Proline-rich Tyrosine Kinase (Pyk2) Promotes Tumor Progression in Multiple Myeloma

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Introduction
Proline-rich tyrosine kinase (Pyk2) is a non-receptor tyrosine kinase which belongs to the focal adhesion kinase (FAK) family. However, its role in modulating multiple myeloma (MM) biology and disease progression remains unexplored. In our study, we describe the tumor-promoting role of Pyk2 in MM, thus providing molecular evidence for a novel tyrosine kinase inhibitor.

Methods
- GEP (Gene profiling) and CCLE (Cancer Cell Line Encyclopedia) analysis and immunohistochemistry were performed to evaluate Pyk2 expression in MM.
- Gain- and loss-of-function assays were performed on indicated MM cells to confirm tumor-promoting role of Pyk2 in MM.
- In vivo tumor growth was observed by Bioluminescent Imaging.
- Pyk2-dependent modulation of β-catenin signaling activity was identified by using immunoblotting.
- Inhibitory effects of VS-4718 on FAK and Pyk2 activity were evaluated by both biochemical and cellular assays.

Results
Figure 1. Highly expressed Pyk2 in MM disease was described by GEP analysis (GSE6477) (A) and immunohistochemistry (B). Based on analysis of 27 MM cell lines enrolled in CCLE database, the copy numbers of genes harbored in the region from 8p21.1 to 8p22, where Pyk2 locates, are shown in the heatmap (C). Pyk2 mRNA levels are positively associated with the copy number (D), suggesting 8p21 stability is important for Pyk2 expression. Note U266 cells, which have a loss of 8p21 (C), show low/absent expression of Pyk2 (D, E).

Figure 2. Pyk2 silencing decreased MM tumor growth and prolonged survival in a mouse xenograft model (A). In vitro function assays showed that, Pyk2 silencing decreased MM cell proliferation (B), increased G1 phase population (C), decreased adhesion to bone marrow mesenchymal stromal cells (MM-BMSCs) (D) and fibronectin (E). Pyk2 silencing led to suppression of Wnt/b-catenin signaling: MM-BMSCs enhanced Pyk2 phosphorylation and b-catenin nuclear translocation and upregulated nuclear c-Myc were abolished by inhibition of Pyk2.

Figure 4. Effects of FAK/Pyk2 dual inhibitor VS-4718 treatment on MM tumor growth. In vitro kinase assays were performed to measure the inhibitory activity of Vs-4718 (A). VS-4718-dependent induction of apoptosis (B), suppression of cellular migration (C), modulation of cell cycle (D) in Pyk2/high (MM.15) versus Pyk2/low (U266) MM cells. VS-4718 impaired BMSCs-induced cell migration of MM.15 but not U266 (C). VS-4718 reduces survival of primary myeloma bone marrow derived CD138+ cells without affecting viability of peripheral blood-derived mononuclear cells (E). VS-4718 treatment effectively reduced MM tumor growth compared with vehicle control (F). The dose-dependent reduction of p-Pyk2 by VS-4718 in the MM.15 cell line was confirmed (G). VS-4718 inhibited b-catenin expression, resulting in downregulation of Cyclin D1 and c-Myc (G).

Conclusion
We demonstrate the tumor-promoting role of Pyk2 in MM, describe the implication of Pyk2 in facilitating the oncogenic Wnt/β-catenin pathway, and support the potential clinical development of a FAK/Pyk2 inhibitor, such as VS-4718, for the treatment of MM.

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