Identification of a novel *RUNX1-TACC1* fusion transcript in acute myeloid leukaemia

Transforming acidic coiled-coil 1 (TACC1) interacts with proteins involved in the centrosome, microtubule dynamics, transcription and mRNA processing and plays roles in cell division, cell proliferation, and gene regulation (Ha, Kim, & Breuer, 2013). TACC1 is also a component of aurora A and aurora B complexes, which are essential for a successful mitosis (Delaval *et al.*, 2004; Nikonova, Astsaturov, Serebriiskii, Dunbrack, & Golemis, 2013). In tumours, *TACC1* expression significantly associates with lymph node metastasis and poor prognosis (Ding *et al.*, 2013; Lv *et al.*, 2014). A *FGFR1-TACC1* fusion capable of constitutive tyrosine kinase activation was found in extraventricular neurocytoma and glioblastoma (Lasorella, Sanson, & Iavarone, 2017; Sievers *et al.*, 2018). However, the role of *TACC1* has not been described in leukaemia so far. Here, we report the first case of acute myeloid leukaemia (AML) with a novel *RUNX1-TACC1* fusion transcript and its associated histological, immunophenotypical, and genetical features.

A 50-year-old male presented with skin ecchymosis for five months, which had become more severe in the last two weeks. Complete peripheral blood cell count demonstrated pancytopenia with $2{\cdot}8 \times 10^9$/l of leukocytes (34·7% of neutrophils, 50·7% of lymphocytes and 14·6% of monocytes), $3{\cdot}4 \times 10^{12}$/l of erythrocytes, 83 g/l of haemoglobin, and 8×10^9/l of platelets. A bone marrow aspirate smear showed significantly increased cellularity with erythroid hyperplasia. Most of the increased erythrocytic series were polychromatic and orthochromatic erythroblasts. Differential cell counts showed 18·5% of blasts, 28·5% of granulocytes (G), 53% of erythrocytes (E), and a G/E = 0·54/1 (Fig 1A). Flow cytometry of bone marrow aspirate found 24% of blasts, which were positive for HLA-DR, CD13, CD34, CD38, CD117 and CD123. A 118 gene DNA NGS (next generation sequencing)-targeted panel for hematopoietic malignancies showed *SETBP1* Asp868Asn (41·7%), *SETBP1* Gly870Ser (3·8%), *U2AF1* Gln157Arg (43·8%) and *U2AF1* Ser34Phe (46·7%). Bone marrow cytogenetic analysis showed a translocation between chromosomes 8 and 21 in each of 20 metaphases as the sole change. Different from the traditional t(8;21) AML, the chromosome 8 breakpoint was located at 8p11, instead of 8q22 (the *RUNX1T1* gene locus)(Fig 1B). FISH analysis with *RUNX1-RUNX1T1* dual fusion probes did not show *RUNX1-RUNX1T1* rearrangement. However, part of the *RUNX1* signal was translocated to the derivative chromosome 8, consistent with a *RUNX1* rearrangement (Fig 1C). The interphase FISH evaluation showed 21% of nuclei with *RUNX1* rearrangement, similar to the blast cell number in this sample (18·5% by smear and 24% by flow cytometry). Because 8p11 harbours the *FGFR1* gene, an oncogene known to be involved in myeloid tumours, we performed FISH analysis with a split-apart *FGFR1* probe, which was negative for *FGFR1* rearrangement. The intact *FGFR1* signal was translocated to the derivative chromosome 21, indicating that an unknown gene centromeric to *FGFR1* on 8p was involved in our case (Fig 1D). A targeted RNA NGS panel specific for myeloid tumours, which includes *RUNX1* as one of the bait genes, showed two fusion transcripts between *RUNX1-TACC1*, containing either the first five exons or the first six exons of *RUNX1*, and the last nine exons of *TACC1* (Fig 2A). The breakpoints of the two *RUNX1-TACC1* fusion gene transcripts were shown in Figure S1. Because *TACC1* is located just centromeric to *FGFR1* at chromosome 8p11, these sequencing results are consistent with both the karyotype and FISH findings. The two *RUNX1-RUNX1T1* fusion transcripts were further confirmed by RT-PCR with primers specific to *RUNX1* and *TACC1* (Fig. 2B,C, Table SI for primer sequences). A western blot analysis of the patient's bone marrow sample with an anti-RUNX1 antibody showed two additional bands at 58 and 51 kD, in addition to the wild-type RUNX1 (Fig. 2D). These extra bands are consistent with the presence of the RUNX1 Ex5-TACC1 Ex6 and the RUNX1 Ex6-TACC1 Ex6 based on molecular weight.

The patient was diagnosed with AML, not otherwise specified, according to the 2016 WHO classification. A standard 7 + 3 regimen (daunorubicin 90 mg/m^2 at days 1–3 and cytarabine 100 mg/m^2 q12h at days 1–7) achieved minimal results, with similar blast counts, karyotype changes and mutation frequencies seen in a subsequent bone marrow aspirate. He was then treated with a HAG regimen (harringtonine 1 mg/m^2 at days 1–8, cytarabine 20 mg/m^2 q12h at days 1–8, granulocyte colony-stimulating factor (G-CSF) 300 μg/m^2 at days 1–4, and G-CSF 150 μg/m^2 at days 5–8). Again, remission was not achieved. The patient was then treated with decitabine (20 mg/m^2 at days 1–5) and, yet again, no clinical response was observed. The patient succumbed to the disease soon after.

RUNX1 is a transcription factor essential to haematopoiesis. *RUNX1* alterations, including both point mutations and intragenic rearrangement, are frequently seen in haematopoietic malignancies (Gaidzik *et al.*, 2011; Bidet *et al.*, 2016). *RUNX1* germline mutations are also associated with inherited

doi: 10.1111/bjh.16444

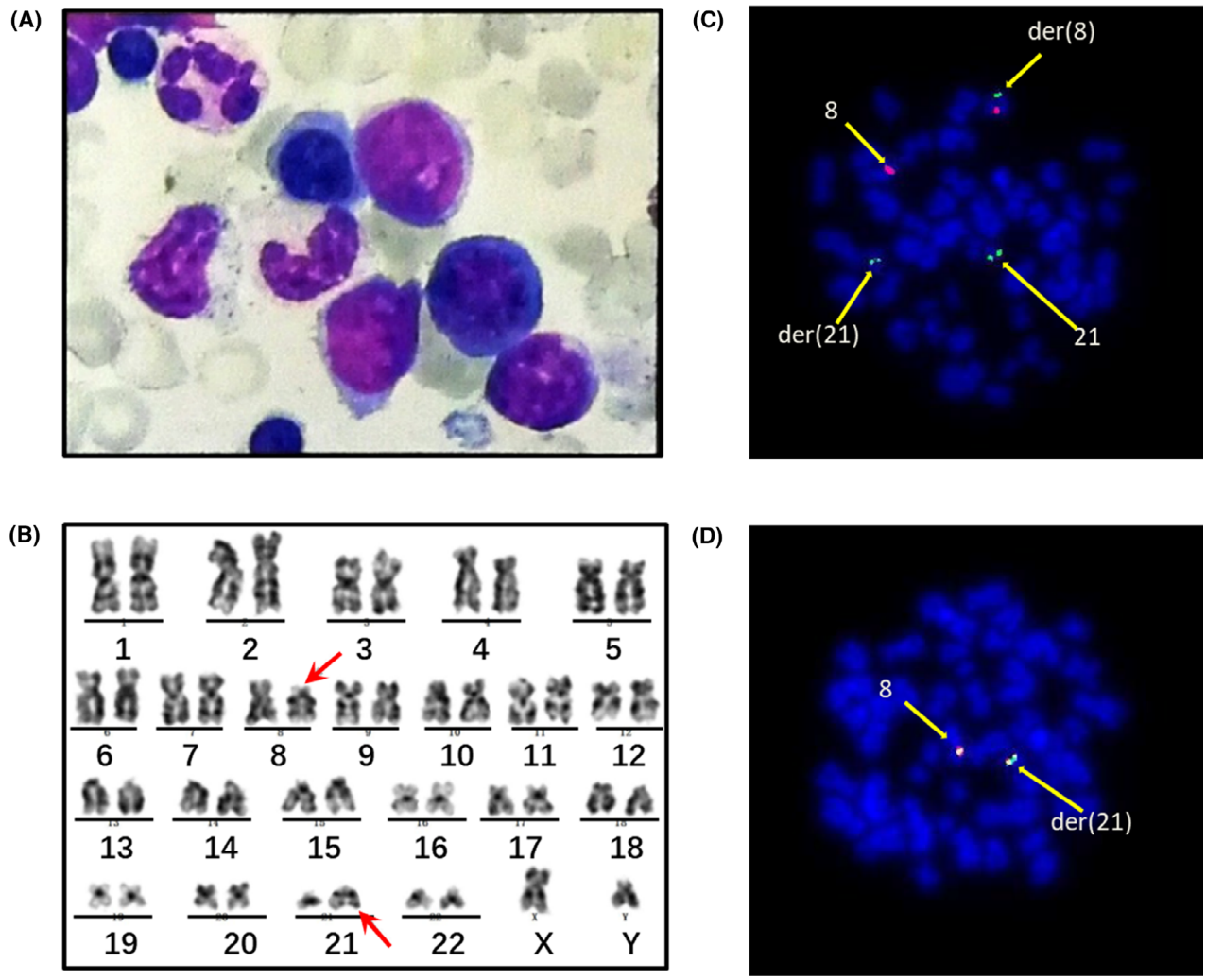
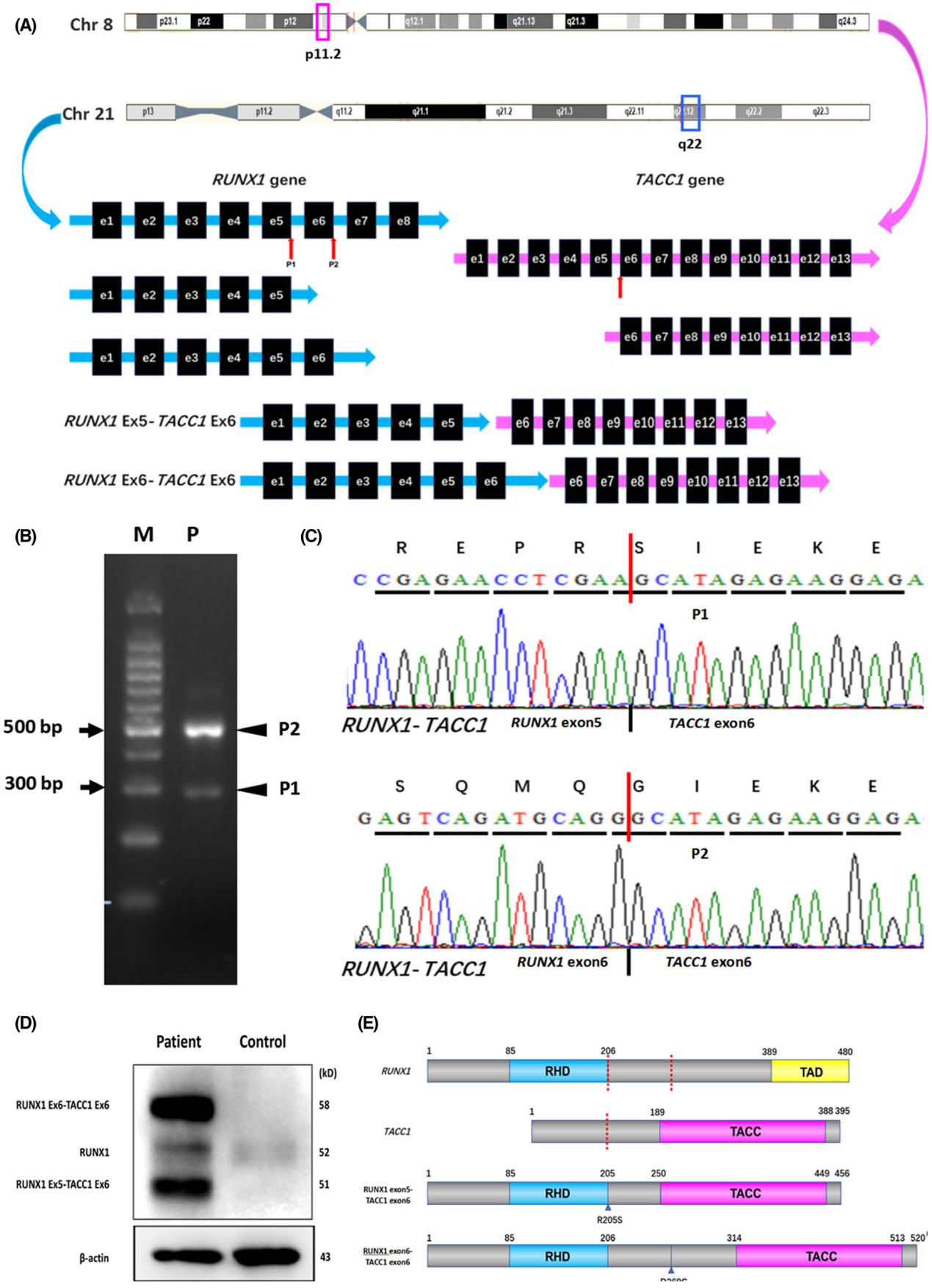


Fig 1. Morphologic features and cytogenetics results of an acute myeloid leukaemia patient with a clonal translocation t(8;21). (A) May-Grünwald-Giemsa staining of bone marrow aspirate showed medium-sized blasts with azurophilic granules of various sizes and 1-4 nucleoli. (B) Karyotypic analysis of unstimulated bone marrow specimen showed 46,XY,t(8;21)(p11;q22) as the sole change. (C) Metaphase FISH using *RUNX1* probe (green) and *RUNX1T1* probe (red) showed that one of the *RUNX1* signals was split-apart and translocated to the der(8) chromosome. (D) Metaphase FISH using 5'*FGFR1* (green) and 3'*FGFR1* probe (red) was negative for the *FGFR1* rearrangement.

leukaemia (Walker *et al.*, 2002). Two common chromosome translocations involving *RUNX1*, i.e., the t(8;21) (*RUNX1-RUNX1T1)* and the t(12;21) (*ETV6-RUNX1)*, are found in AML and ALL, respectively (Sood, Kamikubo, & Liu, 2017). RUNX1 has two defined functional domains, the runt-homology domain (RHD) responsible for DNA-binding and CBFβ interaction, and the transactivation domain (TAD) (Fig. 2E). Various RUNX1 fusion proteins retain RHD only or both RHD and TAD. In the classic *RUNX1-RUNX1T1*, the *RUNX1T1*-encoded protein CBFA2T1 contributes an oligomerisation domain, which is critical for leukaemogenesis (Kwok, Zeisig, Qiu, Dong, & So, 2009). In *RUNX1-TACC1*, *TACC1* contributes a carboxy-terminal transforming acidic coiled coil (TACC) domain. Coiled coil domains function as oligomerisation domains for a wide variety of proteins and are capable of both homo-oligomerisation and hetero-

Fig 2. Molecular characterisations of the *RUNX1-TACC1* fusion. (A) Schematic illustration shows the breakpoints of the *RUNX1-TACC1* fusions and their chromosome localisation. (B and C) RT-PCR assays and Sanger sequencing of the patinet's bone marrow confirmed two types of *RUNX1-TACC1* fusion transcripts, with one containing the first five exons of *RUNX1* and the last nine exons of *TACC1,* and the other containing the first six exons of *RUNX1* and the last nine exons of *TACC1,* likely due to alternative splicing. (D) Western blotting assays with an anti-RUNX1 antibody showed two additional bands at 58 and 51 kD, in addition to the wild-type RUNX1 (D). These extra bands are consistent with the presence of the *RUNX1* Ex5-*TACC1* Ex6 and the *RUNX1* Ex6-*TACC1* Ex6 based on molecular weight. The control was from a non-neoplastic bone marrow specimen. (E) Schematic illustration of RUNX1-TACC1 fusion. RHD, runt-homology domain, TAD, transactivation domain, TACC, transforming acidic coiled-coil.



oligomerisation (Lupas, 1996). Three human TACC proteins, including TACC1, TACC2 and TACC3, are all capable of homo-oligomerisation (Gergely *et al.*, 2000). We propose an oncogenic model, that RUNX1-TACC1 homo-oligomerisation, driven by the TACC domain, leads to leukaemogenesis.

In conclusion, we described a novel *RUNX1-TACC1* fusion in AML. This is the first report of *TACC1* rearrangement in leukaemia, which may provide a unique opportunity to study the functions of these very interesting proteins.

Acknowledgements

This study was supported by Xinjiang Joint Fund of National Natural Science Foundation of China (U1903117), the National Natural Science Foundation of China (81500118), the China Postdoctoral Science Foundation funded project (M600443), Jiangsu Society and Science Development Program (BE2016678).

Disclosure of financial interests

There are no relevant conflicts of interest to disclose.

Authors' contributions

C.X.Y., X.P.L., and W. Z. performed experiments; R.Y.Y., L.J.D., and R.J.W. provided AML patient samples and clinical data; T.T.Q., L.F., and S.X. analysed the data; S.X., G.S.W., and Y.H.L. commented on the paper; T.T.Q. wrote the paper; R.Y.Y., T.T.Q., S.X. and L.Y. designed the research. All authors contributed to writing the paper by providing guidance and comments on its content.

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Keywords: *RUNX1-TACC1*, *RUNX1*, *TACC1*, *RUNX1-TACC1* fusion, acute myeloid leukemia

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Targeted RNA NGS showed two *RUNX1-TACC1* fusion transcripts. (A) *RUNX1 Ex5 -TACC1 Ex6*. (B) *RUNX1 Ex6 -TACC1 Ex6*.

Table SI. Primer sequences used for *RUNX1-TACC1* fusions.

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