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LETTER TO THE EDITOR

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Increased TOX expression associates with exhausted T cells in patients with multiple myeloma

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Abstract

Previous studies have shown increased aberrant expression of immune checkpoint (IC) proteins, such as programmed cell death receptor-1 (PD-1) and T cell immunoglobulin mucin-domain-containing-3 (Tim-3) on T cells from patients with multiple myeloma (MM), which result in T cell exhaustion and dysfunction. However, little is known about the mechanism regulating aberrant IC protein expression. In this study, we analyzed the expression of TOX (thymocyte selection-associated HMG BOX), a crucial transcription factor involved in T cell exhaustion, and its co-expression with PD-1, Tim-3, and CD244 in T cell subsets by multi-color fluorescent flow cytometry in peripheral blood (PB) and bone marrow (BM) samples from patients with MM. Significantly, the percentage of TOX + CD3 +/CD4 +/CD8 + T cells was increased, and similarly, higher numbers of TOX co-expression with PD-1, Tim-3, and CD244 on CD3 +/CD4 +/CD8 + T cells were found. Interestingly, the numbers of TOX +, TOX + PD-1 +, and TOX + Tim-3 + regulatory T (Treg) cells also significantly increased in both the PB and BM of MM patients. In summary, we for the first time observed increased TOX expression concurrent with PD-1, Tim-3, and CD244 on T cells, which may contribute to T cell exhaustion and impair their function in MM. Thus, TOX may be considered a potential target for reversing T cell exhaustion and improving T cell function in MM.

Keywords: TOX, Multiple myeloma, PD-1, Tim-3, CD244, T cell exhaustion

To the Editor,

Multiple myeloma is an aggressive, malignant, and incurable disease characterized by neoplastic plasma cell clone proliferation [1]. Poor prognoses of MM patients may be related to T cell immunodeficiency [2]. Recent findings have indicated that aberrant expression of immune checkpoint (IC) proteins such as programmed cell death receptor-1 (PD-1) and T cell immunoglobulin mucin-domain-containing-3 (Tim-3) is a key reason for T cell immune suppression though the promotion of T cell exhaustion [2, 3]. Up-regulation of PD-1 and other IC proteins, such as Tim-3, on CD4 + and CD8 + T cells has been detected in PB from patients with MM [2, 4]. Immunotherapy based on targeting ICs, such as PD-1 blockade, improves the clinical outcome of solid tumors and lymphoma in clinical trials, and the underlying mechanism is thought to reverse the immunosuppressive status of T cells and restore their anti-tumor ability in patients [5]. However, even with PD-1 over-expression on exhausted T cells, the effects of PD-1 blockade appear to be limited

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and heterogeneous for MM patients [6, 7]. These observations may be related to different immunosuppressive microenvironments and the expression pattern of ICs between solid tumors and MM [2]. Recently, it has been reported that over-expression of TOX (thymocyte selection-associated HMG BOX), a crucial transcription factor involved in T cell exhaustion, is detected in CD8 + tumor-infiltrating lymphocytes (TILs) in bladder cancer, and this is related to PD-1 expression on T cells [8, 9]. To further characterize the alternative expression profile of IC proteins and co-expression with their regulatory factors, we analyzed the expression of TOX and TOX co-expression with PD-1, Tim-3, and CD244 in T cells by multi-color fluorescent flow cytometry in peripheral blood (PB) and bone marrow (BM) samples from 16 patients with MM (Additional file 1: Supplementary Methods and Additional file 3: Table S1). Significantly, the percentages of TOX + CD3 +/CD4 +/CD8 + T cell subsets were all increased, and higher numbers of TOX co-expressed with PD-1, Tim-3, or CD244 in CD3 +/CD4 +/CD8 + T cells were found in both PB and BM from patients with MM in comparison with healthy controls (Fig. 1A, B). This result is consistent with the finding of up-regulation of TOX in solid tumors and lymphomas [10]. However, as the heatmap shows in Fig. 1C, the frequency of TOX and co-expression with PD-1, Tim-3, and CD244 in CD3 +, CD4 +, and CD8 + T cells relatively varied between different MM patients, and did not appear to be associated with different stages of MM. Interestingly, a higher frequency of the TOX + T cell subset can be also found in stage I MM (Fig. 1C). The global distribution and frequency of different phenotypes of T cells in the BM and PB of patients with MM and HI can be represented by tSNE clusters (Fig. 1D). Our previous study demonstrated that the level of PD-1 + Tim-3 + CD3 +/CD4 +/CD8 + T cells was high in the BM when compared with PB [2]. In this study, we also compared the percentage of the TOX + T cell subsets in 16 pairs of PB and BM samples from MM patients. Overall, a high percentage of TOX + T cell subsets could be found in the BM in comparison with that in PB in most cases; however, these were not statistically significant (Fig. 1B, Additional file 2: Figure S1) except for TOX + Tim-3 + regulatory T (Treg) cells, which were significantly higher in BM than in PB (Fig. 2C). Interestingly, the numbers of TOX + Tregs and TOX + PD-1 +/Tim-3 + Tregs significantly increased in the PB and BM (Fig. 2A, B). Our previous study also revealed an increase in TOX + Treg cells in patients with lymphoma [11]. However, the role of Treg cells with higher TOX and PD-1 or Tim-3 in MM pathogenesis, prognosis, and treatment remains unclear. Unlike high expression of PD-1 on CD8 + T cells induces exhaustion, PD-1 expression on Tregs negatively impacts immunosuppressive functions [11], moreover, TOX promotes the exhaustion of antitumor CD8 + T cells by preventing PD-1 degradation due to the binding of TOX to PD-1 in the cytoplasm and maintaining abundant PD-1 expression at the T cell surface [12]. Suggesting that the role of TOX may contribute to maintain PD-1 expression on Treg cells which may enhance negative immune regulatory in MM. In this case, whether targeting TOX has a dual inhibitory function remains an open question.

Taken together, first, our findings indicate increased TOX expression in T cells in MM patients. Second, TOX co-expression with PD-1, Tim-3, and CD244 in T cells may be involved in promoting T cell exhaustion and impairing their function in MM. Third, higher TOX + Treg subsets in the BM, may contribute to mediating the BM immunosuppressive microenvironment, which may also be a reason why the effects of PD-1 blockade are relatively different in different MM patients. Understanding the exhausted phenotype pattern of T cells in different MM patients may help guide precision immunotherapy for MM patients.

In summary, we characterized the distribution of TOX expression in T cell subsets in MM patients. Increased

(See figure on next page.)

Fig. 1 Distribution and frequency of TOX expression and co-expression with PD-1, Tim-3, and CD244 in T cell subsets in PB and BM from patients with MM. **A** The analytic logic of flow cytometry detection of TOX expression and co-expression with PD-1, Tim-3, and CD244 in CD3 +, CD4 +, and CD8 + T cell subsets in PB from a patient with MM and a healthy individual (HI). **B** Comparison of the percentage of TOX + CD3 + T cells (median: BM: 13.2 vs 2.06, $P = 0.008$, PB: 15.9 vs 6.145, $P < 0.001$), TOX + CD4 + T cells(median: BM: 25.2 vs 4.57, $P = 0.004$, PB: 21.85 vs 12.95, $P = 0.019$), TOX + CD8 + T cells (median: BM: 16.2 vs 2.23, $P = 0.002$, PB: 21.6 vs 7.51, $P < 0.001$), TOX + PD-1 + CD3 + /CD4 + /CD8 + T cells (median: BM: 5.575/9.19/5.6 vs 0.51/1.02/0.6, $P = 0.008$, $P = 0.002$, $P = 0.004$, respectively, PB: 5.04/7/4.985/ vs 1.495/3.76/1.555, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively), TOX + Tim-3 + CD3 + /CD4 + /CD8 + T cells (median: BM: 0.425/0.51/0.58 vs 0.00706/0.03/0.00747, $P = 0.002$, $P = 0.002$, $P = 0.002$, respectively, PB: 0.295/0.34/0.455 vs 0.063/0.0865/0.068, $P < 0.001$, $P = 0.004$, $P < 0.001$, respectively), and TOX + CD244 + CD3 + /CD4 + /CD8 + T cells (median: BM: 11.35/6.535/14.8 vs 1.71/0.81/1.85, $P = 0.004$, $P = 0.004$, $P = 0.002$, respectively, PB: 11.2/3.645/20.25 vs 4.36/2.435/6.755, $P < 0.001$, $P = 0.171$, $P < 0.001$, respectively) in BM and PB from patients with MM and HIs. **C** Heatmap representing the frequency of TOX +, TOX + PD-1 +, TOX + Tim-3 +, and TOX + CD244 + cells in T cell subsets in PB from 16 patients (stage I (2 cases), stage II (7 cases) and stage III (7 cases) with MM compared with HIs. **D** tSNE clusters of the global distribution and frequency of different phenotypes of T cells in the BM and PB of patients with MM and HIs. Note: P1–P16: MM patients who are numbered according to collection time

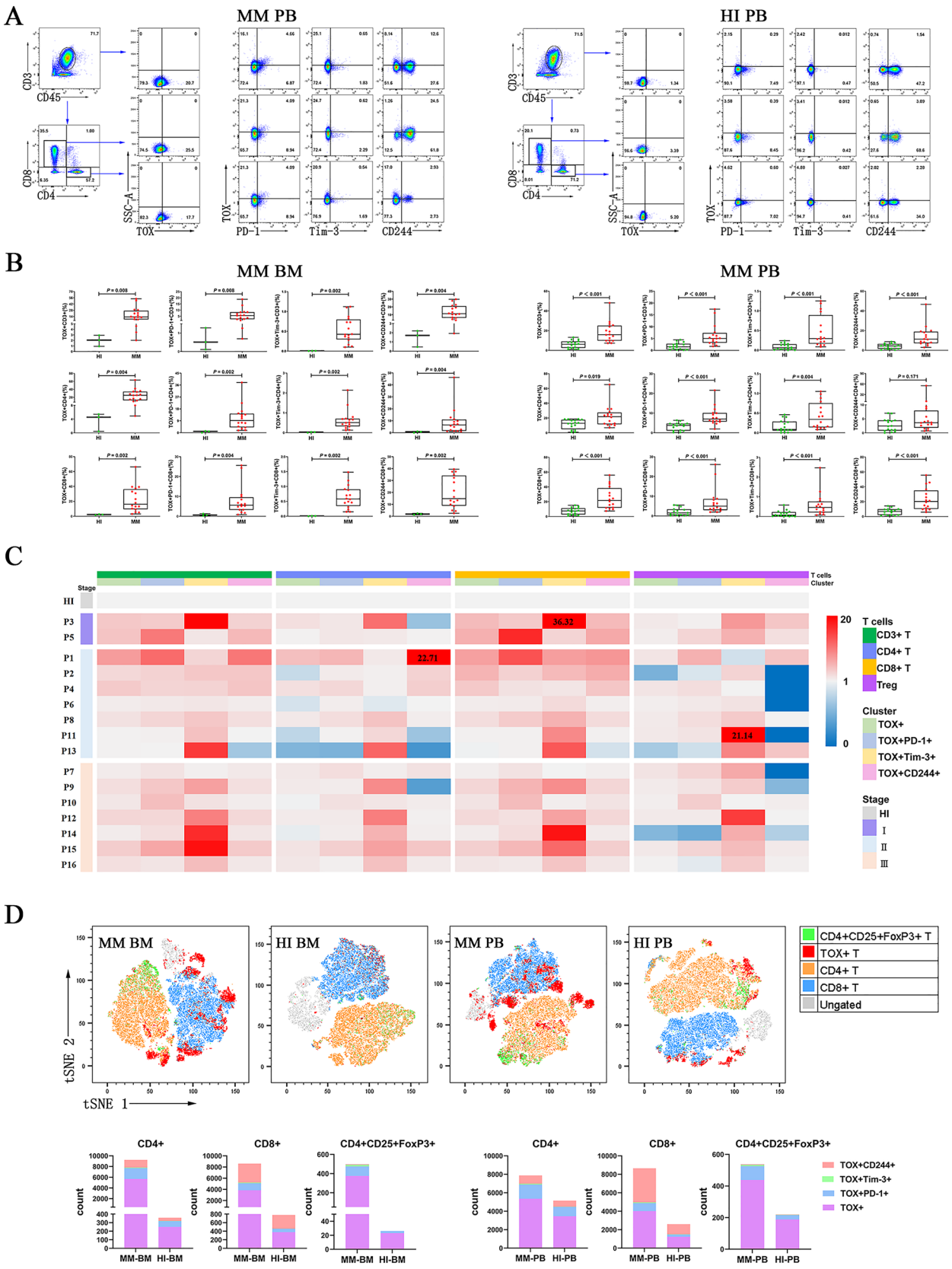

Fig. 1 (See legend on previous page.)

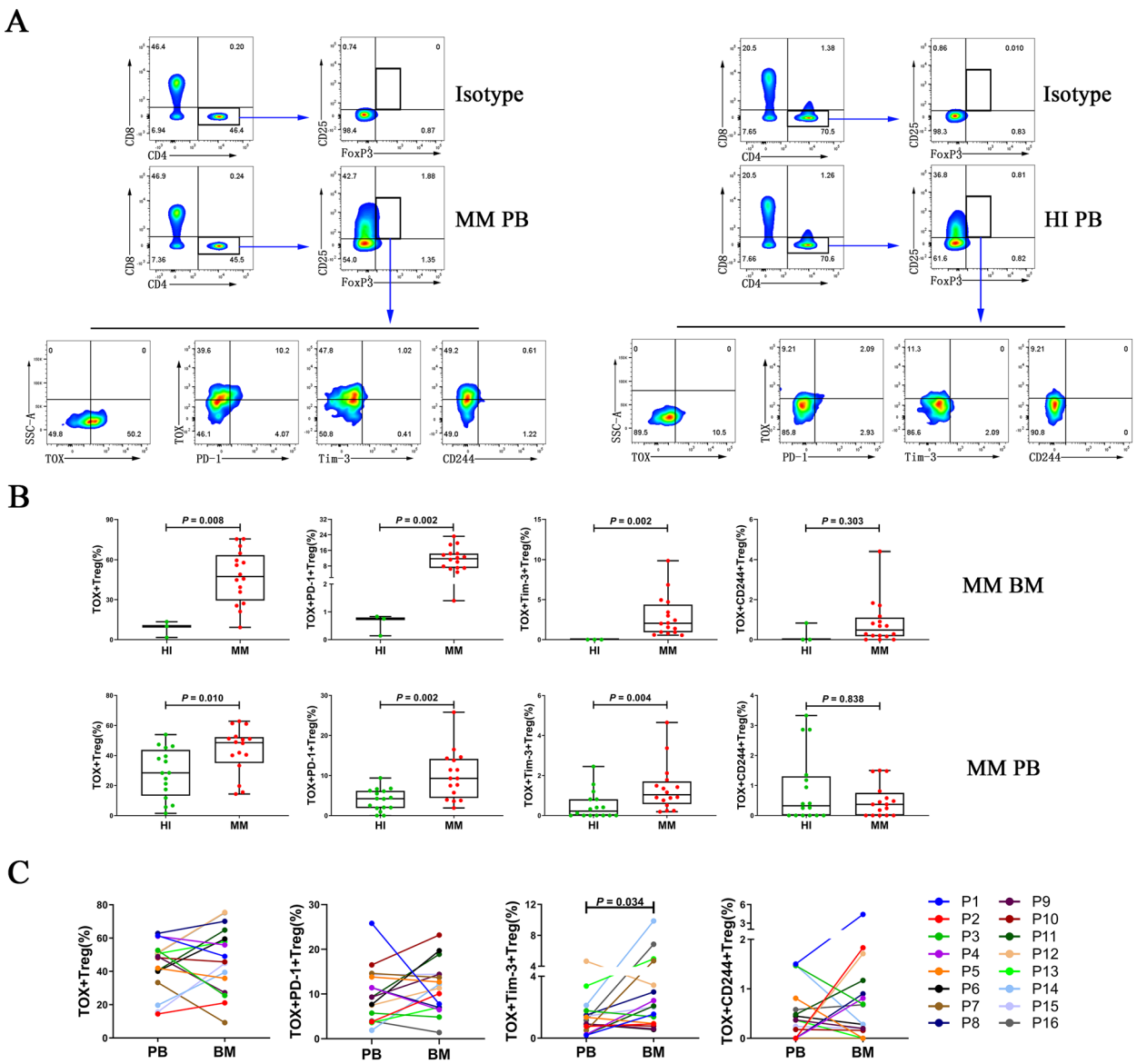

Fig. 2 Distribution and frequency of TOX expression and co-expression with PD-1, Tim-3, and CD244 on Treg cells in BM and PB from MM patients. **A** The analytic logic of flow cytometry detection of TOX expression and co-expression with PD-1, Tim-3, and CD244 in Treg cells in PB from a patient with MM and a HI. **B** Comparison of the percentage of TOX + Treg cells (median: BM: 47.35 vs 10, $P = 0.008$, PB: 48.55 vs 28.45, $P = 0.010$), TOX + PD-1 + Treg cells (median: BM: 11.75 vs 0.75, $P = 0.002$, PB: 9.3 vs 4.21, $P = 0.002$), TOX + Tim-3 + Treg cells (median: BM: 2.045 vs 0, $P = 0.002$, PB: 1.04 vs 0.22, $P = 0.004$), and TOX + CD244 + Treg cells (median: BM: 0.48 vs 0, $P = 0.303$, PB: 0.37 vs 0.325, $P = 0.838$) in BM and PB from patients with MM and HIs. **C** Comparison of the percentage of TOX +, TOX + PD-1 +, TOX + Tim-3 +, and TOX + CD244 + Treg cells between PB and BM from 16 patients (P1–P16) with MM

TOX concurrent with PD-1, Tim-3, and CD244 in T cells may be considered a potential target for reversing T cell exhaustion and improving T cell function in MM.

Abbreviations

BM: Bone marrow; IC: Immune checkpoint; MM: Multiple myeloma; PB: Peripheral blood; PD-1: Programmed cell death receptor-1; TILs: Tumor-infiltrating lymphocytes; Tim-3: T cell immunoglobulin mucin-domain-containing-3; TOX: Thymocyte selection-associated HMG BOX; Treg: Regulatory T.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40164-022-00267-0.

Additional file 1: Supplementary methods.

Additional file 2: Figure S1. Comparison of the percentage of the TOX +, TOX + PD-1 +, TOX + Tim-3 +, and TOX + CD244 + CD3 +/CD4 +/CD8 + T cell subsets between PB and BM from 16 patients (P1–P16) with MM.

Additional file 3: Table S1. Clinical information of MM patients used in the study.

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Authors' contributions
LZ, SC, and YL contributed to the concept development and study design. YZ, SH, YH, and TD performed the experiments. PL, JT, and HZ diagnosed and treated the patients and collected the clinical data. YZ, PL, and SH contributed to the data analysis and figure preparation. YL, YZ, SC, and LZ drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The materials supporting the conclusions of this research article are included within the article.

Declarations

Ethics approval and consent to participate
This study was performed according to the Declaration of Helsinki principles and approved by the Ethics Committee of Guangdong Provincial People's Hospital. All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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