LETTER TO THE EDITOR

MMSET is the key molecular target in t(4;14) myeloma

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The t(4;14)(p16.3;q32.3) is found in 15% of presenting multiple myeloma (MM) cases and is associated with a significantly worse prognosis than other biological subgroups. As a consequence of the translocation, two genes are aberrantly expressed, the fibroblast growth factor receptor 3 (FGFR3) and a multiple myeloma SET domain containing protein, MMSET (WHSC1/NSD2), both of which have potential oncogenic activity. Importantly, FGFR3 shows only weak transforming activity and is eventually lost in 30% of patients, suggesting that it is not the main oncogenic factor. In contrast, MMSET gene overexpression is universal, and when it is knocked down experimentally, there is inhibition of proliferation, induction of apoptosis and alteration of cell adhesion, suggesting it is central to the pathogenesis of this subtype of MM. MMSET is known to have histone methyl transferase activity and is deregulated early on in the genesis of developing myeloma, and could therefore constitute a good therapeutic target. The MMSET locus in t(4;14) myeloma patients has a complicated genomic structure and after translocation events and RNA splicing, a number of different transcripts are generated (Figure 1). This genetic complexity of MMSET has been added to recently by the discovery of the H/ACA box RNA ACA11 (SCARN22), that has been found within intron 20 of MMSET and is also overexpressed in the t(4;14) subgroup. Small RNA has been suggested to be key to the pathogenesis of t(4;14) MM, raising the question that it may constitute the main therapeutic target.

The box H/ACA RNAs are a group of small nucleolar RNA (snoRNA) conserved from Archea to mammals. These RNAs are generally associated with a multi-protein complex, and usually function as a guide to the site-specific pseudouridylation of rRNA and spliceosomal small nuclear RNAs. However, they are also involved in other regulatory complexes, such as telomerase, but their full biological roles have not been completely elucidated. More than 90% of human snoRNAs are encoded within spliced introns, and their expression is closely linked to the splicing of the host gene. After host gene transcription, the intronic snoRNAs are trimmed to a mature form by exonucleolytic activities. The mRNA splicing machinery may chaperone snoRNA post-transcriptional maturation steps, but in some cases, these steps are splicing-independent. However, there are a few cases in mammals where snoRNAs are independently transcribed, for instance, the gene for the telomerase RNA component (terC) or the RNAs involved in the pre-rRNA endonucleolytic processing, but this is a rare event.

In a series of experiments it has been shown that ACA11 knockdown impairs cell proliferation and deregulates the oxidative stress response, and its overexpression downregulates the transcription of ribosomal protein genes. The same group, on the basis of experiments showing that t(4;14) cell lines knocked out for MMSET, either on the translocated allele (TKO) or on the non-translocated allele (NTKO), have lower ACA11 levels compared with their parental cell line (KMS11) with a normal overexpressed MMSET, suggested that ACA11, rather than MMSET, is the key pathogenic gene in t(4;14) MM. In previous studies, the same TKO model system had been used to prove the oncogenic effect of MMSET in myeloma; however, in these more recent experiments where ACA11 was knocked down, the key implication of the work seemed to shift the pathogenic importance from MMSET to ACA11.

However, when we examined these results in detail, some of the results do not seem to accurately reflect what is understood about the TKO cell line system biology. In particular, the MMSET gene was erroneously reported to be deleted, whereas, in fact, the TKO and NTKO cell lines were generated from the parental KMS11 t(4;14) line, by deleting MMSET exon 7 only (Figure 1). This deletion creates a frameshift that introduces an earlier STOP codon, leaving the level of MMSET mRNA unaltered, and in theory, ACA11 untouched. Moreover, both TKO and NTKO cell lines express the MMSET isoform REIIBP. REIIBP mRNA is transcribed from an intronic promoter downstream of both the TKO and NTKO knockout mutation (Figure 1), and harbors intron 20, the location of the ACA11 gene. In order to reconcile the observations reported in the paper with what we understand about the biology of MMSET and other human intronic snoRNAs, we would have to hypothesize that ACA11 biogenesis in myeloma is different. It could be possible that ACA11, even though it is localized within an MMSET intron, could have an independent transcription start site distinct from the MMSET/REIIBP gene, or alternatively it may be regulated at the post-transcriptional level. However, as TKO, NTKO and KMS11 are con-isogenic lines, differing only for the level of functional MMSET, differential ACA11 regulation could only be due to a direct or indirect role of MMSET.

In order to resolve these discrepancies, we investigated MMSET and ACA11 expression in 153 myeloma patients as well as in the same set of con-isogenic cell lines. Statistical analysis showed that there is a good correlation between the expression of both genes (Figure 2a), suggesting that their regulation is interdependent. A similar result was found by an independent study recently. This observation was also corroborated by ENCODE RNA sequencing (RNA-seq) data, in which MMSET and ACA11 RNAs expression in non-myeloma lines were also frequently co-expressed. The results of this work are consistent with ACA11 biogenesis being via a classical intronic mechanism. Further, we went on to show that ACA11 expression was indeed lower in TKO cells, using RNA-seq on TKO and its parental KMS11 cell lines. We found that both lines had equal levels of signal coming from MMSET exons, (Figure 2b). As an internal control, we were able to demonstrate that exon 7 signal levels were reduced in TKO. We describe an intronic signal peak present in both cell lines, derived from intron 20 (Figure 2b), which is of comparable intensity and perfectly overlapping with the position of the ACA11 gene. This result demonstrates that in both lines the snoRNA is intact and is present in equal amount.

To confirm and better characterize this result, we designed and used a qRT-PCR test and applied it to total RNA from KMS11, TKO and NTKO looking at the expression of ACA11 and MMSET. In this experiment, we compared these values to those from HeLa, RPMI-8226 (t(4;14) negative MM line) and TKO::MMSET (TKO line virally transduced with the intronless cDNA for MMSET, and hence not carrying ACA11), (Figure 2c). We demonstrated a marked difference in MMSET and ACA11 expression between t(4;14) positive and negative lines. However, in contrast, we could not demonstrate a difference in MMSET mRNA in TKO, KMS11 and NTKO lines, even if these lines have different level of MMSET protein.
Taking the RNA-seq and qRT-PCR data as whole, they show that ACA11 expression directly mirrors MMSET expression level, highlighting the correlation demonstrated in primary patient material (Figure 2a). We also show that ACA11 expression is neither deregulated in the TKO nor in the NTKO cell line compared with parental KMS11. This result is compatible with the ACA11 snoRNA being generated by excision from the MMSET mRNA. Alternatively, if ACA11 had an independent promoter from MMSET isoforms, it must be under the control of the same enhancer that regulates MMSET and REIIBP expression (the IgH enhancer or the MMSET enhancer in non-t(4;14) samples). Interestingly, we saw that in TKO:MMSET, the ACA11 level was very close to those of TKO and KMS11, even if MMSET levels were virally transduced to be four-times higher. This observation shows that MMSET has no role in directly regulating ACA11 gene expression or in regulating its post-transcriptional maturation steps.

Overall, the data presented here are consistent with MMSET being the key pathologic mediator in t(4;14) myeloma. We do, however, think that ACA11 has an important role in t(4;14) pathogenesis, because evolutionary pressure seems to have kept ACA11 inside the MMSET locus, and often, intronic RNA are found to be involved in the same biochemical pathway as their host gene.

CONFLICT OF INTEREST
The authors declare no conflict of interest.
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F Mirabella, P Wu, CP Wardell, MF Kaiser, BA Walker, DC Johnson and GJ Morgan
Haemato-Oncology Research Unit, Division of Molecular Pathology, The Institute of Cancer Research, London, UK
E-mails: gareth.morgan@icr.ac.uk or fabio.mirabella@icr.ac.uk

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