Letter to the Editor

Clinical Cancer Research

CAF-Derived IL6 and GM-CSF Cooperate to Induce M2-like TAMs–Response

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We read with great interest the letter by Iorio and colleagues entitled "Cancer-stimulated CAFs enhance monocyte differentiation and pro-tumoral TAM activation" in this issue of *Clinical Cancer Research*, which provides valuable context and additional discussion of our recent article in this journal. In their letter, the authors reevaluated their recent work on the role of BAG3, a regulator of chaperone-assisted selective autophagy, which inhibits tumorigenesis and metastasis in pancreatic ductal adenocarcinoma (1, 2). They report reductions in cancer-associated fibroblast (CAF) activation and lowered collagen deposition in both syngeneic and patient-derived xenograft models of pancreatic cancer treated with BAG3 antibodies.

In light of these new findings, we have also assessed CAF activation in our orthotopic syngeneic colon carcinoma model. We previously demonstrated that dual blockade with anti-IL6 and anti-granulocyte macrophage colony-stimulating factor (GM-CSF) decreased tumor growth, metastasis, and the presence of M2-like tumor-associated macrophages (TAM; ref. 3). Using similar analytical techniques as Iorio and colleagues we can report that CAF activation is also decreased by anti-IL6 and anti-GM-CSF treatment. Expression of the CAF activation marker, α-smooth muscle actin (α-SMA), was reduced in the colon tumor tissue after dual blockade (Fig. 1A and B). In addition, collagen deposition in the tumors was decreased (Fig. 1C and D). These results from our colon carcinoma model are in accordance with the downregulation of CAF activation reported by Iorio and colleagues in their model of pancreatic ductal adenocarcinoma. As described by Iorio and colleagues, TGFβ is a TAM-secreted cytokine involved in CAF activation. We also observed that TGFβ can activate normal fibroblasts into the CAF phenotype, whereas IL10, a major component of the M2-like TAMs secretome, had no significant effect (Fig. 1E). In addition, TGFβ expression can be induced in bone marrow–derived monocytes by treatment with IL6 or GM-CSF (Fig. 1F). These additional data compliment the findings of Iorio and colleagues and suggest that blockade strategies targeting TAM activation by CAF (such as anti-BAG3, dual anti-IL6/anti-GM-CSF) or the reciprocal CAF activation by TAMs (via TGFβ secretion) can perturb this positive feedback loop within the tumor ecologic niche (Fig. 1G). Particularly, our finding that IL6 and GM-CSF induces TGFβ expression in differentiating monocytes can be significant, because TGFβ-mediated programming of

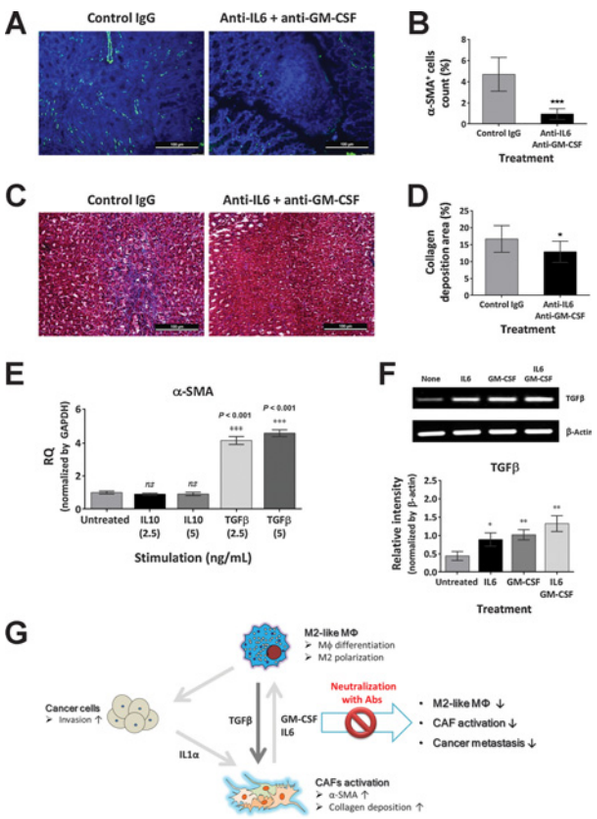

Figure 1.

A, Representative images of IHC for α-SMA in our orthotopic syngeneic colon carcinoma mouse model treated with control IgG or anti-IL6 and anti-GM-CSF, as previously described (3). $n = 5$; error bars = SD. **B,** Quantification of α-SMA–stained cells; ***, $P < 0.001$ compared with control IgG treatment, as quantified by ImageJ software (ver.1.52). **C,** Representative Masson trichrome staining for collagen deposition in the tumor-bearing tissues (blue color). **D,** Quantification of collagen staining; $n = 6$; error bars = SD; *, $P < 0.05$ compared with control IgG treatment. **E,** qPCR analysis of α-SMA expression in murine primary fibroblasts treated with TGFβ or IL10 for 72 hours. $n = 3$; error bars = SD. **F,** RT-PCR analysis of TGFβ induction in bone marrow–derived monocytes treated with 12.5 ng/mL GM-CSF and/or 12.5 ng/mL IL6 treatment for 72 hours. Representative gel image is shown. $n = 3$; error bars = SD; *, $P < 0.05$; **, $P < 0.01$ compared with untreated. **G,** Schematic diagram of the reciprocal relationship between CAF-induced monocyte differentiation into M2-like TAMs (via IL6 and GM-CSF) and fibroblast activation into CAF (via TGFβ signaling).

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the stroma initiates metastasis in colorectal cancer (4). Because of the importance of CAF in protumorigenic signaling, immunologic responses, and drug resistance (5, 6), current studies in our laboratory are focused on further deciphering the broad implications of CAF–TAM interactions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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