Loss of Smad4 From Colorectal Cancer Cells Promotes CCL15 Expression to Recruit CCR1+ Myeloid Cells and Facilitate Liver Metastasis
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Yoshiro Itatani1,2, Kenji Kawada2*, Teruaki Fujishita1,4, Fumihiko Kakizaki1, Hideyo Hirai3, Takuya Matsumoto2, Masayoshi Iwamoto2, Susumu Inamoto2, Etsuro Hatano2, Suguru Hasegawa2, Taira Maekawa3, Shinji Uemoto2, Yoshiharu Sakai2 and Makoto Mark Taketo1*

Departments of Pharmacology1, Surgery2, and Transfusion Medicine & Cell Therapy3, Graduate School of Medicine, Kyoto University, Kyoto, Japan. Division of Molecular Pathology4, Aichi Cancer Center, Aichi, Japan.

ABSTRACT

BACKGROUND & AIMS: Loss of the tumor suppressor SMAD4 is correlated with progression of colorectal cancer (CRC). In mice, colon tumors that express CCL9 recruit CCR1+ myeloid cells, which facilitate tumor invasion and metastasis by secreting matrix metalloproteinase (MMP) 9 (1)(2).

METHODS: We used human CRC cell lines to investigate the ability of SMAD4 to regulate expression of CCL15, a human ortholog of mouse CCL9. We employed immunohistochemistry to compare levels of CCL15 and other proteins in 141 samples of human liver metastases.

RESULTS: In human CRC cell lines, knockdown of SMAD4 increased CCL15 expression, whereas overexpression of SMAD4 decreased it. SMAD4 bound directly to the promoter region of CCL15 gene to negatively regulate its expression; transforming growth factor-β (TGF-β) enhanced binding of SMAD4 to the CCL15 promoter and transcriptional repression. In livers of nude mice, SMAD4-deficient human CRC cells upregulated CCL15 to recruit CCR1+ cells
and promote the metastatic colonization. Analysis of clinical specimens showed a strong inverse correlation between levels of CCL15 and SMAD4; metastases that expressed CCL15 contained 3-fold more CCR1+ cells than those without CCL15. Patients with CCL15-expressing metastases showed significantly shorter disease-free survival (DFS) than those with CCL15-negative metastases. CCR1+ cells in the metastases expressed the myeloid cell markers CD11b and myeloperoxidase, and also MMP9.

**CONCLUSIONS:** In human CRC cells, loss of SMAD4 leads to upregulation of CCL15 expression. Human liver metastases with CCL15 expression contain higher numbers CCR1+ cells and these patients are associated with shorter DFS. Therapeutics that block CCL15 recruitment of CCR1+ cells may prevent metastasis of CRC to liver.

**REFERENCES**
