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SKP2 knockout in Rb1/p53 deficient transgenic mouse models of osteosarcoma improves survival via induction of macrophage infiltration

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Background: Osteosarcoma (OS) is an aggressive cancer of the bone with a poor prognosis and limited improvements in treatment for several decades. OS patients frequently display loss-of-function mutations in the tumor suppressors *RB1* and *TP53*. One putative proto-oncogene in OS is *SKP2*, which codes for a substrate-recognition factor of the SCF E3 ubiquitin ligase. We have previously demonstrated that germline knockout of *Skp2* in a transgenic mouse model of OS doubly deficient in *Rb1* and *Trp53* improved survival in mice, along with delaying tumorigenesis, inducing apoptosis, and decreasing stemness in tumors. However, the role that *SKP2* plays in OS progression remains unclear.

Questions / Purposes: We set out to study the regulatory and oncogenic mechanisms of *SKP2* in OS. Surprisingly, we found that immune infiltration is dramatically increased in the tumor microenvironment of *SKP2*-knockout OS mouse models compared to controls. Therefore, we set out to answer the following questions: 1) What types of immune cells are present and altered in the tumor microenvironment in OS after SKP2-knockout? 2) Do the levels of infiltration of different immune cell types provide any survival benefit in OS patients?

Method: We performed bulk RNA-sequencing on pre-clinical transgenic mouse models of OS. In one model, we generated a conditional double-knockout line of *Trp53* and *Rb1* mutations induced in cells of the bone-forming osteoblast lineage via Cre-Lox recombination ("DKO": Osx1-Cre;$Rb1^{lox/lox}$;$p53^{lox/lox}$). In the other model, we additionally introduced a germline *Skp2* knockout ("TKO": Osx1-Cre;$Rb1^{lox/lox}$;$p53^{lox/lox}$;$SKP2^{-/-}$). We compared tumors from the TKO and DKO models via bulk RNA-sequencing (n=3 per group) to study differential expression, with a focus on how immune cell gene signatures were altered. To link the mouse model work to patients, we studied immune and stromal cell infiltration levels, using a "digital pathology" bulk RNA-seq deconvolution method called Microenvironment Cell Population Counter (MCP-Counter), in both the mouse data and a human OS patient cohort of 87 participants (NCI TARGET OS). In addition, we analyzed the expression of genes differentially expressed between our two mouse models in the patient cohort. The data were then used for Kaplan-Meier analysis, univariate Cox regression, and multivariate Cox regression, controlling for metastasis at diagnosis as a stand-in for stage. Model diagnostics for regression analysis detected no abnormalities.

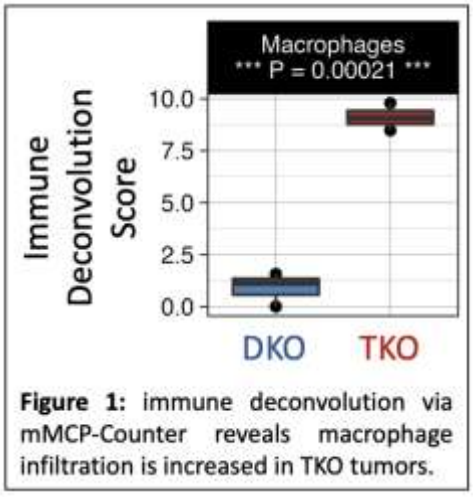

Figure 1: immune deconvolution via mMCP-Counter reveals macrophage infiltration is increased in TKO tumors.

Result: We found a large difference in gene expression between the two models. Surprisingly, we observed a dramatic increase in immune microenvironment infiltration in TKO tumors compared to DKO (Figure 1). Macrophage transcriptomic signatures were the strongest signal detected (t-test, $p<0.001$), but significant increases in B cells, endothelial cells and T cell signatures were also observed. Further analysis indicated that naïve/M0-like macrophages were predominant in the TKO, rather than M1 or M2 macrophages. In the NCI TARGET OS cohort, high expression of genes up-regulated in TKO was significantly correlated with 5-year overall survival (HR[95%CI]=0.39[0.17-0.91]). This effect was driven by macrophage transcriptomic signatures. This finding was supported by an immune microenvironment deconvolution analysis, which showed that macrophages were associated with improved overall survival in the patient cohort (HR[95%CI]=0.37[0.17-0.83], Figure 2). T cell infiltration scores were also associated with improved overall survival (HR[95%CI]=0.38[0.17-0.85]). We are currently using immunohistochemistry and flow cytometric analysis to validate our findings of immune infiltration, and performing single-cell RNA-sequencing analysis to study immune infiltration and cellular heterogeneity in more detail.

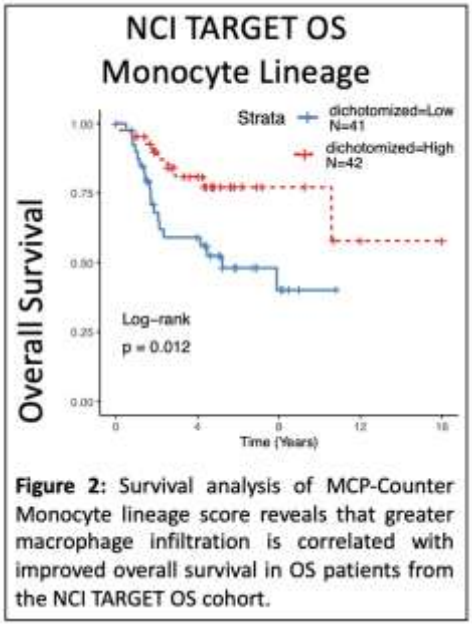

Figure 2: Survival analysis of MCP-Counter Monocyte lineage score reveals that greater macrophage infiltration is correlated with improved overall survival in OS patients from the NCI TARGET OS cohort.

Conclusion: Taken together, these findings indicate that *SKP2* modulation in OS may induce vigorous anti-tumor immune activation especially in the form of macrophage and lymphocyte infiltration into the tumor microenvironment. Limitations of this study include its small sample size in bulk RNA-seq and the limited resolution of bulk RNA-seq in studying the tumor microenvironment.

This study adds to a growing body of work on the anti-tumor role of macrophages, both within OS and in cancers generally. In OS, prior work has shown that macrophage infiltration may be correlated in with improved metastasis-free survival. More generally, macrophages have been shown to be capable of anti-tumor activity in the context of blockade of the “don’t eat me” signal Cd47. Future work on SKP2 and macrophages in OS may reveal potent combination therapeutics harnessing critical antineoplastic mechanisms of these innate immune cells.