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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

SDC2 Expression Is Increased in Myeloma Cells in Response to Loss of Pro-Survival Surface Proteins, CD28 and CD86

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Introduction: While recent advances in multiple myeloma (MM) have improved overall survival, most patients will succumb to late-stage and drug resistant disease. Plasma cell (PC) survival outside of the bone marrow is characteristic of late-stage myeloma and pivotal to disease progression. PCs are typically restricted to the bone marrow microenvironment, as they are dependent on stromal interactions for survival. One such interaction is the engagement of CD28 and its ligand CD86, proteins which are best understood for their roles in T-cell activation by antigen presenting cells. However, myeloma cells can express both CD28 and CD86 on the cell surface. Notably, high CD28 expression is seen in 59% of medullary recurrences, and over 90% of extramedullary relapses. Moreover, high CD86 expression is more common in malignant plasma cells as compared to precursor stages and has a reported role in driving expression of IRF4. We have previously shown that expression of CD28 and CD86 is associated with worse outcomes than that observed in patients with low expression in the MMRF CoMMpass study (NCT01454297). We have also shown that silencing of CD28 or CD86 results in cell death and loss of IRF4 protein expression in human myeloma cell lines (HMCL). We confirmed these studies using CRISPR/Cas9 editing and demonstrated loss of proliferation and induction of cell death in HMCLs. However, the mechanism(s) by which CD28 and CD86 contribute to MM cell survival is not fully understood.

Methods: To shed light on the mechanistic basis of CD28 and CD86 in the survival of MM, we used CRISPR/Cas9 editing to disrupt expression from RPMI8226 myeloma cells. We then passaged transduced cells to select for populations that can survive loss of CD28 or CD86. These passaged cells were sorted into populations that either retained CD28 or CD86 expression as transduction controls (High), or populations that underwent CRISPR/Cas9 depletion (Low). Surface expression of CD28 $^{High/Low}$ and CD86 $^{High/Low}$ populations was confirmed via flow cytometry and were then processed for RNA isolation and cDNA library preparation. RNA-sequencing was performed using paired-end sequencing, and data were aligned using the STAR aligner. Differentially expressed genes were determined using edgeR with an FDR $\leq$ 0.05 and a fold-change $\geq$ 2.0. Validation of RNA-sequencing results was further assessed via western blot and flow cytometry.

Results: Analysis of RNA-sequencing results indicated that 57 genes were differentially expressed between High and Low cells, with 23 genes common to both CD28 and CD86 High/Low comparisons. (FDR<0.05). Notably, our results reveal that loss of CD28 or CD86 is associated with an increase in mRNA expression of Syndecan-2 (SDC2). In addition, our preliminary experiments evaluating SDC2 surface expression via flow cytometry suggest that both CD28 ^{Low} and CD86 ^{Low} populations have increased expression of SDC2. Additionally, in the RNA-seq analysis we also observed that CD28 ^{Low} and CD86 ^{Low} cells were also able to sustain mRNA expression of IRF4. This result was later confirmed via western blot, demonstrating CD28 ^{Low} and CD86 ^{Low} cells sustain protein expression of IRF4.

Conclusions: Our results demonstrate that RPMI8226 cells that lack CD28 or CD86 expression have increased mRNA and protein expression of SDC2, a member the heparan sulfate proteoglycan family. Since SDC2 expression has also been associated with a number of cancers including colorectal, lung, and pancreatic cancers, these results provide insight to potential alternative pathways that contribute to myeloma survival independent of CD28 and CD86 signaling. We also confirmed that CD28- and CD86-depleted populations were able to maintain IRF4 mRNA/protein levels. This indicates myeloma cells have mechanisms to sustain IRF4 expression in addition to CD28 and CD86 signaling. These results are also consistent with previ-

ous findings illustrating importance of IRF4 in myeloma. Finally, we have developed CRISPR/Cas9-depleted CD28 and CD86 populations in additional HMCLs. We are currently in the process of isolating CD28 $^{High/Low}$ and CD86 $^{High/Low}$ populations for additional analysis and will present these upcoming results.

Disclosures Boise: *Astra Zeneca:* Consultancy, Honoraria; *Abbvie:* Consultancy.

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