LETTER

# Inhibitory signaling through signal regulatory protein-α is not sufficient to explain the antitumor activities of CD47 antibodies

CD47 is a receptor for the secreted protein thrombospondin-1 and a counterreceptor for two members of the signal regulatory protein (SIRP) family. These interactions play important roles in hemostasis, cardiovascular pathophysiology, ischemic injuries, inflammation, radiation injuries, and cancer. Correspondingly, antibodies that block thrombospondin-1 or SIRPα binding and antisense oligonucleotide analogs that suppress CD47 expression have shown efficacy in treating rodent and porcine models of these diseases.

In this context, some conclusions recently presented by Willingham et al. (1) are inconsistent with existing knowledge about CD47. Most of the therapeutic data presented rely on administration of the anti-human CD47 antibody B6H12 to mice bearing human xenograft tumors. The authors interpreted reduced growth of these tumors as evidence that tumor cell CD47 inhibits their clearance by macrophages via inhibitory signaling through the cytoplasmic domain of SIRPα expressed on these macrophages. Similar conclusions were drawn from xenograft studies previously and subsequently published by the same group, but a recent publication questions this logic (2). Zhao et al. showed that B6H12 mediates antibody-dependent tumor cell killing, contradicting evidence to the contrary by Willingham. Furthermore, if blocking inhibitory signaling through SIRPα on macrophages was sufficient to activate innate tumor immunity, deletion of the cytoplasmic tail of SIRPα in host mice should decrease tumor growth. However, Zhao observed no such response. Notably, Zhao and others have reported that CD47 antibodies enhance nonphagocytic tumor cell killing by neutrophils and NK cells. These results suggest that the antitumor activity of B6H12 in the present xenograft models results from opsonization of the human tumors by this antibody and other undefined mechanisms in addition to inhibiting macrophage SIRPα binding to tumor cell CD47.

Other data in Willingham et al. (1) further indicated that SIRPα signaling is not involved in the antitumor activity of CD47 antibodies. Movie S2 in Willingham et al. (1) showed that B6H12 enhances human tumor cell phagocytosis by macrophages that express a form of murine SIRPα that cannot bind human CD47. Figure 6 in Willingham et al. (1) presented a syngeneic tumor model using MT1A2 mouse breast cancer cells. Injecting the tumor-bearing mice with the CD47 antibody miap301, which blocks murine CD47 interactions with SIRPα (3) and functionally blocks CD47 in vivo in murine soft tissue ischemia and skin graft models, yielded no significant inhibition of tumor growth relative to an IgG control. In contrast, the CD47 antibody miap410, which does not block SIRPα interaction (4), dramatically inhibited tumor growth. Therefore, targeting of CD47 in a syngeneic tumor model can inhibit tumor growth, as reported previously in conjunction with irradiation using antisense suppression of CD47 in syngeneic melanoma and squamous carcinoma models (5), but such antitumor activity is clearly independent of CD47–SIRPα interactions.

We have much yet to learn about the signaling functions of CD47 and its roles in cancer and other diseases. The correlations between tumor CD47 expression and patient survival presented by Willingham et al. are important and provide further incentive to define these pathways and develop therapeutics to target CD47, but mechanistic data must be carefully evaluated in the context of existing knowledge to achieve these goals.

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