Aminopeptidase Gene Expression in Myeloma

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INTRODUCTION

A hallmark of myeloma is high-level production of immunoglobulins leading to a heavy load on protein folding and homeostasis in tumor cells. The aminopeptidase gene family catalyze the hydrolysis of amino acid residues from proteins or peptides and are last in line for protein degradation. They are thus an important group of metalloenzymes implicated in cellular functions such as differentiation, cell cycle, DNA repair, and apoptosis. However, there is limited information about the expression of aminopeptidases in myeloma and their possible utilization for novel therapeutic approaches including peptide conjugated drugs.

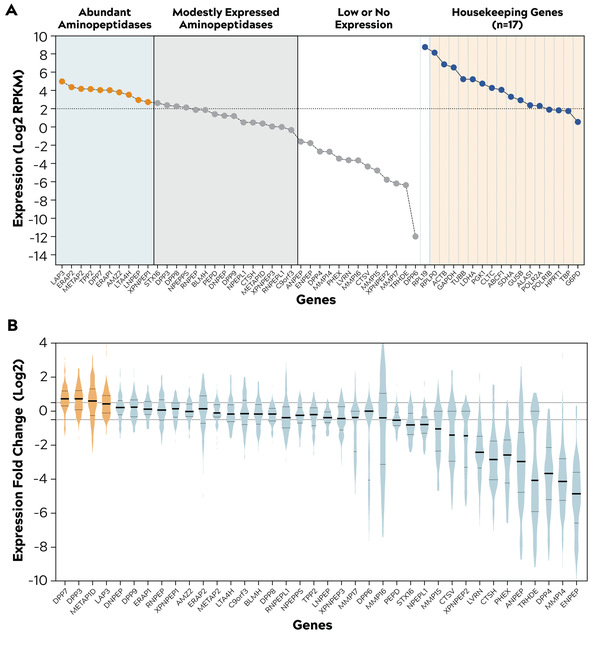
METHODS

103 bone marrow aspirates from myeloma patients and 2 healthy donors were obtained after written informed consent and following approved protocols in compliance with the Declaration of Helsinki. CD138+ cells were enriched and used for RNA or protein preparation. Illumina-compatible RNA sequencing libraries were prepared and sequenced. Proteomic analysis was performed using Q-Exactive MS/Dionex Ultimate 3000 instruments. Contribution of aminopeptidase gene expression to survival outcome was estimated by Kaplan-Meier analysis. Significance of survival curves between 2 groups (high vs. normal expression) was deduced using a log rank test (Mantel-Cox).

RESULTS

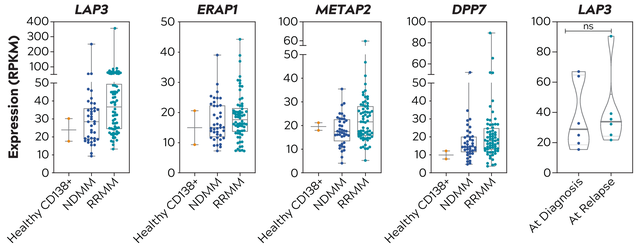
- Aminopeptidase gene expression levels were ranked based on abundance levels in all samples (**Figure 1A**)
- Aminopeptidases were differentially expressed compared to heathy plasma cells (**Figure 1B**)
  - The majority of the genes in patient samples showed related expression patterns or were modestly overexpressed (*DPP7*, *DPP3*, *METAP2* and *LAP3*) compared to healthy plasma cells
  - Decreased expression was detected for several aminopeptidases including *MMP14*, *MMP15*, *ANPEP*, *ENPEP*, and *CTSH*

Figure 1. Gene Expression Profiles of Aminopeptidases



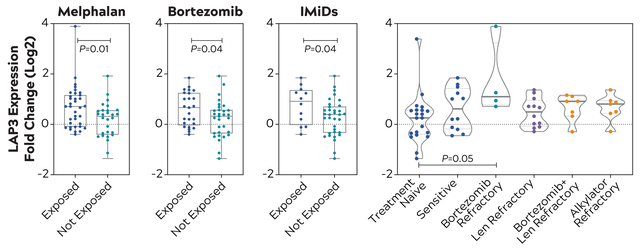
- We also investigated whether any aminopeptidase could be linked to disease progression and found no significant difference (**Figure 2**)
  - Expression levels of *LAP3*, *ERAP1*, *METAP2*, and *DPP7* (P>0.005) appeared higher in relapsed/refractory multiple myeloma (RRMM) than in newly diagnosed multiple myeloma (NDMM) samples
  - A comparison of expression levels in 6 paired NDMM and RRMM samples showed a trend for increased *LAP3* at relapse

Figure 2. Gene Expression Changes During Disease Progression



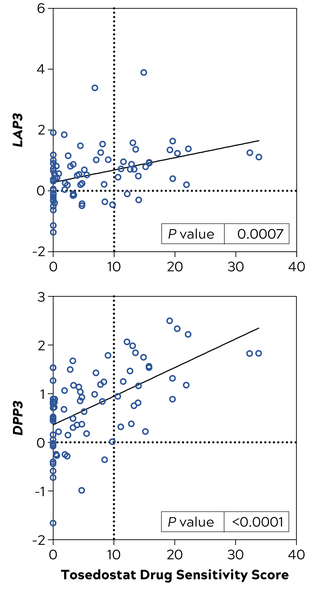
- Association of prior exposure to treatments with *LAP3* expression was analyzed in the patients (**Figure 3**)
  - Here, we compared expression between samples exposed and naïve to melphalan, bortezomib class, or immunomodulatory drugs (IMiDs)
  - Elevated expression of *LAP3* was detected in all treated groups (melphalan P=0.01, bortezomib P=0.04, and IMiDs P=0.04)

Figure 3. *LAP3* Expression Underlying Disease Progression



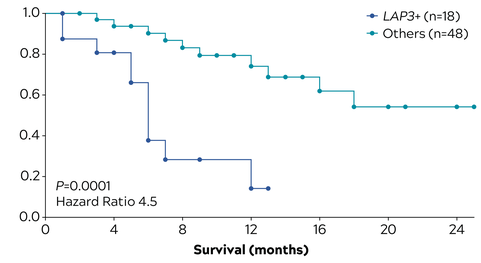
- Ex vivo testing of patient cells with the aminopeptidase inhibitor tosedostat showed that the viability of approximately 30% of relapsed myeloma samples was reduced (**Figure 4**)
  - RNA expression of *LAP3*, *DPP3*, *METAP1D*, *CTSV*, *TPP2*, and *XNPEP1* was found to significantly correlate with tosedostat response (Spearman P<0.005)
  - The figure demonstrates *LAP3* and *DDP3* RNA expression in relation to tosedostat treatment

Figure 4. Inhibition of Aminopeptidases



- Survival analysis revealed patient samples exhibiting 2× or higher *LAP3* expression had poorer prognosis with a median survival of 6 months from the sampling date (P=0.0001, HR 4.5; 95% CI 1.45-14.05) (**Figure 5**)

Figure 5. *LAP3* Expression Is Associated With Poor Prognosis



CONCLUSIONS

- The majority of aminopeptidase genes in myeloma patient samples showed similar expression patterns or were modestly overexpressed compared to healthy plasma cells
- *DPP7*, *DPP3*, *METAP2*, and *LAP3* were most frequently expressed in this sample set
- *LAP3* was identified as a potential poor prognostic marker for myeloma
- Ex vivo treatment of myeloma patient CD138+ cells with the aminopeptidase inhibitor tosedostat results in cell death and thus strengthens the role of these genes in myeloma biology

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