Abstract

Multiple myeloma (MM) remains an incurable malignancy due, in part, to the influence of the bone marrow microenvironment on survival and drug response. Identification of microenvironment-specific survival signaling determinants is critical for the rational design of therapy and elimination of MM. We have shown that collaborative signaling between β3 integrin-mediated adhesion to fibronectin (FN) and Interleukin-6 (IL-6) confers a more malignant phenotype via amplification of STAT3 activation. Further characterization of the events modulated under these conditions with quantitative phospho-protein profiling identified 193 differentially phosphorylated peptides. Seventy-seven phosphorylations were up-regulated upon adhesion, including PYK2/JAK2, Paxillin, Cas11, and p53CAAX consistent with focal adhesion (FA) formation. We hypothesized that the collaborative signaling between β3, integrin and gp130 is mediated by FA formation and PYK2 kinase activity. Both pharmacological and molecular targeting of PYK2 attenuated the amplification of STAT3 phosphorylation under co-stimulatory conditions. Further, co-culture of MM cells with patient bone marrow stromal cells (BMSCs) showed similar β3, integrin-specific enhancement of PYK2, JAK1, STAT3 signaling. Importantly, molecular and pharmacological targeting of PYK2 specifically reduced cell death and reduced clonogenic growth in BMSC-adherent myeloma cell lines, ALDH+ MM cancer stem cells, and patient specimens. These data identify a novel PYK2-mediated survival pathway in MM cells and MM cancer stem cells activated within the context of microenvironmental cues, suggesting that PYK2 is a putative therapeutic target. Moreover, these data provide preclinical support for the use of the clinical stage FA/JAK inhibitors for treatment of MM, especially in an MDR setting.

Is PYK2 involved in the Enhanced Signaling?

Pyk2 mediates enhanced survival in the bone marrow microenvironment

Introduction

We have previously demonstrated that collaborative signaling between β3 integrin and IL-6 is involved in the enhanced activity of STAT3 and confers a more malignant phenotype to MM cells (Javaheri et al., Cancer Res. 2010). We also demonstrated that the presence of BMSCs as a co-culture with these MM cells results in enhanced proliferation and survival signaling in this microenvironment environment. Furthermore, we identified the focal adhesion kinase, PYK2/JAK2 as a key component of enhanced STAT3 signaling and a role-specific myeloma cell in this context.

Hypothesis

Myeloma cell adhesion modulates signaling pathways elicited by soluble determinants of the microenvironment promoting MM cell pathogenesis, contributing to therapy resistance, and minimal residual disease (MRD). Improved understanding of the dominant pathways elicited under constraint conditions will lead to improved understanding of myeloma/niche biology and improved clinical outcomes.

Results

Pyk2 mediates enhanced survival in the bone marrow microenvironment

Four different cell lines derived from MM patients were cultured in the absence of BMSCs or in the presence of BMSCs. These cells were treated with increasing concentrations of VSP6062. The control experiments were carried out using Tacrolimus, which is an inhibitor of JAK1/2. The number of viable cells, as measured by trypan blue exclusion, was determined by the MTT assay. A threefold increase in the number of viable cells was observed in the presence of BMSCs compared to the absence of BMSCs. This effect was significantly reduced in the presence of Tacrolimus, which is an inhibitor of JAK1/2. These results suggest that the increased survival in the presence of BMSCs is mediated by JAK1/2 signaling.

Figures 1 and 2: The preclinical evaluation of the bone marrow microenvironment on increasing complexity and clinical outcomes. A) The percentage of viable cells in the presence of BMSCs was significantly higher than the absence of BMSCs. B) The percentage of viable cells in the presence of Tacrolimus was significantly lower than the absence of Tacrolimus. C) The percentage of viable cells in the presence of BMSCs and Tacrolimus was significantly lower than the absence of BMSCs and Tacrolimus.

Figures 3 and 4: Does adhesion lead to large changes in the tyrosine-phosphoproteome?

The tyrosine-phosphoproteome of MM cells was analyzed in the absence and presence of BMSCs and Tacrolimus. The results showed a significant increase in the tyrosine-phosphoproteome in the presence of BMSCs compared to the absence of BMSCs. This effect was significantly reduced in the presence of Tacrolimus, which is an inhibitor of JAK1/2. These results suggest that the increased survival in the presence of BMSCs is mediated by JAK1/2 signaling.

Figures 5 and 6: Does the Pyk2/JAK/STAT3 axis play a role in more complex modes of the bone marrow niche?

The expression of Pyk2/JAK/STAT3 axis was measured in the absence and presence of BMSCs and Tacrolimus. The results showed a significant increase in the expression of Pyk2/JAK/STAT3 axis in the presence of BMSCs compared to the absence of BMSCs. This effect was significantly reduced in the presence of Tacrolimus, which is an inhibitor of JAK1/2. These results suggest that the increased survival in the presence of BMSCs is mediated by JAK1/2 signaling.

Summary

Pyk2 mediates the amplification of Jak/JAK3/STAT3 signaling in adherent myeloma cells and in OS31-enriched myeloma patient specimens. The Pyk2/JAK1/STAT3 axis is similarly enhanced in myeloma cell lines and OS31-enriched MM patient specimens upon adhesion-dependent co-culture with MM patient derived BMSCs.

Does the Pyk2/JAK/STAT3 axis play a role in myeloma survival?

The expression of Pyk2/JAK/STAT3 axis was measured in the absence and presence of BMSCs and Tacrolimus. The results showed a significant increase in the expression of Pyk2/JAK/STAT3 axis in the presence of BMSCs compared to the absence of BMSCs. This effect was significantly reduced in the presence of Tacrolimus, which is an inhibitor of JAK1/2. These results suggest that the increased survival in the presence of BMSCs is mediated by JAK1/2 signaling.

Conclusion

These data demonstrate that within the context of multiple stromal interactions (collaborative signaling between soluble and physical factors) Pyk2/JAK/STAT3/STAT signaling is preferentially modulated. These findings are significant as they identify a novel niche-specific Pyk2/JAK/STAT3 signaling axis that is critical for adherent MM cell and ALDH+ MM stem cell survival. Further, these data provide preclinical evidence and rationale for the clinical use of Pyk2/JAK inhibitors, such as VS6062, for the treatment of MM in the setting of minimal residual disease where cooperative signaling between physical and soluble effectors of the marrow microenvironment and promote MM cell and ALDH+ stem cell growth and survival.

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References

3. Verastem. 2015

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