Letter to the Editor

Clinical Cancer Research

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

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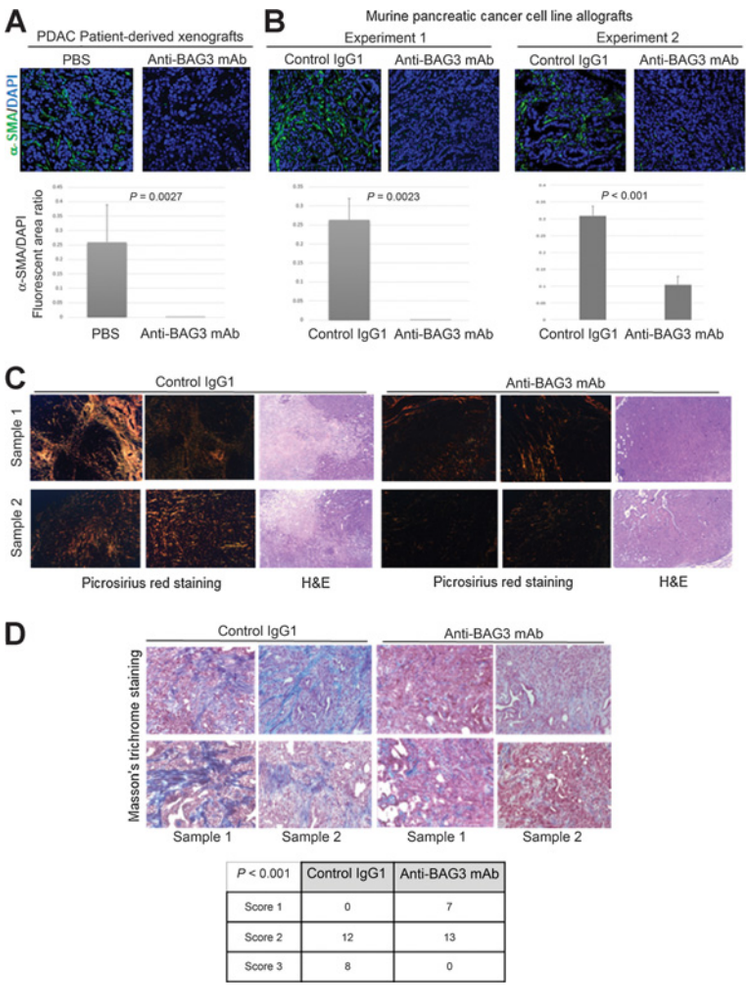
In a recent article, Cho and colleagues highlight the connection between cancer-associated fibroblasts (CAF) and tumor-associated macrophages (TAM) in tumor progression. They show that cancer cell–activated CAFs induced monocyte differentiation into M2-like TAMs via IL6 and GM-CSF release, and that CAFs cotransplantation with tumor cells enhances TAM infiltration and the metastatic process in a syngeneic colon carcinoma mouse model (1).

This and other recent evidence on CAFs/TAMs relationship (1–3) help to better understand the complex functioning of tumor



P < 0.001	Control IgG1	Anti-BAG3 mAb
Score 1	0	7
Score 2	12	13
Score 3	8	0

Figure 1.

A and **B,** Immunofluorescence analysis of CAF α-SMA expression in tumor tissues from a PDAC patient derived xenografts (control $n = 4$, anti-BAG3 mAb $n = 4$; at least 5 analyzed fields per group; **A**), or from allografts of the murine pancreatic cancer cell line mt4-2D (exp.1: control $n = 3$, anti-BAG3 mAb $n = 3$, at least 5 analyzed fields per group; exp.2: control $n = 3$, anti-BAG3 mAb $n = 3$, at least 10 analyzed fields per group; **B**). Tumor- bearing mice were treated with anti-BAG3 antibody and compared with tumor-bearing controls injected with PBS (**A**) or a control IgG1 (**B**). Samples were analyzed using a confocal laser scanning microscope (Leica SP5, Leica Microsystems). Images were acquired in sequential scan mode by using the same acquisitions parameters when comparing anti-BAG3–treated and control materials. Relative fluorescence area of α-SMA–positive cells was calculated as ratio to DAPI staining using ImageJ software from at least three images at ×10 field magnification. Error bars, SD. *P* values in figure are calculated by Dunnett *post hoc* test versus control group. **C** and **D,** Collagen amount analysis by Picrosirius red staining (**C**) or Masson trichrome staining (**D**) in tumor tissues from the allografts described in **B**. Percentages of collagen (blue) positive areas were assessed on 2 anti-BAG3–treated and 2 control tissues. At least 10 different fields were analyzed for each sample and quantified using a semiquantitative scoring system: score 1 [low/negative: <20% positivity], score 2 [patchy/focal expression: $\geq$ 20% $\leq$ 50% positivity] or score 3 [high: diffuse expression throughout the tumor: >50% positivity]. Data are expressed as means and SE in each group. *P* value in figure is calculated by Fisher exact test for the contingency table.

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stroma. Furthermore, they can induce a rereading of many experimental results obtained with TAM-targeting drugs, to consider the eventual involvement of CAFs in the investigated mechanisms.

In this vision, we have reconsidered some results from our laboratory on the role of BAG3 protein in pancreatic ductal adenocarcinoma (PDAC). In several mice models, BAG3, released by PDAC cells, binds to TAMs, stimulating their protumor activity; a BAG3-blocking antibody impairs tumor growth and the metastatic spread (4, 5). We have now verified whether CAFs are also involved in the anti-BAG3 effect, in two of the previously studied models (4, 5): two experiments in allografts of a murine pancreatic cancer cell line in syngeneic mice; and an experiment in patient-derived PDAC xenografts in immunodeficient mice. In both models, we have detected a high downmodulation in the expression of the CAF activation marker α-SMA in anti-BAG3–treated mice compared with controls (Fig. 1A and B). We have also observed a marked reduction of collagen fibers in anti-BAG3–treated tumors (Fig. 1C and D).

Reduction of α-SMA expression and collagen deposition documents the anti-BAG3–inhibitory effect on CAF activation. Several TAM-released cytokines, including TGFβ, TNFα, IL17A, and others, are involved in CAF activation. By acting on TAMs, anti-BAG3 mAb decreases the levels of TAM-produced cytokines in tumor stroma (4). Such decrease can likely explain the observed inhibition of CAF activation, although the mechanism deserves detailed investigation.

These results are an example of how CAFs–TAMs connections can emerge in deepened studies of tumor stroma functioning. This consideration particularly applies to the design and study of therapeutic molecules. Indeed, limitations in the effects of some drugs that address TAMs can be due to unconsidered consequences on CAFs (and vice versa). This is the case, for example, of 2 CSF1 agonists, whose activity reduces protumorigenic TAMs but enhances PMN-MDSCs recruitment by CAF-derived chemokines (2). Innovative strategies for tumor therapy have to pay due attention to the circuits that involve CAFs and TAMs in a complex interplay.

Disclosure of Potential Conflicts of Interest

M. De Marco, A. Basile, L. Marzullo, A. Rosati, V. De Laurenzi, and M.C. Turco hold ownership interest (including patents) in BIOUNIVERSA s.r.l. that provided anti-BAG3 antibodies. No potential conflicts of interest were disclosed by the other authors.

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