

High Expression of Ligands for Chemokine Receptor CXCR2 in Alveolar Epithelial Neoplasia Induced by Oncogenic Kras

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Abstract

CXCL8, a ligand for the chemokine receptor CXCR2, was recently reported to be a transcriptional target of Ras signaling, but its role in Ras-induced tumorigenesis has not been fully defined. Here we investigated the role of KC and MIP-2, the murine homologues of CXCL8, in KrasLA1 mice, which develop lung adenocarcinoma owing to somatic activation of the KRAS oncogene. We first investigated biologic evidence of CXCR2 ligands in KrasLA1 mice. Malignant progression of normal alveolar epithelial cells to adenocarcinoma in KrasLA1 mice was associated with enhanced intralumenal vascularity and neutrophilic inflammation, which are hallmarks of chemoattraction by CXCR2 ligands. In vitro migration assays, supernatants of bronchoalveolar lavage samples from KrasLA1 mice chemoattracted murine endothelial cells, alveolar inflammatory cells, and the LKR-13 lung adenocarcinoma cell line derived from KrasLA1 mice, an effect that was abrogated by pre-treatment of the cells with a CXCR2 neutralizing antibody. CXCR2 and its ligands were highly expressed in LKR-13 cells and premalignant alveolar lesions in KrasLA1 mice. Treatment of KrasLA1 mice with a CXCR2-neutralizing antibody inhibited the progression of premalignant alveolar lesions and induced apoptosis of vascular endothelial cells within alveolar lesions. Whereas the proliferation of LKR-13 cells in vitro was resistant to treatment with the antibody, LKR-13 cells established as syngeneic tumors were sensitive, supporting a role for the tumor microenvironment in the activity of CXCR2. Thus, high expression of CXCR2 ligands may contribute to the expansion of early alveolar neoplastic lesions induced by oncogenic KRAS.

Figure 1. Malignant progression in KrasLA1 mice is associated with infiltration of neutrophils and endothelial cells.

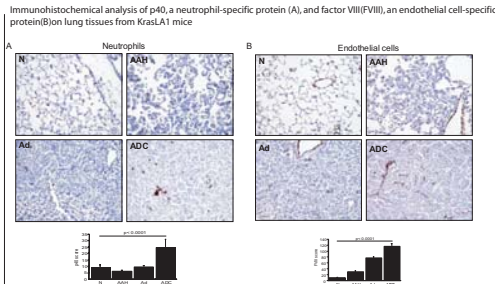


Figure 2. CXCR2 neutralization inhibits the migration of epithelial cells, endothelial cells, and inflammatory cells in vitro.

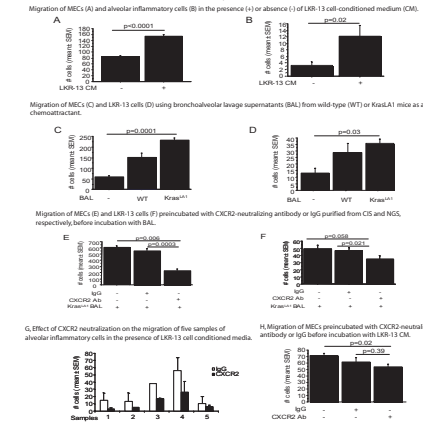


Figure 3. Alveolar lesions in KrasLA1 mice have high expression of KC and MIP-2, murine functional homologues of CXCL8.

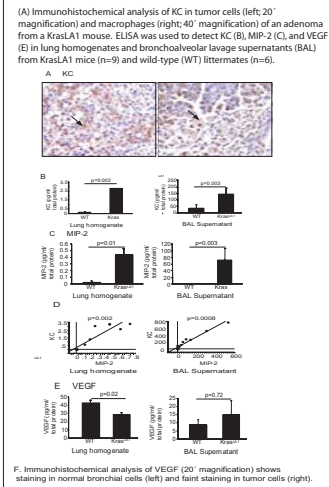


Figure 4. Mutant KRAS increases CXCL8 expression in human bronchial epithelial cells (HBECS).

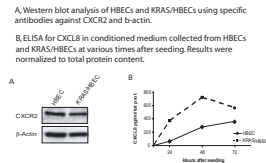


Figure 5. CXCR2 neutralization inhibits malignant progression in KrasLA1 mice.

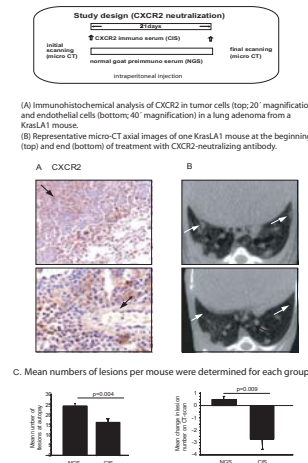


Figure 6. CXCR2 inhibition induces apoptosis of vascular endothelial cells in alveolar lesions.

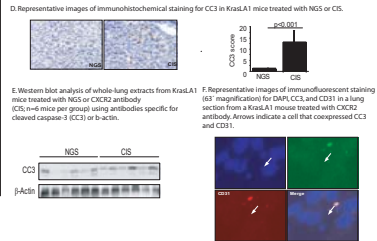
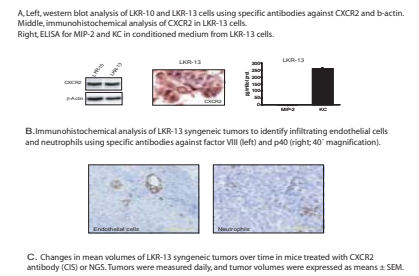


Figure 7. CXCR2 inhibition shrinks LKR-13 syngeneic tumors.



Summary

Malignant progression in KrasLA1 mouse was associated with enhanced neutrophil inflammation and neovascularization and high expression of CXCR2 ligands.

Alveolar epithelial cells transformed by oncogenic KRAS have high expression of CXCR2 ligands, which recruit inflammatory and endothelial cells, creating a milieu that promotes the progression of early neoplasia.

CXCR2 neutralization reduced the numbers of early lesions and induced apoptosis of vascular endothelial cells.

These findings suggest that high expression of CXCR2 ligands and the accompanying neovascularization are required for early alveolar neoplasia.

Our finding that oncogenic KRAS triggers tumor-host interactions that are crucial to the promotion of tumor growth provides a rationale to target CXCR2 or its ligand, CXCL8, in the treatment of oncogenic KRAS-induced lung malignancy.

Animal model

K-ras^{LA1} mice, a model in which oncogenic K-ras is activated through somatic recombination and recapitulates features of lung adenocarcinoma progression.

Mice	Mean number of cells/4 ± SEM (% ± SEM of total cells)			
	Total Cells	Alveolar macrophages	Neutrophils	Lymphocytes
Wild type (n=3)	106.6 ± 52.3 (94.3 ± 1.2)	93.3 ± 47.7 (2.7 ± 1.6)	1.7 ± 1.3 (2.2 ± 2.1)	5.5 ± 5.3 (4.6 ± 1.4)
Kras ^{LA1} (n=4)	180.0 ± 58.4 (79.6 ± 2.6)	136.4 ± 41.7 (18.3 ± 1.4)	25.7 ± 9.3 (0.03)	9.3 ± 4.2 (0.7)
P value	0.7	0.4	0.03	0.28

Statistical analysis was performed using the Mann-Whitney nonparametric test.