS component (top left) is positive for LCA, and the MPT component (bottom right) is negative. The MS cells are positive for CD13 (H), CD14 (I), CD4 (J), PG-M1 (K) and vimentin (L). Original magnification:  $\times 40$  (A, B, G),  $\times 100$  (E),  $\times 400$  (C, D, F left, H–L),  $\times 1000$  (F right).

Viewer (<http://www.broadinstitute.org/igv/>). Taken together, these observations support our hypothesis that the MS component may have developed from the pre-existing MPT component. The exact mechanisms of progression are not clear, but the alterations observed only in the MS component and

or other unidentified alterations, such as mutations in non-coding regions, epigenetic modifications and translocations causing fusion genes, may be involved.

Similar phenomena of trans-differentiation have been reported in histiocytic/dendritic cell sarcomas and

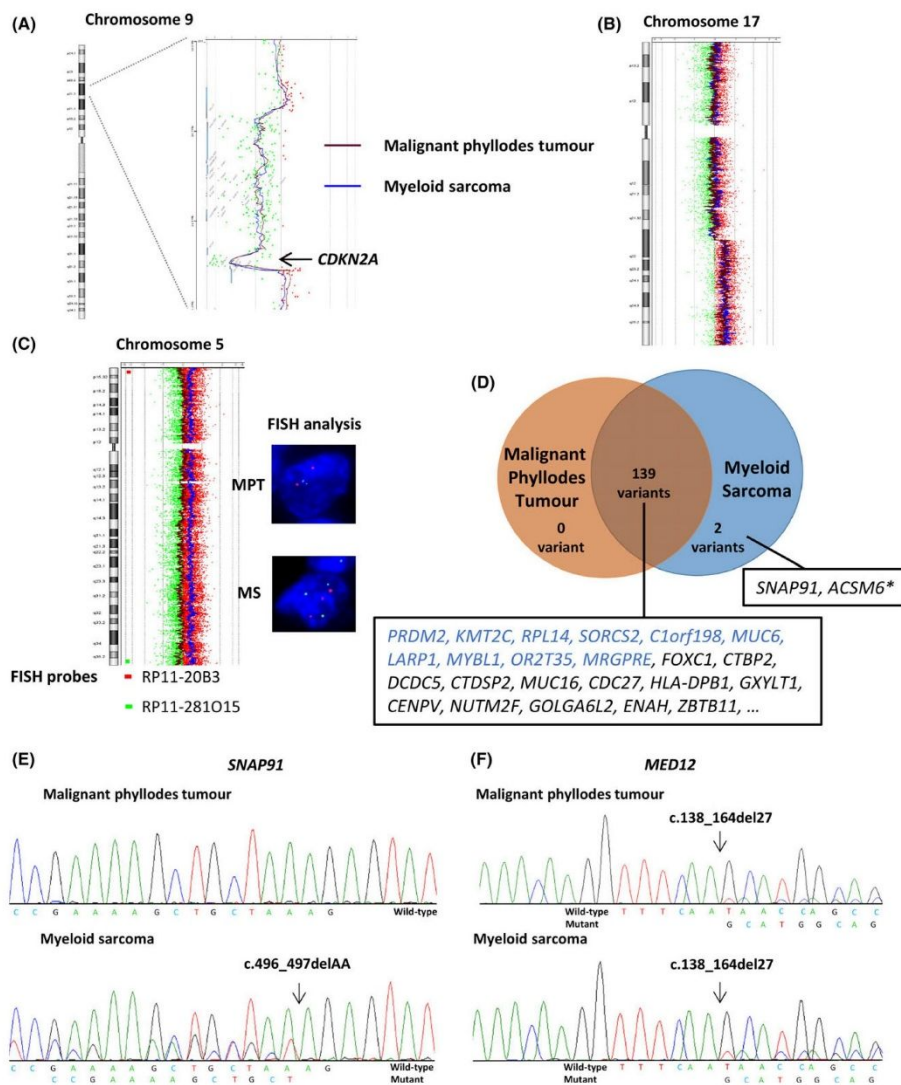


Fig 2. Molecular analyses performed separately for the malignant phyllodes tumour (MPT) and myeloid sarcoma (MS) components. Examples of copy number alterations common to both MPT and MS components analysed by array comparative genomic hybridization (A, B) and a gain of the entire chromosome 5 observed only in the MS component (C). A. Combination of heterozygous and homozygous deletions in chromosome 9p21-3, with the latter encompassing *CDKN2A*. B. A gain in chromosome 17q22-q25.3. C. A gain of the entire chromosome 5 observed only in the MS component was validated by fluorescence *in situ* hybridization analysis. D. Number of variants detected by whole-exome sequencing. Variants in genes in blue font are archived in the Catalogue of Somatic Mutations in Cancer database. \*The variant in *ACSM6* could not be confirmed by Sanger sequencing. E. A variant in *SNAP91* (c.496\_497delAA) detected only in the MS component was confirmed by Sanger sequencing. F. A *MED12* mutation (c.138\_164del27) in both components.

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Langerhans cell neoplasms secondarily developing from haematopoietic and lymphoid tumours, and these secondary histiocytic/dendritic cell tumours shared the same molecular or cytogenetic abnormality as the precursor tumours (Feldman *et al*, 2008; Kumar *et al*, 2011; Shao *et al*, 2011; Hure *et al*, 2012; West *et al*, 2013; Michonneau *et al*, 2014). Although we were unable to find any reported histiocytic/dendritic cell tumours arising from non-lymphoid/hematologic neoplasms, Rocca *et al* (2012) reported a case of MS coexistent with a colon adenocarcinoma. The authors concluded that the coexistence was probably incidental and these two different tumours arose from different mechanisms, but that molecular studies were needed to clarify the pathogenetic relationship between them.

In conclusion, our results suggest that MS can develop from other non-lymphoid/haematological tumours. More cases should be tested to prove this progression, elucidate the molecular mechanisms involved, and to explore clinicopathological and molecular differences associated with MS developing from precursor neoplasms.

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## Author contributions

HA and SN conceived and designed the experiments; HA, TS, MK and J. Hirato performed the pathological analysis; HA, SN, RK-I, SR and TY performed the laboratory research; TH and J. Horiguchi managed the patient; HA, SN and TY analysed and interpreted the data; HA, SN and TH wrote the manuscript; MN, HY, J. Hirato and TO participated in construction of the manuscript and revised it critically; and all authors accepted the final version of the manuscript.

## Conflict of interest

Masahiko Nishiyama received a research grant from Yakult Honsha Co. Ltd. The other authors declare that they have no conflict of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Supplementary methods.

**Fig S1.** Computed tomography, macroscopic, and loupe images.

**Table S1.** Abberations common to both the malignant phylloides tumour and myeloid sarcoma components detected by array comparative genomic hybridization.

**Table S2.** Variants common to both the malignant phylloides tumour and myeloid sarcoma components detected by whole-exome sequencing.

**Table S3.** Variants only in the myeloid sarcoma component detected by whole-exome sequencing.

**Table S4.** Antibodies used for immunohistochemistry and results.

**Table S5.** Primer sequences.

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