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COMMENTARY

# PRL-3 promotes a positive feedback loop between STAT1/2-induced gene expression and glycolysis in multiple myeloma

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Over 34 000 patients are diagnosed yearly with multiple myeloma (MM), which remains a fatal malignancy. Expression of the phosphatase PRL-3 is associated with poor prognosis in MM patients, and Vandsemb *et al*. have demonstrated that PRL-3 contributes to enhanced MM cell fitness through activation of a glycolysis-associated feedback loop. PRL-3 resulted in increased expression of signal transducer and activator of transcription 1 (STAT1) and 2 (STAT2) and increased glycolysis. Increased glucose metabolism in turn activated STAT1/2 and interferon 1-related genes. This discovery advances the MM field by providing a new potential treatment avenue.

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## Introduction

Multiple myeloma (MM) is a rare disease that begins with the presence of rapidly dividing plasma cells in the bone marrow. The tumor microenvironment (TME) within the bone marrow plays a critical supporting role in MM progression, contributing to differentiation, migration, survival, and therapeutic resistance of MM cells [1]. There are many factors in the bone marrow TME that provide optimal growth conditions for MM, including the interplay of cytokines, chemokines, enzymes, and adhesion molecules [2]. Interleukin-6 (IL-6) is one cytokine provided by the TME that has been extensively studied as a potential drug target in MM. Normally, IL-6 plays multiple roles in inflammation and immunity and is well known for its role in driving antibody production in plasma cells. However, over 20 years ago, IL-6 was shown to play a role in kick-starting rapid proliferation in malignant plasma cells in the bone marrow [2,3]. Currently, there are multiple FDA-approved IL-6 inhibitors on the market, yet MM will frequently become resistant to IL-6 inhibitors and relapse. A major focus in MM research is to identify and target signaling pathways that are activated downstream of IL-6 in MM, with the goal of decreasing MM tumor burden and ameliorating the protumorigenic effects of the bone marrow TME.

The dual-specificity phosphatase PRL-3 is highly expressed in MM cells, compared to normal plasma cells

**Abbreviations**
IFN-1, type 1 interferon; IL-6, interleukin-6; ISG, interferon-stimulated genes; MM, multiple myeloma; PRL-3, phosphatase of regenerating liver 3; TME, tumor microenvironment.

[4]. PRL-3 expression is also significantly correlated with poor prognosis in MM patients [4]. As a phosphatase, PRL-3 can be enzymatically inhibited, suggesting that it may be a viable therapeutic target in MM. Excitingly, Slordahl *et al*. [5] showed PRL-3 to be an effector protein of IL-6. Specifically, when PRL-3 is overexpressed in an IL-6-dependent MM cell line (INA-6), cells can survive in the absence of IL-6. In its prosurvival role downstream of IL-6, PRL-3 stabilized MCL-1, an anti-apoptotic factor. The same research team also linked PRL-3 to the regulation of metabolism in MM [6]. Increased PRL-3 expression in MM cell lines was found to promote glucose uptake and enhance the expression of proteins that regulate glycolysis [6]. These findings have been quite exciting as they present a novel avenue to introduce chemotherapies to target cell metabolism in PRL-3-expressing tumors. Vandsemb *et al*. [7] have now taken the next step to define the signaling mechanisms that link PRL-3 to enhanced glycolysis in MM.

To determine how PRL-3 can modulate metabolism and survival in MM cells, PRL-3 was overexpressed in both IL-6-dependent (INA-6) and IL-6-independent (JJN-3) MM cell lines. Interestingly, RNAseq analysis showed that PRL-3 induced expression of factors involved in type 1 interferon (IFN-1) signaling, a key signaling pathway induced by IL-6. In particular, signal transducer and activator of transcription 1 (STAT1) and 2 (STAT2) were highly induced by PRL-3 expression. Interestingly, PRL-3 induced similar expression changes in both IL-6-dependent and IL-6-independent MM cells, and whether cells were treated with IL-6 or not. These data indicate that PRL-3 expression can independently promote IL-6-induced signaling cascades to enhance MM stability and growth, making it a potential drug target.

## PRL-3 induces a feedback loop between STAT1/2 and glycolysis

PRL-3 expression in MM cell lines led to increased levels and activation of STAT1. These data were supported by findings in MM patient samples, where STAT1 expression was increased in patients with higher expression of PRL-3. Classically, upregulation of STAT1 and STAT2 in MM cells is regulated by IFN-1, which would obviate the need for PRL-3 expression. Therefore, it was necessary to determine whether the expression of PRL-3 could bypass the need for IFN-1 to activate STAT1 and STAT2. Expression of PRL-3 in both IL-6-dependent and IL-6-independent MM cells showed an increase in STAT1 phosphorylation and total STAT1 expression when PRL-3 was overexpressed, and a decrease in STAT1 phosphorylation and expression was observed when PRL-3 was inhibited through PRL-3 inhibitor 1 and siRNA treatments. These findings further specify that PRL-3 may be acting outside of normal IL-6 signaling mechanisms.

After observing this initial relationship between PRL-3 and STAT activation, there was a need to determine whether PRL-3-induced STAT1 and STAT2 mediated prosurvival mechanisms in MM cells. However, knocking down STAT1 and STAT2 via siRNA individually and in combination did not change cell growth rates in MM cells that overexpressed PRL-3. Studies on solid tumors have shown that STAT1 expression is associated with resistance against DNA damage [8,9], so researchers hypothesized that PRL-3-induced STAT1 in MM may have a similar effect. Yet, doxorubicin treatment of PRL-3-overexpressing MM cells showed no distinct differences in cell viability or apoptosis when compared to mock transfected cells. These data show that STAT1 and STAT2 do not regulate survival of MM cells and PRL-3 does not change their susceptibility to DNA-damaging MM therapeutics.

PRL-3 promotes glycolysis in MM cells in part by increasing glucose uptake [6]. Interestingly, STAT1/2 knockdown in PRL-3-overexpressing cells showed decreased glycolysis and glycolytic capacity measured by a Seahorse XF Bioanalyzer, demonstrating that PRL-3 influences MM cell metabolism through STAT1 and STAT2. Additionally, treatment of MM cells with glucose increased both total and phosphorylated levels of STAT1 and STAT2 in a dose-dependent manner. This increase in STAT1/2 activation only occurred in MM cells with high PRL-3 expression. When measured by qPCR, it was determined that overexpression of PRL-3 coupled with glucose stimulation led to a significant increase in expression of six different interferon-stimulated genes (ISGs), or STAT1/2 effector proteins. These findings ultimately lead to the conclusion that STAT1 and STAT2 are activated by PRL-3 expression and help regulate glycolysis output in MM. The subsequent increase in glucose metabolism then further stimulates STAT1 and STAT2 expression and phosphorylation, creating a positive feedback circuit that leads to increased expression of ISGs, which are involved with MM cell survival.

## Conclusion

While there have been advances in treatment options for MM, it is still an incurable disease. There is an intense need to define signaling pathways involved in MM pathology that may have therapeutic potential. In their manuscript, *Vandsemb* et al. have described a novel signaling loop where PRL-3 enhances expression

and activation of the STAT1 and STAT2 transcription factors to promote glycolysis; glucose in turn activates STAT1/2 and the expression of ISGs that can enhance MM proliferation and survival. Importantly, PRL-3 was found to induce STAT1/2 independently of IL-6, a key cytokine involved in MM malignancy that is well known to be involved in JAK/STAT signaling. The discovery of this novel feedback loop between STAT1/2 and glucose production in MM cells provides the field with two new features to interrogate in MM therapy: PRL-3 as a druggable target and modulation of pathways involved in MM glycolysis to ultimately halt cancer cell growth.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

CNS wrote and JSB edited the manuscript.

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