A novel E-cadherin/SOX9 axis regulates cancer stem cells in multiple myeloma by activating Akt and MAPK pathways

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Abstract
Cancer stem cells (CSCs) have been identified in multiple myeloma (MM) and are widely regarded as a key driver of MM initiation and progression. E-cadherin, in addition to its established role as a marker for epithelial-mesenchymal transition, also plays critical roles in controlling the aggressive behaviors of various tumor cells. Here, we show that depletion of E-cadherin in MM cells remarkably inhibited cell proliferation and cell cycle progression, in part through the decreased prosurvival CD138 and Bcl-2 and the inactivated Akt and MAPK pathways. CSC features, including the ability of the cells to form clonogenic colonies indicative of self-renewal and side population, were greatly suppressed upon the depletion of E-cadherin and subsequent loss of SOX9 stem-cell factor. We further provide evidence that SOX9 is a downstream target of E-cadherin-mediated CSC growth and self-renewal—ectopic re-expression of SOX9 in E-cadherin-depleted cells rescued its inhibitory effects on CSC-like properties and survival signaling. Collectively, our findings unveil a novel regulatory mechanism of MM CSCs via the E-cadherin/SOX9 axis, which could be important in understanding the long-term cell survival and outgrowth that leads to relapsed/refractory MM.

Keywords: Multiple myeloma, E-cadherin, SOX9, Cancer stem cells, Self-renewal

To the editor,
Novel therapies for multiple myeloma (MM), such as proteasomal inhibitors, immunomodulatory drugs, and CAR-T cell therapy, have improved palliation and response rates, providing a longer disease-free period; however, MM inevitably progresses in the vast majority of patients [1]. Cancer stem cells (CSCs), also known as tumor initiating cells, are believed to be the root cause of tumor recurrence for most if not all malignancies, including MM [2]. Identification of molecular pathways that contribute to CSCs is essential to understanding how MM progression is regulated. E-cadherin (encoded by CDH1) is known to have a pivotal role in the regulation of embryonic and normal adult stem cell survival and self-renewal [3, 4]. In solid tumors, loss of E-cadherin has traditionally been viewed as a hallmark of the occurrence of epithelial-to-mesenchymal transition, linking to metastasis. The role of E-cadherin in solid tumor growth, however, remains controversial and appears to be cell type- and tumor stage-dependent [5, 6]. E-cadherin protein level is significantly higher in MM tissues compared to normal tissues [7], and its increased mRNA expression has been correlated with symptomatic MM [8] and plasma cell leukemia, an aggressive variant of MM (Additional file 2: Figure S1). We have previously reported the...
decreased E-cadherin level in poorly disseminated MM cells mediated by hyper-O-GlcNAcylation [9].

CSC phenotypes include their self-renewal and proliferative properties. To investigate the functional role of E-cadherin in regulating MM CSCs, we first established E-cadherin-depleted cells in human MM-derived cell lines RPMI 8226 and NCI-H929 using the CRISPR/Cas9 system (Additional file 2: Figure S2) and examined its effects on cell growth and cell cycle. Detailed methods can be found in Additional file 1. Figure 1A – C shows that both E-cadherin-depleted cells were less proliferative than wild type (WT) control cells, corresponding to the increased CD138-negative subpopulation and decreased prosurvival Bcl-2, but not Mcl-1. Our findings were consistent with a previous study reporting the prosurvival effect of CD138 in MM [10]. We also found that loss of E-cadherin caused either G0/G1 or G2/M arrest, depending on the cellular context, by controlling its key cell cycle regulators in each phase (Fig. 1D). The PI3K/Akt and MAPK pathways have been reported to regulate the proliferation and survival of MM cells [11].

Herein, we showed that E-cadherin activates Akt, p38, and p44/42 (ERK1/2), but not SAPK/JNK, via protein phosphorylation (Fig. 1E). Altogether, these results support the positive regulatory role of E-cadherin in MM cell growth and survival.

We hypothesized that E-cadherin may be involved in CSC self-renewal. To investigate, colony-forming ability, the potential of a single cell to indefinitely grow and survive [12], was evaluated by clonogenic assay. Figure 1F shows that depletion of E-cadherin resulted in a reduction in both the number and size of MM colonies when compared to WT cells (Additional file 2: Figure S3), which could be reactivated by the restoration of E-cadherin (Additional file 2: Figure S4). Additionally, we found that depletion of E-cadherin reduced the side population (SP) phenotype, a common feature of CSCs related to the ABCG2 multidrug efflux transporter (Fig. 1G and H; Additional file 2: Figure S5). Profiling of stemness-related genes, i.e., SOX2, SOX9, NANO, and OCT4, pointed out that SOX9 could be a key regulator of E-cadherin-mediated MM CSCs (Fig. 1I; Additional file 2: Figure S6). To first test whether SOX9 is functionally linked to CSCs, SOX9 was depleted in RPMI 8226 cells using shRNA. Similar to E-cadherin, depletion of SOX9 reduced Akt and MAPK activity, colony-forming capacity, and SP cells and its corresponding ABCG2 when compared to WT cells (Fig. 2A – D; Additional file 2: Figures S7 and S8), indicating the critical role of SOX9 in MM CSCs. To further validate that SOX9 is downstream of E-cadherin, rescue experiments were conducted in which SOX9 plasmid was ectopically overexpressed in E-cadherin-depleted cells. Figure 2E and F shows that the reduced SOX9 and ABCG2 as well as the reduced Akt and MAPK signaling in E-cadherin depleted cells could be rescued by ectopic SOX9 (see also Additional file 2: Figures S9 and S10). This SOX9 restoration also reversed the inhibitory effects of E-cadherin depletion on the colony forming capacity and SP cells (Fig. 2G and H; Additional file 2: Figures S11 and S12), thus confirming that E-cadherin mediates MM CSCs via SOX9. We also found that SOX9 is, in turn, necessary for maintaining E-cadherin level (Additional file 2: Figure S13), indicating a positive feedback loop that controls MM CSCs.

(See figure on next page.)
In summary, we revealed a novel regulatory mechanism of MM CSCs via the E-cadherin/SOX9 axis (Fig. 2I), which could be important in understanding the long-term cell survival and outgrowth that leads to relapsed/refractory MM. Our findings provided a potential rationale for targeting E-cadherin/SOX9 axis, while in vivo studies are warranted to further validate this hypothesis.
EA SOX9 E-cadherin

Multiple myeloma cells

Cell proliferation and growth
Activating Akt and MAPK signaling
Stemness markers
Regulating cell cycle
Bcl-2
Prosurvival proteins
G2/M
G0/G1

CDH1-KO SOX9-CDH1-KO

Side population
Myeloma stem cells
Regulating self-renewal capability

Fig. 2 (See legend on previous page.)
Additional file 1: Detailed methods.

Additional file 2: Figure S1. Analysis of CDH1 mRNA expression in clinical samples using publicly available microarray data. Figure S2. Successful depletion of E-cadherin by the CRISPR/Cas9 system in the human MM-derived cell lines RPMI 8226 and NCI-H929. Figure S3. Depletion of E-cadherin reduces the clonogenic potential of MM cells. Figure S4. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S5. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S6. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S7. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S8. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S9. Depletion of SOX9 decreases the proportion of the SP subpopulation in MM cells. Figure S10. Re-expression of SOX9 in E-cadherin-depleted MM cells rescues the ABCG2 level. Figure S11. SOX9 regulates E-cadherin-mediated clonogenic growth in MM cells. Figure S12. Re-expression of SOX9 induces the acquisition of the SP subpopulation in E-cadherin-depleted MM cells. Figure S13. Depletion of SOX9 suppresses E-cadherin level in MM cells.

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Author contributions
PS designed research, carried out experiments, analyzed data, and drafted the manuscript. SL supervised the project and provided resources. SL conceived the study, designed research, participated in the data analysis, coordinated the project, and drafted and edited the manuscript. All authors read and approved the final manuscript.

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Abbreviations
CSC: Cancer stem cells; MM: Multiple myeloma; p-: Phosphorylated; RT-qPCR: Quantitative real-time PCR; SP: Side-population; WT: Wild type.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s40164-022-00294-x.

Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Additional file information is available in Additional files 1 and 2.

Declarations

Ethics approval and consent to participate
This study was approved by the Siriraj Institutional Review Board (COA No. Si 101/2015) and was in accordance with the Helsinki Declaration of 1975. The cell lines used in this study were purchased from American Type Culture Collection (ATCC).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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