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Article

Autocrine Effects of Tumor-Derived Complement

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SUMMARY

We describe a role for the complement system in enhancing cancer growth. Cancer cells secrete complement proteins that stimulate tumor growth upon activation. Complement promotes tumor growth via a direct autocrine effect that is partially independent of tumor-infiltrating cytotoxic T cells. Activated C5aR and C3aR signal through the PI3K/AKT pathway in cancer cells, and silencing the *PI3K* or *AKT* gene in cancer cells eliminates the progrowth effects of C5aR and C3aR stimulation. In patients with ovarian or lung cancer, higher tumoral C3 or C5aR mRNA levels were associated with decreased overall survival. These data identify a role for tumor-derived complement proteins in promoting tumor growth, and they therefore have substantial clinical and therapeutic implications.

INTRODUCTION

Complement proteins in plasma are mainly synthesized in hepatocytes, but endothelial cells, white blood cells, and epithelial cells also secrete complement proteins (Peng et al., 2008; Pratt et al., 2002; Raedler et al., 2009; Strainic et al., 2008). There are three pathways to activate the complement system: the *classical*, *alternative*, and *lectin* pathways. The initial steps in complement activation pathways are different, but all of them result in deposition of C3 degradation products on target surfaces and generation of anaphylatoxins (C3a and C5a) and membrane attack complex (MAC; C5b-9). Complement activation on the surface of pathogens in the blood stream helps to eradicate them from circulation. In extravascular tissues, complement proteins also participate in cell-to-cell communications and are involved in organ regeneration, angiogenesis, epithelial-mesenchymal transition, and cell migration. Despite the presence of an extensive range of responses to complement activation in normal tissues, the effect of complement activation in neoplastic tissue is not well understood. Here, we have identified a role for complement, whereby tumor-derived C3 enhances tumor growth via an autocrine pathway.

RESULTS

Biological Effects of Tumor-Derived C3 in Ovarian Cancer Cells

To address the question of whether host-derived complement proteins affect tumor growth, we first used a syngeneic mouse model of ovarian cancer in which ID8-VEGF murine ovarian cancer cells were injected into the peritoneal cavity of wild-type (WT) or C3-deficient ($C3^{-/-}$) B6 mice. After 6 weeks, there was no difference in the growth of implanted tumors between the two groups of mice (average tumor weight of 0.5 g in WT versus 0.53 g in $C3^{-/-}$ mice, n = 7 in each group; p = 0.84, t test) (Figure 1A). Surprisingly, C3 immunostaining of tumor specimens showed comparable C3 deposition in tumors resected from WT and $C3^{-/-}$ mice (Figure 1B). Because $C3^{-/-}$ mice do not produce C3, we investigated whether C3 was being produced by cancer cells. We examined a large panel of ovarian cancer cell lines for C3 mRNA levels using quantitative real-time PCR. C3 mRNA was present in all murine and in 30% of human (h) ovarian cancer cell lines (Figure 1C). To determine whether C3 is secreted by cancer cells, we measured C3 concentration in cell culture media of ovarian cancer cell lines. Supernatant of serum-free media incubated for 72 hr with normal murine ovarian endothelial cells (MOEC), murine (ID8, ID8-VEGF, and IG10), or human (SKOV3) ovarian cancer cell lines was collected and used to determine the concentration of C3 by ELISA. Ovarian cancer cells secrete much more C3 into cell culture media than control MOECs (70 ng/ml for MOECs, 4,504 ng/ml for SKOV3ip1, 332 ng/ml for ID8, 2,411 ng/ml for ID8-VEGF, and 1,329 ng/ml for IG10, Figure S1A). To determine the effects of C3 secreted by the cancer cells on the growth of implanted ovarian tumors, we reduced production of C3 in cancer cells by small interfering RNA for C3 (C3 siRNA). We used hC3 siRNAs on SKOV3ip1 ovarian cancer cells that reduced

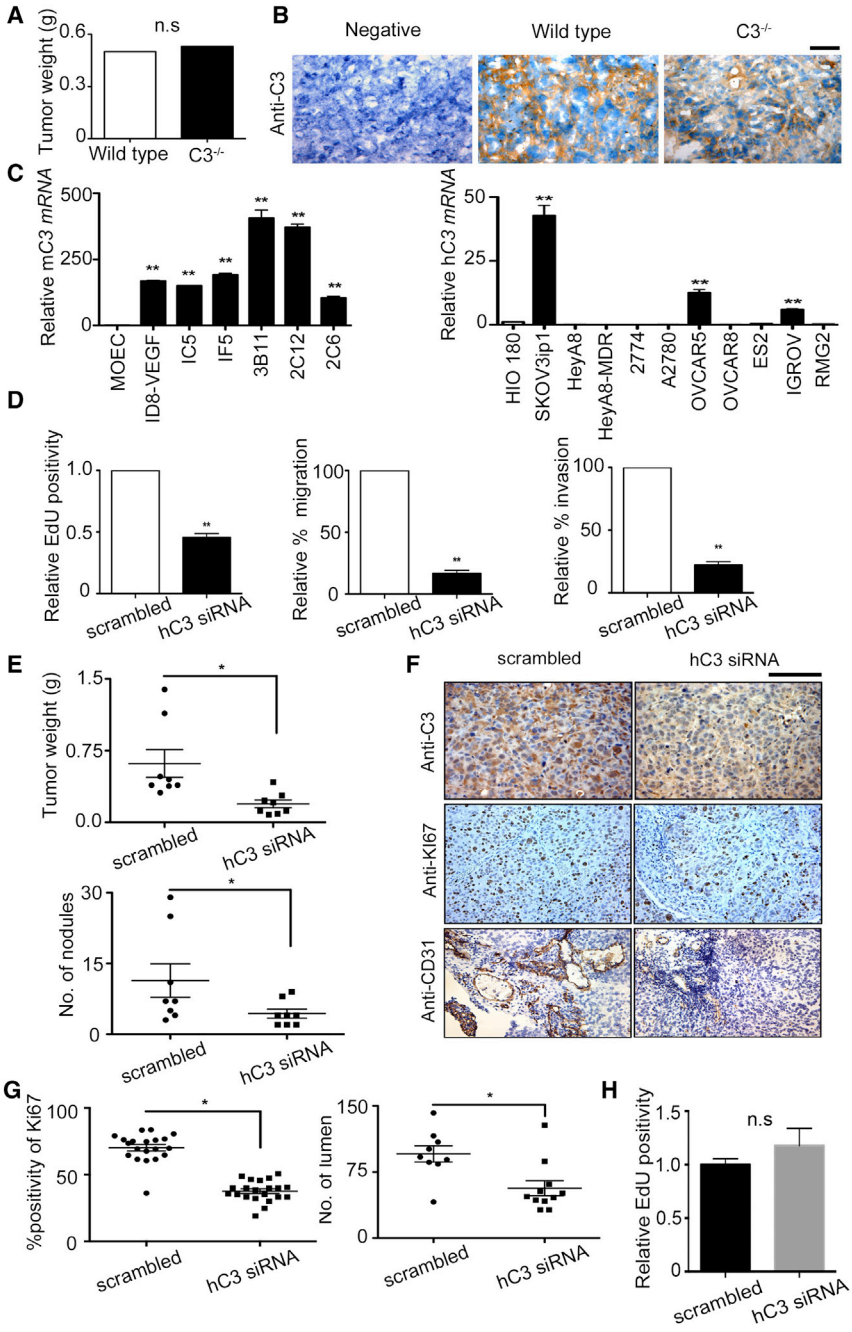

Figure 1. Ovarian Cancer Cells Secrete Complement Proteins, which Enhance Tumor Growth

(A) We measured total tumor weight in an orthotopic murine model of ovarian cancer induced by ID8-VEGF murine ovarian cancer cells in *C3*$^{-/-}$ and WT control mice, both in C57BL/6 background. n.s, not significant.

(B) Immunostaining of tumors induced by ID8-VEGF in WT and *C3*$^{-/-}$ mice, using anti-C3 antibody compared to negative control stain (secondary antibody alone). Scale bar length is 100 μm.

(C) Quantitative real-time PCR for C3 mRNA on RNA isolated from murine and human ovarian cancer cell lines. Expression of C3 mRNA in cancer cell lines was compared to that in MOECs and normal human ovarian surface epithelial cell lines (HIO 180) (n = 3; $^{**}p \leq 0.01$, t test).

(D) C3 gene knockdown in SKOV3ip1 human ovarian cancer cells using C3 siRNA, reduced proliferation, migration, and invasion of these cells in vitro. Results of three independent experiments (each of them in triplicate) are summarized as bar graphs ($^{**}p \leq 0.01$, t test).

(E) C3 gene knockdown in SKOV3ip1-induced tumors by intraperitoneal injection of hC3 siRNA into tumor-bearing NU/NU mice reduced total weight ($^{*}p = 0.017$) and number of tumor nodules ($^{*}p = 0.05$).

(F) Representative immunostaining for C3, Ki67, and CD31 in tumors resected from hC3 siRNA-injected and scrambled siRNA-injected mice. Scale bar, 100 μm.

(G) The proliferation index in resected tumors was quantified as the percentage of Ki67 positivity shown in dot plots (39% in C3 siRNA versus 74% in scrambled siRNA, n = 5 mice in each group; $^{*}p = 0.05$, t test). The number of blood vessels in resected tumors was quantified by counting the number of CD31+ lumen structures in five high-power fields (HPFs) per section and in five sections per tumor nodule and in five mice per group. Average number of CD31+ lumens per HPF is shown as dot plots (22/HPF in C3 siRNA versus 42/HPF in scrambled siRNA; $^{*}p = 0.05$, t test).

(H) We investigated the effect of complement on proliferation of endothelial cells by measuring the proliferation rate of RF24 endothelial cells after transfection with C3 siRNA. C3 knockdown did not reduce the proliferation rate in RF24 endothelial cells (n = 3; p = 0.07, t test).

C3 mRNA and protein level by >99% (Figures S1B and S1C). Next, we examined whether C3 knockdown would have direct effects on tumor cell proliferation, migration, and invasion (Figure 1D). C3 silencing in SKOV3ip1 reduced the proliferation rate at the 48 hr time point by 55%, migration at 6 hr by 84%, and invasive potential at 24 hr by 78% compared to cancer cells transfected with scrambled siRNA. The effects of C3 silencing on migration and invasion were measured using short-term assays and were likely to be independent of the effects on proliferation.

C3 Silencing in Ovarian Cancer Cells Reduces Tumor Growth In Vivo

To evaluate the in vivo effects of C3 knockdown on tumor growth, we used hC3 siRNA in tumor-bearing mice. We selected the most efficient hC3 siRNA in vitro (Figure S1B), conjugated it with 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) nano-liposomes, and injected it into the peritoneal cavity of SKOV3ip1 tumor-bearing mice twice per week for 4 weeks starting 1 week after injection of cancer cells. Control mice underwent the same procedures except that they received scrambled