TARGETING OF NOTCH LIGAND JAGGED2 IN LUNG CANCER CELLS DRIVES ANTI-TUMOR IMMUNITY VIA NOTCH-INDUCED FUNCTIONAL REPROGRAMMING OF TUMOR-ASSOCIATED MACROPHAGES

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Background Signaling through the Notch family of receptors after engagement with ligands, Jagged (JAG1-2) or Delta-like ligands (DLL1-4), promotes the development and progression of several tumors and modulates anti-tumor immunity. However, whether expression of Notch ligands in tumor cells impacts anti-tumor immunity remains practically unknown. Herein, we sought to determine the impact of the expression of Notch ligands, Jagged1 and Jagged2, on lung tumors in the modulation of anti-tumor immunity.

Methods We evaluated the correlation between JAG1-2 expression in lung tumors along with clinical outcome and intra-tumoral T-cell infiltration. Furthermore, we eliminated Jagged forms in murine lung tumors via Crispr-based approaches followed by studies identifying the tumor immune-landscape and functional changes in T-cells and macrophages.

Results Analyses of the TCGA lung adenocarcinoma datasets showed that augmented expression of JAG2 in tumors, but not JAG1, correlated with reduced patient survival. Additionally, tumor tissue microarray-based studies showed a correlation between lower Jagged2 levels in lung cancer cells and increased intra-tumor T-cell frequency. Consistently, elimination of Jag2, but not Jag1, in murine lung cancer cells activated anti-tumor T-cell immunity and delayed tumor growth in mice. Elimination of T-cells restored growth of Jag2KO tumors in vivo. Moreover, analysis via single-cell RNA sequencing (scRNAseq) and flow cytometry indicated the expansion of effector T-cells and, perhaps more importantly, a subset of macrophages in Jag2KO tumors. The expanded subset of macrophages in Jag2KO tumors displayed a further differentiated phenotype, exhibited increased antigen presentation, and consequently promoted lymphocyte-driven anti-tumor immunity. Moreover, transfer of macrophages from Jag2KO tumors into wild-type tumors reduced growth in immunocompetent mice but not in Rag1KO mice. Prevention of macrophage expansion using anti-CSF1 receptor blockade, treatment with clodronate, or ablation of Ccr2 rescued growth in Jag2KO tumors and blocked intra-tumoral accumulation of T-cells. Mechanistically, Notch1/2 signaling on macrophages promoted Irf4-dependent immunostimulatory reprogramming. Subsequent knockout of Irf4 in myeloid populations abrogated the development of this subset of macrophages and reinstated growth of Jag2KO tumors.

Conclusions Our findings demonstrate an underlying, pivotal role for Jagged2 in lung tumor cells in directing immune evasion. Our experiments provide mechanistic opportunities to reprogram immunosuppressive myelopoiesis in vivo for the benefit of cancer immunotherapy.