Genetic and Epigenetic Analyses Reveal Transcriptional Silencing of SOX7 due to Deletion in a Multiple Myeloma Case with Double Relapse

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To the Editor,

Multiple myeloma is a plasma cell disorder accounting for 10% of all hematological malignancies.[1] Relapse is a frequent observation associated with poor prognosis in multiple myeloma.[2] The genetic and epigenetic aberrancies of cancer-related genes associated with multiple myeloma (MM) relapse are largely unknown. Homozygous deletions of 8p23.1 genomic locus, which includes many genes including *SOX7*, was reported in diagnostic MM tumor samples using Affymetrix SNP6.0 arrays.[3] Importantly, *SOX7* tumor suppressor gene was reported to be silenced through promoter hypermethylation in different cancer types.[4,5] No study has been performed to date investigating genetic or epigenetic aberrancies of *SOX7* in relapsed MM.

In this study, we evaluated *SOX7* copy number, transcript expression, and promoter methylation in a MM case with two consequential relapses. Our patient was diagnosed with IgG-kappa MM, and staged III based on ISS. He underwent four cycles of adriamycin-dexamethasone and five cycles of bortezomib-dexamethasone as the induction chemotherapy. Having achieved partial response, we collected patient's hematopoietic stem cells (6.07×10^6 $CD34^+$ cells/kg) with cyclophosphamide and granulocyte colony-stimulating factor. High-dose melphalan (200 mg/m^2) conditioning regime was applied in two separate doses before autologous stem cell transplantation. When patient relapsed at 30 months post-diagnosis, lenalidomide-dexamethasone treatment was started. The 2nd relapse was observed 42 months after 1st relapse, and patient died 2 months later due to pneumonia.

$CD138^+$ $CD38^+$ $CD19^-$ tumor cells were sorted with BD FACSAria III from bone marrow (BM) aspirates at both 1st and 2nd relapse of the MM patient. Genomic DNA and total RNA were simultaneously isolated from FACS-sorted bone marrow tumor cells using AllPrep DNA/RNA Mini Kit (Qiagen, Germany) for *SOX7* deletion, promoter methylation, and transcript expression analyses. CD38-bright plasma cells[6] from reactive tonsils (RT-PC), and $IgD^-/CD38^+$ plasma cells from tonsils of obstructive sleeping apnea patients (OSA-PC), which are expected to have no copy number abnormality, were used as normal controls for *SOX7* deletion and transcript expression analysis.

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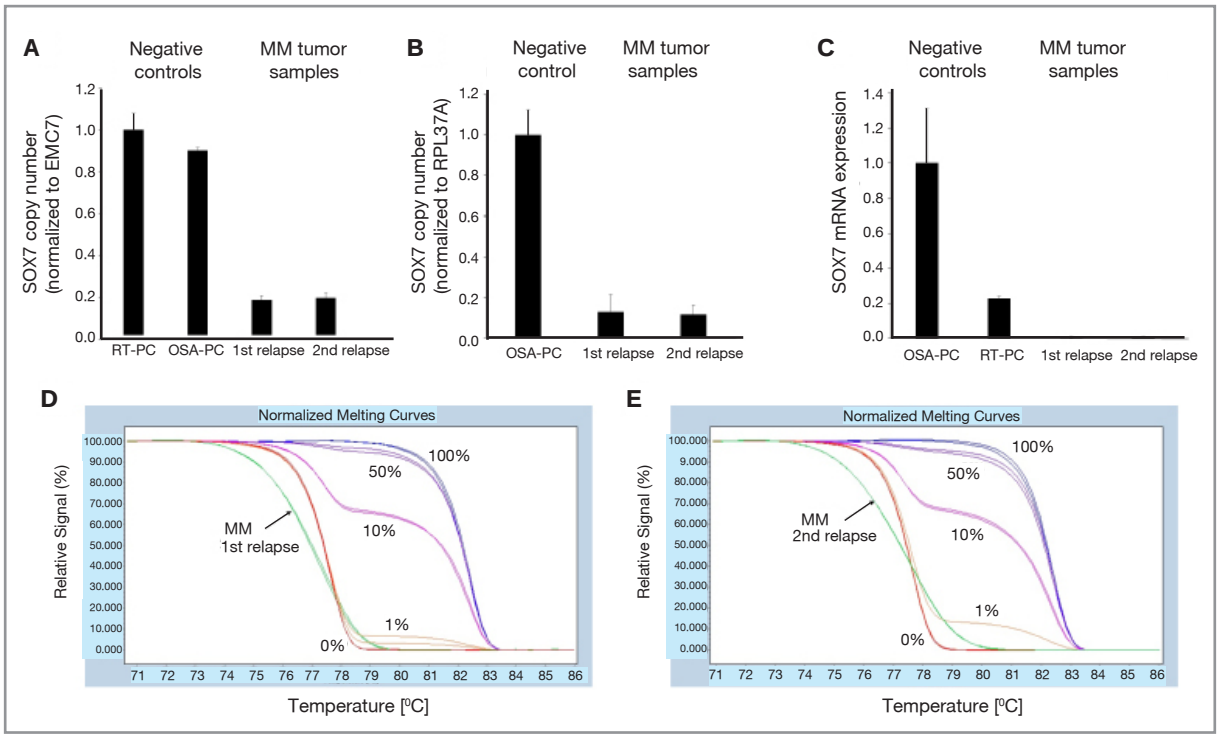

Figure 1. *SOX7 deletion is associated with transcriptional silencing in relapsed multiple myeloma tumor samples*

SOX7 copy number was evaluated with qPCR by normalization of the gene copy number either to that of *EMC7* or *RPL37A* as the reference gene as described before.[7]

After normalization with the *EMC7* reference gene, which codes for a component of the endoplasmic reticulum membrane protein complex involved in insertion of newly synthesized membrane proteins into membranes of endoplasmic reticulum, *SOX7* copy number in 1st and 2nd relapse samples were 18% and 19%, respectively, compared to RT-PC sample (Figure 1A). Similarly, after normalization with RPL37A, *SOX7* copy number in 1st and 2nd relapse samples were 13% and 11%, respectively, compared to OSA-PC sample (Figure 1B). Observed copy number values suggested a clonal heterogeneity of FACS-sorted tumor cells with biallelic *SOX7* deletions in most tumor cells. We measured *SOX7* mRNA levels with qRT-PCR as described previously[8], and observed transcriptional silencing of *SOX7* mRNA expression in both 1st and 2nd relapse tumor samples (Figure 1C). As the *SOX7* copy number analyses suggested lack of deletion in minor subclone(s) of tumor cells, we also evaluated the possibility of epigenetic silencing of *SOX7* through CpG island hypermethylation. Bisulfite converted DNA samples were analyzed with methylation-sensitive high-resolution melting curves.[9] The samples representing different levels of methylations were prepared by mixing fully methylated and unmethylated, bisulfite converted gDNA samples at predefined ratios and used as controls. We did not observe hypermethylation of *SOX7* promoter (chr8:10730260-10730424, hg38) in tumor samples during 1st (Figure 1D) and 2nd (Figure 1E) relapse. However, normalized melting curves of the MM tumor samples were different than controls, suggesting the possibility of low levels of heterogeneous methylation among tumor cells.[10] To sum up, these observations suggest that *SOX7* may have a tumor suppressive role associated with MM relapse, and can potentially be used as a biomarker for predicting relapse. However, further studies need to be performed to evaluate the presence of genetic and epigenetic aberrancies of *SOX7* using several diagnostic and relapsed MM cases to address these possibilities.

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