CSNK1A1 mutations and gene expression analysis in myelodysplastic syndromes with del(5q)

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The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal haematopoietic stem cell (HSC) malignancies characterized by ineffective haematopoiesis and peripheral blood cytopenias (Heaney & Golde, 1999). MDS patients typically have a hypercellular bone marrow. Approximately 40% of MDS cases progress to acute myeloid leukaemia (Heaney & Golde, 1999).

Deletion of the long arm of chromosome 5 [del(5q)] occurs in approximately 10–20% of patients with de novo MDS (Giagounidis et al, 2004; Boultwood et al, 2010) and is the sole karyotypic abnormality in patients with the 5q- syndrome, the most distinctive of the MDS (Giagounidis et al, 2004; Boultwood et al, 2010). The commonly deleted region (CDR) of the 5q- syndrome was identified and narrowed to a 1.5 Mb interval at 5q32 (Boultwood et al, 2002). Several candidate genes map to the CDR, including CSNK1A1, which was found to be haploinsufficient (i.e. down-regulated by approximately 50%) in the CD34+ cells of 5q- syndrome.

Summary

Mutations of CSNK1A1, a gene mapping to the commonly deleted region of the 5q- syndrome, have been recently described in patients with del(5q) myelodysplastic syndromes (MDS). Haploinsufficiency of Cska1 in mice has been shown to result in β-catenin activation and expansion of haematopoietic stem cells (HSC). We have screened a large cohort of 104 del(5q) MDS patients and have identified mutations of CSNK1A1 in five cases (approximately 5%). We have shown up-regulation of β-catenin target genes in the HSC of patients with del(5q) MDS. Our data further support a central role of CSNK1A1 in the pathogenesis of MDS with del(5q).

Keywords: CSNK1A1, mutation, haploinsufficiency, 5q- syndrome, del(5q).
patients in a gene expression profiling (GEP) study (Boul-wood et al, 2007). In this setting the remaining copy of the gene does not compensate the loss of the other allele. Sanger sequencing-based screening of all 40 genes within the CDR did not identify any mutations in a cohort of ten 5q- syndrome patients. However, in a recent study (Schneider et al, 2014), mutations of CSNK1A1 were identified using whole-exome sequencing in 2 of 19 del(5q) cases and a further CSNK1A1 mutation was found in an additional cohort of 22 MDS cases with isolated del(5q) using next-generation targeted sequencing, giving an overall frequency of approximately 7% in the MDS del(5q) cases analysed. This is the first report of mutations in a gene mapping to the CDR of the 5q- syndrome, although these mutations are found in a small subset of del(5q) MDS patients (Schneider et al, 2014). CSNK1A1 encodes a serine/threonine kinase (CK1α), which has a regulatory role in the Wnt/β-catenin and p53 signalling pathways (Elyada et al, 2011). Schneider et al (2014) showed that expression of mutant CSNK1A1 resulted in β-catenin activation and HSC cell cycle progression.

Heterozygous inactivation of Csnk1a1 in mice also resulted in β-catenin activation and expansion of HSCs, suggesting that CSNK1A1 haploinsufficiency may be the mechanism underlying the initial clonal expansion in patients with the 5q- syndrome (Schneider et al, 2014).

In this study, firstly we have screened a large cohort of MDS cases with del(5q) for mutations in CSNK1A1. Secondly, we have investigated the impact of CSNK1A1 haploinsufficiency and mutation on the expression of β-catenin-related genes in the CD34+ cells from MDS patients with del(5q) using GEP.

Materials and methods

Patient samples

A total of 104 MDS cases with del(5q) were included in this study (Table SI). Genomic DNA was isolated using phenol-chloroform extraction from bone marrow samples or from peripheral blood neutrophils isolated using Histopaque (Sigma-Aldrich, Gillingham, UK) and pelleted after hypotonic lysis of erythrocytes.

Sanger sequencing

Sanger sequencing was performed following polymerase chain reaction (PCR) amplification using the following primers: exon 3 of CSNK1A1 forward primer 5'-TCCTTTTGTTCGTTAGGTGTT-3' and reverse primer 5'-AAGGTAAAATAGTGATGCACAGGA-3', exon 4 forward primer 5'-GCCAACACAGCAGGTA-3' and reverse primer 5'-CAGCACAATCTACTATG-3'.

GEP and data analysis

Gene expression profiling data on CD34+ cells from a group of MDS patients with del(5q) and healthy controls were obtained from a dataset previously published by our group (Pellagatti et al, 2010). The microarray platform used was the Affymetrix GeneChip Human Genome U133 Plus 2.0 (47 000 transcripts) (Affymetrix, Santa Clara, CA, USA). Analysis of gene set up- or down-regulation was performed using Gene Set Enrichment Analysis (GSEA) as previously described (Papaemmanuil et al, 2011).

Real-time quantitative PCR

Real-time quantitative PCR reactions were run on a LightCycler 96 Real-Time PCR System (Roche Diagnostics, Lewes, UK). Pre-developed TaqMan Assays were used (Assays-on-Demand, Applied Biosystems, Foster City, CA, USA) and the expression level of the beta-2-microglobulin gene (B2M) was used to normalize for differences in input cDNA. Each sample was performed in triplicate and the expression ratios were calculated using the ΔΔCt method.

Results and discussion

We have determined the frequency of CSNK1A1 mutations in a large cohort of 104 cases of MDS with del(5q) using

| Patient ID | Diagnosis | Karyotype | CSNK1A1 mutation | PolyPhen-2 prediction/score | SIFT prediction/score |
|------------|------------|-----------|------------------|----------------------------|----------------------|
| MDS05      | RA         | 46,XX,T(1;3)(p33;p14),del(5)(q14q34)[21]/46,XX[4]| c.401A>G, p.H134L | Probably damaging/1 | Damaging/0 |
| MDS07      | RA         | 46,XX,del(5)(q14q34),inv(9)(p11q13)c[30] | c.293A>G, p.E98G | Probably damaging/0.999 | Damaging/0 |
| MDS14      | (5q- syndrome) | 46,XX,del(5)(q13q33)[26]/46,XX[4] | c.419A>G, p.D140A | Possibly damaging/0.877 | Damaging/0 |
| MDS36      | (5q- syndrome) | 46,XX,del(5)(q2)[30] | c.292G>A, p.E98K | Probably damaging/0.999 | Damaging/0 |
| MDS72      | RA         | 46,XX,del(5)(q2),del(7)(q2)[30] | c.292G>A, p.E98K | Probably damaging/0.999 | Damaging/0 |

MDS, myelodysplastic syndrome; RA, refractory anaemia; SIFT, Sorting Intolerant from Tolerant.

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Sanger sequencing. Schneider et al. (2014) identified CSNK1A1 mutations in del(5q) MDS in exon 3, and two previous studies (Graubert et al., 2012; Woll et al., 2014) reported CSNK1A1 mutations in exon 4 of the gene. We therefore focused our investigation on the analysis of the sequences of exon 3 and 4 of CSNK1A1.

We identified missense mutations of CSNK1A1 in five del(5q) MDS cases in our cohort (Table I, Fig 1A). All five patients harbouring CSNK1A1 mutations had refractory anaemia (two of which had the 5q- syndrome). Two of the CSNK1A1 mutations identified caused a previously reported amino acid change, E98K (Schneider et al., 2014). An additional case harboured a different CSNK1A1 mutation affecting amino acid 98 (E98G), previously described in other malignancies (Dulak et al., 2013). Moreover, a previously reported CSNK1A1 mutation at amino acid 140 (D140A) (Graubert et al., 2012) was found in one case of MDS with isolated del(5q) in our study. We identified a novel CSNK1A1 mutation at codon 134 (H134L) that has not been previously reported. The CSNK1A1 mutations identified were analysed using the PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) online tools, in order to predict the effect of the mutations on protein function. All CSNK1A1 mutations, including the newly identified H134L mutation, were reported as damaging by PolyPhen-2 and SIFT analysis (Table I).

The overall frequency of CSNK1A1 mutations in our cohort was approximately 5% (5/104 of cases), which is consistent with the previous report suggesting that CSNK1A1 mutations are rare events in del(5q) MDS (Schneider et al., 2014). Patients with del(5q) show haploinsufficiency of CSNK1A1 (because it maps to the CDR) and a small proportion of these patients also harbour mutation of the remaining allele. Next-generation-based targeted re-sequencing data, using a panel targeting 25 genes mutated in various myeloid malignancies (Fernandez-Mercado et al., 2013), were available for four patients with and 37 patients without CSNK1A1 mutations. The additional mutations found in the cases with CSNK1A1 mutations were a RUNX1 mutation in one patient and a U2AF1 mutation in another patient and we did not therefore observe specific association of CSNK1A1 mutations with other myeloid gene mutations. However, the number of cases analysed is clearly small, and the study of larger cohorts of del(5q) MDS cases with CSNK1A1 mutations is required to determine whether robust associations with other gene mutations exist.

It has been recently reported that lenalidomide, a drug widely used to treat del(5q) MDS (List et al., 2006), induces the ubiquitination and consequent degradation of CSNK1A1 by the CRBN-CRL4 E3 ubiquitin ligase, and that haploinsufficiency of CSNK1A1 might increase lenalidomide sensitivity in del(5q) haematopoietic cells (Fink et al., 2014). Knockdown of CSNK1A1 sensitized primary CD34+ cells to lenalidomide (Fink et al., 2014). Moreover, a previously reported H134L mutation at codon 134 (H134L) that has not been previously reported. The CSNK1A1 mutations identified were analysed using the PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) online tools, in order to predict the effect of the mutations on protein function. All CSNK1A1 mutations, including the newly identified H134L mutation, were reported as damaging by PolyPhen-2 and SIFT analysis (Table I).

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nulling pathway. The average expression fold change (patients versus median of 17 healthy controls) for CSNK1A1 was 0.56 in 16 patients with 5q- syndrome and 0.58 in 30 patients with del(5q) (Fig S1), confirming that patients with 5q- show haploinsufficient levels of this gene. The average expression fold change for CCND1 (encoding cyclin D1), a major downstream effector of Wnt/β-catenin and regulator of cell cycle progression, was 1.42 in patients with 5q- syndrome and 1.58 in patients with del(5q) (Fig S1), showing that expression levels of this gene are increased by approximately 50% in patients with 5q-. These data show that haploinsufficiency of CSNK1A1 is associated with increased expression of Wnt/β-catenin downstream effector genes in the HSC of MDS patients with del(5q) and are consistent with the previous demonstration that Csnk1a1+/− haematopoietic cells transplanted into wild-type mice showed increased expression of cyclin D1 (accompanied by β-catenin nuclear accumulation) (Schneider et al, 2014).

Gene expression profiling data were available for four patients with del(5q) for which we were able to determine the mutation status of CSNK1A1: two patients were mutated and two patients were wild-type for CSNK1A1. We performed GSEA to compare the gene expression profiles of the two patients with CSNK1A1 mutation with those of the two patients without mutations of this gene, in order to determine whether coordinated up-regulation of pathways/processes associated with Wnt/β-catenin function could be observed. The ‘REACTOME_SIGNALING_BY_WNT’ gene set was found to be significantly up-regulated (q < 0.001) in the patients with CSNK1A1 mutation compared with the patients without CSNK1A1 mutations (Fig 1B, C). These data suggest that CSNK1A1 mutations in del(5q) MDS lead to an increase in the expression of genes involved in Wnt signalling.

In summary, we have confirmed the presence of CSNK1A1 mutations in a small proportion of patients with del(5q) MDS and shown up-regulation of β-catenin target genes in the HSC of patients with del(5q) MDS. Our data support a central role for CSNK1A1 in the pathogenesis of MDS with del(5q).

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Author contributions
AP and JB designed the research study; EB, AP and JS performed the research; CM, RK, SK, AG, SR, MJ, PF and JB contributed patient samples and helped with the analysis of the data; EB, AP, JS and JB analysed the data and wrote the paper.

Conflicts of interest
The authors have no competing interests.

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

Table SI. Patient details.

Fig S1. Expression ratios for CSNK1A1 [n = 3 cases with del(5q)] and CCND1 [n = 3 cases with del(5q)] obtained from real-time quantitative PCR (blue bars) and Affymetrix experiments (red bars).

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