Am J Cancer Res 2017;7(8):1714-1723
www.ajcr.us /ISSN:2156-6976/ajcr0060150

Original Article

A human recombinant IL-7/HGFβ hybrid cytokine enhances antitumor immunity in mice

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Received June 23, 2017; Accepted July 5, 2017; Epub August 1, 2017; Published August 15, 2017

Abstract: We purified a hybrid cytokine that contains interleukin-7 (IL-7) and the beta-chain of hepatocyte growth factor (HGFβ) from a unique long-term murine bone marrow culture system. We have cloned and expressed the human form of IL-7/HGFβ in which the IL-7 and HGFβ genes are connected by a flexible linker to produce a single-chain recombinant human IL-7/HGFβ protein (hrIL-7/HGFβ). To determine whether hrIL-7/HGFβ has antitumor activity, we injected this hybrid cytokine into melanoma and colon cancer animal models, and then assessed the local tumor growth and tumor metastasis. We show here that *in vivo* administration of hrIL-7/HGFβ significantly inhibited the growth and metastasis of malignant melanoma and colon cancer in mice. The antitumor activity was involved in a marked increase in the number of tumor-infiltrating $CD4^+$ and $CD8^+$ T cells and activated dendritic cells. The immunological mechanism by which hrIL-7/HGFβ inhibits tumor growth was confirmed by its inability to inhibit tumor growth *in vitro* and in immunodeficient mice. Furthermore, immune cells from hrIL-7/HGFβ-treated cancer-bearing mice can be adoptively transferred into naïve mice to resist same tumor cell challenge. Therefore, hrIL-7/HGFβ has potential applications in the treatment of cancer patients.

Keywords: Tumor immunotherapy, cytokines, mice, T cells, dendritic cells, metastasis

Introduction

We purified a novel hybrid cytokine consisting of IL-7 and HGFβ from a unique long-term bone marrow culture system for the generation of lymphocytes [1, 2]. We have cloned and expressed a single-chain mouse IL-7/HGFβ in which the IL-7 and HGFβ genes are connected by a flexible linker to produce rIL-7/HGFβ protein [3]. We have reported that *in vivo* administration of the murine rIL-7/HGFβ hybrid cytokine inhibits the growth of local tumors and metastatic tumors in mouse models of colon cancer and melanoma [4].

Since it has been shown that murine rIL-7 does not efficiently stimulate human lymphoid cells [5], it was necessary to develop a human form of the hybrid cytokine for potential clinical utility. Therefore, we cloned and expressed the human (h) rIL-7/HGFβ protein containing hIL-7 and hHGFβ [6]. Since hrIL-7 and hrHGF are active in the mouse [5], we were able to conduct preclinical studies of antitumor effects of the human hybrid cytokine in mouse models. We show here that *in vivo* administration of hrIL-7/HGFβ also significantly inhibited the local tumor growth and metastases of melanoma and colon cancer. The antitumor mechanism is immunological, involving the expansion and activation of tumor infiltrating lymphocytes (TILs) and dendritic cells (DCs). We have also expanded our studies to show that hrIL-7/HGFβ-induced immune responses were tumor-specific and could be adoptively transferred to naïve mice to enable these mice to resist same tumor cell challenge.

Materials and methods

Cell lines and mice

Murine CT-26 colon cancer cells and B16-F10 melanoma cells were obtained from the National Cancer Institute (Frederick, MD) and the American Type Culture Collection. BALB/c, C57BL/6 and NU/J nude mice were purchased from Jackson Laboratory. Murine breast cancer

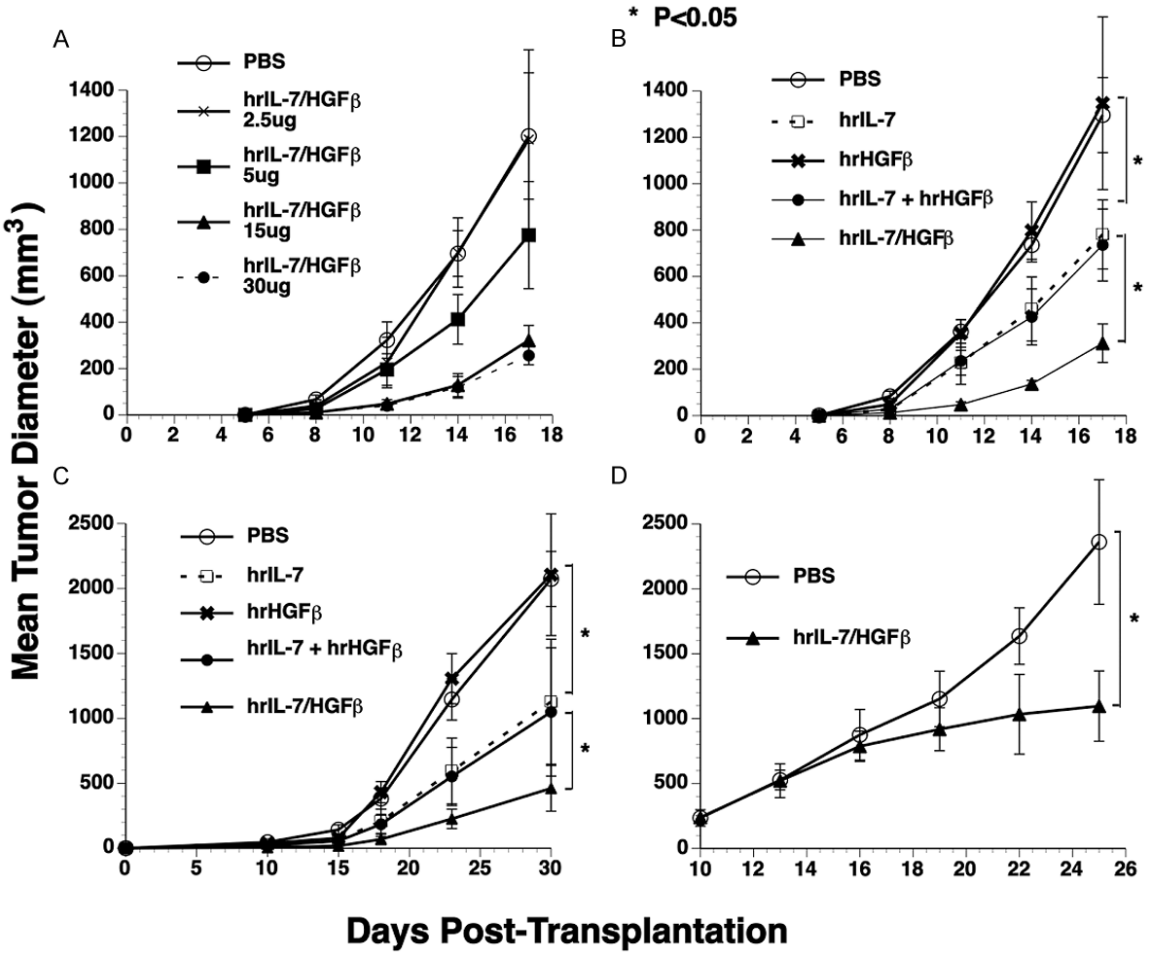

Figure 1. *In vivo* administration of hrIL-7/HGFβ inhibits local growth of melanoma and colon cancer. (A, B) C57BL/6 mice were injected s.c. with 1×10^5 B16F10 melanoma cells followed by intratumoral (i.t.) injections with (A) hrIL-7/HGFβ (2.5, 5, 15, or 30 μg) or PBS, (B) equimolar doses of hrIL-7/HGFβ (15 μg), hrIL-7 (5 μg) and/or hrHGFβ (10 μg) or PBS, at 2-day intervals from days 2-14 after tumor inoculation. (C) BALB/c mice were injected s.c. with 2×10^5 CT-26 colon cancer cells, followed by i.t. injections with equimolar doses of hrIL-7/HGFβ (15 μg), hrIL-7 (5 μg) and/or hrHGFβ (10 μg) or PBS, at 2-day intervals from days 2-24. (D) BALB/c mice were injected s.c. with 2×10^5 CT-26 colon cancer cells, and i.t. with hrIL-7/HGFβ (15 μg) or PBS at 2-day intervals from days 10-24. Tumors were measured twice weekly. The mean tumor volume (mm^3) ± S.D. at the indicated time points are shown. The data are representative of 2 independent experiments with 4-6 mice per group with similar results.

66.1 cell line was kindly provided by Drs. A. M. Fulton and N. Kundu (University of Maryland). Mice were used according to protocols approved by the Institutional Animal Care and Use Committee of the University of Connecticut and were conducted in accordance with NIH guidelines. hrIL-7/HGFβ, hrIL-7 and hrHGFβ were cloned, expressed, and purified as we have described [6].

Evaluation of local tumor growth and pulmonary metastasis

Cancer cells in the exponential growth phase were harvested and washed in PBS before *in vivo* injection. To induce localized tumors, 2×10^5 CT-26 colon cancer cells, or 1×10^5 B16F10 melanoma cells were injected subcutaneously (s.c.) into the flank of syngeneic BALB/c, or C57BL/6 mice, respectively [4]. The indicated doses of hrIL-7/HGFβ, hrIL-7 and/or hrHGFβ (or PBS) were then injected into the tumor injection site at 2-day intervals over the indicated time period. Tumor size (volume) was determined twice weekly by caliper measurements of the shortest (A) and longest (B) diameter, using the formula $V = (A^2B)/2$.

To induce pulmonary metastases, 2×10^5 CT-26 or B16F10 cancer cells were injected into the

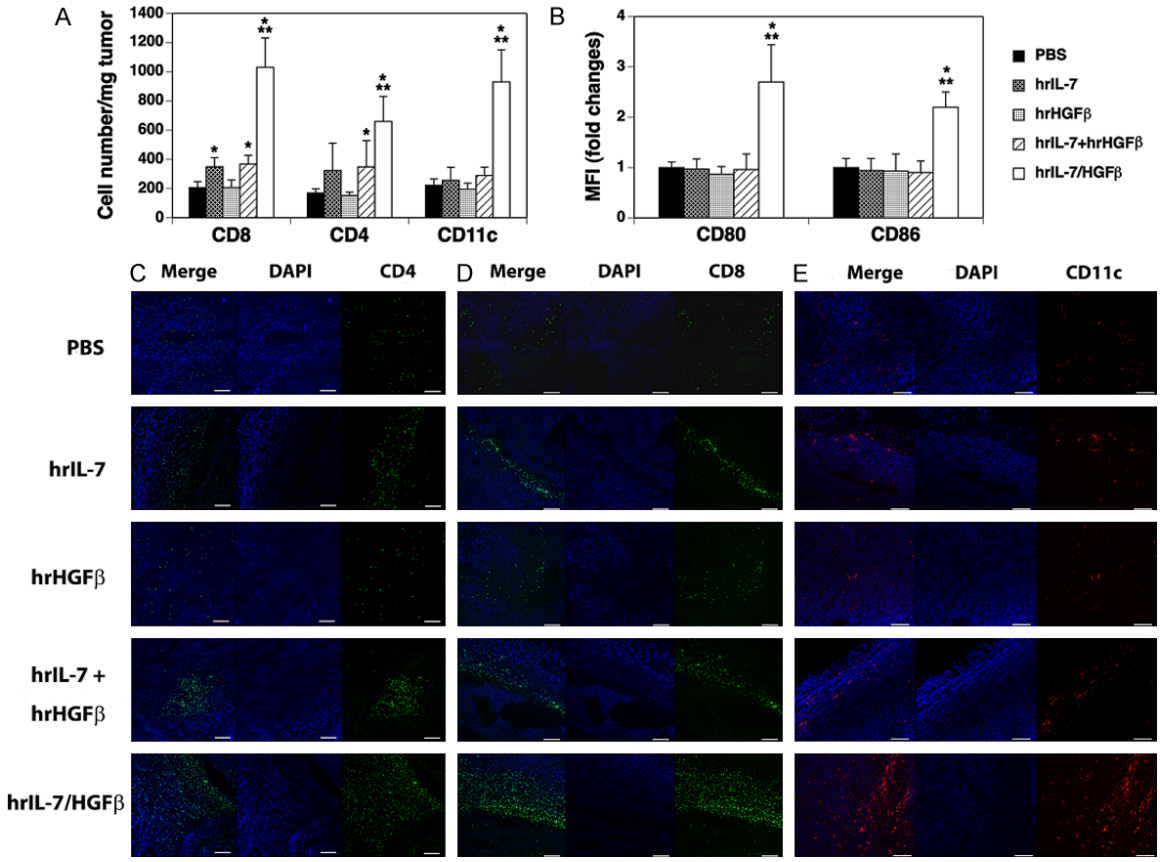

Figure 2. hrIL-7/HGFβ increases the infiltration of T cells and activated DCs in tumors. C57BL/6 mice were injected s.c. with B16F10 melanoma cells and treated with equimolar doses of hrIL-7/HGFβ (15 μg), hrIL-7 (5 μg) and/or hrHGFβ (10 μg) or PBS as in **Figure 1B**. Seventeen days after tumor inoculation, the mice were euthanized and the tumors were removed. Single-cell suspensions from the tumors were analyzed by flow cytometry for (A) $CD4^+$ and $CD8^+$ T cells, and $CD11c^+$ DCs; and (B) the expression levels of CD80 and CD86 on the $CD11c^+$ DCs. Data represent (A) mean numbers ± SD of positive cells per mg tumor tissue from 4 to 6 mice per group; and (B) relative mean ± SD fluorescence intensity (MFI) of CD80 and CD86 on DCs in cytokine or PBS-treated tumors. *P<0.05 compared with PBS-treated group; **P<0.05 as compared with the hrIL-7 and/or hrHGFβ-treated groups. (C-E) Tumor sections were analyzed for presence of (C) $CD4^+$ T cells, (D) $CD8^+$ T cells and (E) $CD11c^+$ DCs by immunofluorescence. DAPI (blue), $CD4^+$ or $CD8^+$ T cells (green) and $CD11c^+$ DCs (red). Scale bar = 100 μm. Representative tumor sections are shown.

tail vein of syngeneic mice, and hrIL-7/HGFβ or PBS were injected intravenously (i.v.) at 2-day intervals from days 2-18. The animals were euthanized at the indicated times after tumor inoculation. Metastatic tumor nodules in the subpleural regions of the lungs were counted under a dissecting microscope.

Evaluation of TILs and DCs

At the indicated time points, the s.c. tumors were excised, weighed, minced into small fragments, and digested in 1 mg/ml collagenase IV (Sigma, St. Louis, MO) and 0.1 mg/ml DNase (Sigma, St. Louis, MO) at 37°C for 1 hour. The dissociated cells were then prepared for phenotypic analysis of immune cells by flow cytometry.

Flow cytometry

Single-cell suspensions from tumors, and draining or non-draining lymph nodes were stained with the following fluorochrome-conjugated antibodies: CD4, CD8, CD11c, CD80, and CD86 (BioLegend or BD Biosciences, San Diego, CA). The samples were analyzed on a FACSCalibur flow cytometer (Becton and Dickinson). Data analysis was done using FlowJo software (Ashland, OR).

Immunofluorescence

Frozen sections of tumor tissue were prepared as described [7]. The sections were stained with the following fluorochrome-conjugated antibodies: CD4, CD8, and CD11c (BioLegend,

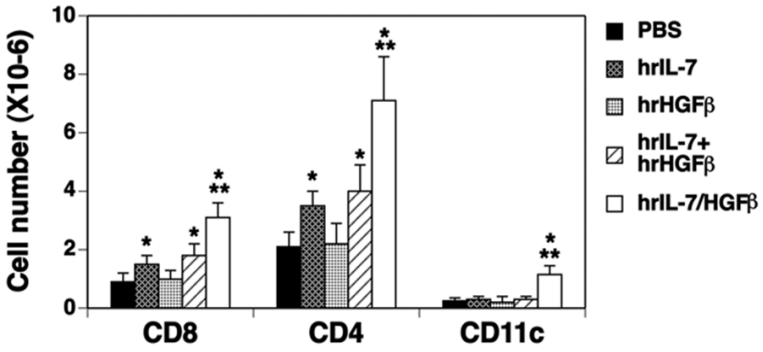

Figure 3. hrIL-7/HGFβ treatment increases the numbers of $CD4^+$ and $CD8^+$ T cells, and $CD11c^+$ DCs in the DLNs of melanoma-bearing mice. Single-cell suspensions of DLNs from the cytokine-treated tumors as in **Figure 1B** were analyzed for the numbers of $CD4^+$ and $CD8^+$ T cells, and $CD11c^+$ DCs. Data represent mean numbers of positive cells ± SD from 4 to 6 mice per group. *P<0.05 as compared with the PBS-treated group; **P<0.05 as compared with the hrIL-7 and/or hrHGFβ-treated groups.

or BD Biosciences, San Jose, CA). All of the sections were then counterstained with 4',6'-diamidino-2-phenylindole (DAPI; Sigma) and observed under a Nikon A1R Spectral Confocal microscope (Nikon, Kanagawa, Japan). A minimum of 6 sections from each tumor tissue were used to evaluate $CD4^+$, $CD8^+$, and $CD11c^+$ cells.

Statistical analysis

P-values were based on the two-sided Student's t test. A confidence level above 95% (P<0.05) was determined to be significant.

Results

hrIL-7/HGFβ inhibits local tumor growth in a dose-response manner

To determine the antitumor activity of hrIL-7/HGFβ, B16F10 melanoma cells were injected s.c. into the flank of syngeneic C57BL/6 mice. The mice were then injected at the tumor site with different doses of hrIL-7/HGFβ (2.5, 5, 15 and 30 μg/injection) at 2-day intervals from days 2-14. Control vehicle (PBS) was used as controls. Tumor volumes in each group were measured over time and compared statistically. As shown in **Figure 1A**, tumor growth was inhibited by hrIL-7/HGFβ in a dose-responsive manner, with no detectable effect seen by day 17 at the 2.5-μg level and greater than 75% inhibition at the 30-μg level.

To compare the antitumor effect of hrIL-7/HGFβ with its component cytokines, C57BL/6 mice were injected at the tumor site with optimal and equimolar amounts of hrIL-7/HGFβ (15 μg/injection), hrIL-7 (5 μg/injection) and/or hrHGFβ (10 μg/injection), according to the above schedule. As shown in **Figure 1B**, hrIL-7 alone or mixed with hrHGFβ inhibited local tumor growth by ~43% by day 17, whereas hrHGFβ alone had no effect. In contrast, the hrIL-7/HGFβ hybrid cytokine inhibited tumor cell growth by ~76% by day 17 (P<0.01). The results indicate that the single-chain hrIL-7/HGFβ hybrid cytokine has a higher antitumor activity than hrIL-7 used alone or mixed with hrHGFβ.

To determine hrIL-7/HGFβ has antitumor effect on other cancers, BALB/c mice were injected s.c. with murine CT-26 colon cancer cells. The mice were then injected at the tumor site with equimolar amounts of hrIL-7/HGFβ (15 μg/injection), hrIL-7 (5 μg/injection) and/or hrHGFβ (10 μg/injection), or PBS at 2-day intervals from days 2-24. Similarly, hrIL-7/HGFβ hybrid cytokine has a higher antitumor activity than hrIL-7 used alone or mixed with hrHGFβ in the colon cancer model (**Figure 1C**).

We then determined whether hrIL-7/HGFβ treatment can inhibit the growth of established local tumors. To this end, BALB/c mice were injected s.c with CT-26 colon cancer cells. After the tumors were visible and palpable, the mice were injected with hrIL-7/HGFβ, or PBS. A significant antitumor activity was observed in mice in which hrIL-7/HGFβ treatment was initiated after cancers had been established (**Figure 1D**).

To determine whether hrIL-7/HGFβ, hrIL-7 and/or hrHGFβ directly affect the growth of tumor cells, CT-26 and B16F10 cancer cells were cultured *in vitro* for 2 to 7 days in the presence of 20 to 150 ng/ml rIL-7/HGFβ, hrIL-7 and/or hrHGFβ (or PBS), a dose range that has been shown to stimulate the proliferation of T cells (4). The rate of tumor cell growth was not significantly different at any dose level of hrIL-7/HGFβ from those observed in in cultures containing PBS, or equimolar amounts of hrIL-7 and/or hrHGFβ (data not shown). Hence, the mechanism by which rIL-7/HGFβ inhibits the growth of

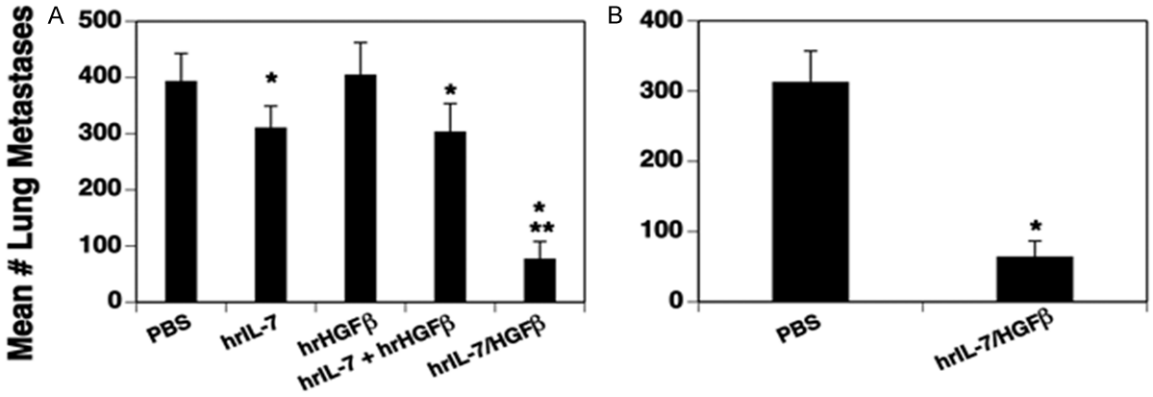

Figure 4. hrIL-7/HGFβ inhibits the formation of pulmonary metastases by melanoma and colon cancer cells. A. C57BL/6 mice were injected i.v. with 2×10^5 B16F10 cells, followed by i.v. injections with equimolar doses of hrIL-7/HGFβ (15 μg), hrIL-7 (5 μg) and/or hrHGFβ (10 μg) or PBS at 2-day intervals between days 2-18. B. BALB/c mice were injected i.v. with 2×10^5 CT-26 colon cancer cells, followed by injections with hrIL-7/HGFβ (15 μg) or PBS at 2-day intervals between days 2-18. A, B. The mice were euthanized on day 21 after tumor cell inoculation, and the total tumor nodules visible at the surface of the lungs were counted under a dissecting microscope. *P<0.05 as compared with PBS-treated group. **P<0.05 as compared with the hrIL-7 and/or hHGFβ-treated groups. The data are representative of 2 independent experiments with 4-6 mice per group with similar results.

the cancers *in vivo* would not appear to involve direct cytotoxic or cytostatic activities.

hrIL-7/HGFβ induces infiltration of T cells and activated DCs

We then determined whether hrIL-7/HGFβ had an effect on TILs and DCs in the tumors. To normalize for differences in tumor size at the time of sacrifice, we assessed the numbers of TILs per mg tumor tissue. On day 17 after tumor inoculation of B16F10 cancer cells, single-cell suspensions of tumor tissue from mice treated with equimolar amounts of hrIL-7/HGFβ, hrIL-7 and/or hrHGFβ, or PBS (see **Figure 1B**) were analyzed for $CD4^+$ and $CD8^+$ T cells, as well as $CD11c^+$ DCs by flow cytometry. As shown in **Figure 2A**, hrIL-7 alone or mixed with hrHGFβ modestly (1.7 to 2.0-fold) increased the numbers of $CD4^+$ and $CD8^+$ T cells (but not DCs) in the tumors, and hrHGFβ alone had no effect. In contrast, hrIL-7/HGFβ tremendously increased the numbers of $CD4^+$ and $CD8^+$ T cells, as well as DCs 3.9 to 5-fold above the levels in PBS-injected controls. Furthermore, like murine rIL-7/HGFβ, hrIL-7/HGFβ also enhanced the expression of CD80 and CD86 by the DCs (**Figure 2B**), indicating that the DCs had undergone activation and maturation after hrIL-7/HGFβ treatment. The increased percentage of tumor infiltrating $CD4^+$ and $CD8^+$ T cells, as well as $CD11c^+$ DCs was confirmed by immunofluorescence (**Figure 2C-E**).

In addition to the tumors themselves, there was a parallel increase in the numbers of $CD4^+$ and $CD8^+$ T cells, as well as activated DCs in the draining lymph nodes (DLNs) (**Figure 3**). Similar results were observed in rIL-7/HGFβ-treated CT 26 colon cancer-bearing mice (data not shown).

hrIL-7/HGFβ inhibits pulmonary metastases of melanoma and colon cancer

Having established that hrIL-7/HGFβ inhibited local tumor growth, we wanted to assess whether hrIL-7/HGFβ could also inhibit metastatic tumors. To this end, C57BL6 mice were injected i.v. with B16F10 melanoma cells to establish pulmonary metastases. The mice were then treated with 15 μg hrIL-7/HGFβ or equimolar doses of hrIL-7 and/or hrHGFβ (or PBS). The mice were euthanized on day 21, the lungs were removed and weighed, and tumor colonies on the surface of the lung were counted. hrIL-7/HGFβ treatment reduced the numbers of metastatic nodules on the lungs by approximately 5.1-fold, as compared with 1.3-fold after hrIL-7 treatment (**Figure 4A**). The trend observed for lung weights mirrored the observed hrIL-7/HGFβ-mediated reduction in the numbers (and size) of lung metastases (data not shown). Similar antimetastatic activity was observed in the lungs of hrIL-7/HGFβ-treated BALB/c mice after i.v. injection of CT-26 colon cancer cells (**Figure 4B**). Similar to those

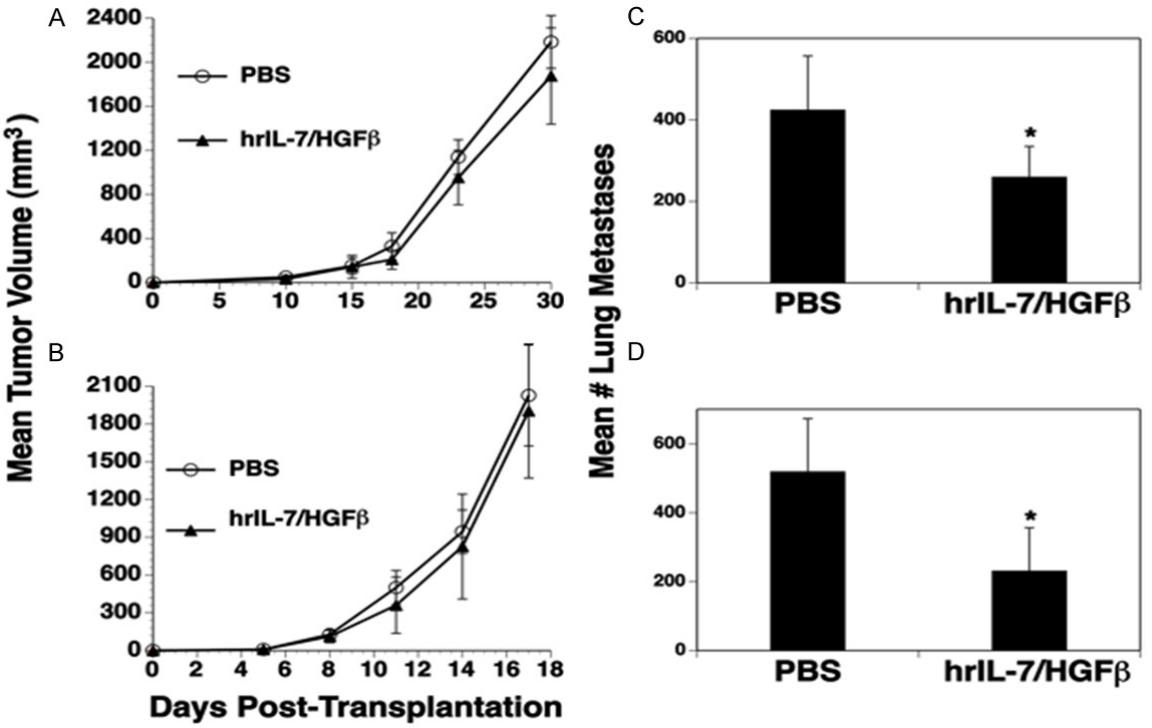

Figure 5. The antitumor and antimetastatic activities of hrIL-7/HGFβ in nude mice. Nude mice were injected s.c. with (A) 1×10^5 B16F10 melanoma cells or (B) 2×10^5 CT-26 colon cancer cells, followed by i.t. injections with hrIL-7/HGFβ (15 μg) or PBS at 2-day intervals between (A) days 2-14, or (B) days 2-24 after tumor cell inoculation. Data represent mean tumor volume (mm^3) ± S.D. is shown; 5 mice per group. Nude mice were injected i.v. with 2×10^5 (C) B16F10 melanoma cells or (D) CT-26 colon cancer cells, followed by the i.v. injection of hrIL-7/HGFβ (15 μg) or PBS at 2-day intervals between days 2-18. Data represent mean ± S.D. numbers of tumor nodules in the lungs on day 21; 5 mice per group. *P<0.05 as compared with the PBS-treated group.

observed in the DLNs after the local administration of hrIL-7/HGFβ, randomly sampled peripheral lymph nodes also contained significantly increased numbers of $CD4^+$ and $CD8^+$ T cells, and activated DCs following the systemic administration of hrIL-7/HGFβ (data not shown). These results indicate that these immune cells may also be involved in the antimetastatic activity of hrIL-7/HGFβ.

The antitumor activity of hrIL-7/HGFβ is lost in immunodeficient mice

To confirm that immune cells were involved in the antitumor activity of hrIL-7/HGFβ, the previous experiments were repeated in nude mice that lack functional T cells. In contrast to its effects in immune-competent BALB/c and C57BL/6 mice, hrIL-7/HGFβ failed to inhibit local tumor growth in nude mice by both CT-26 colon cancer cells (**Figure 5A**) and B16F10 melanoma cells (**Figure 5B**). However, the antimetastatic activity of hrIL-7/HGFβ was only partly lost in nude mice (**Figure 5C**, **5D**). These results suggested that T cells play a critical role in the inhibition of local tumor growth by hrIL-7/HGFβ. However, other immune cells are also involved in the antimetastatic activity of hrIL-7/HGFβ.

hrIL-7/HGFβ-induced tumor immunity prevents recurrence of the same tumor

To determine whether hrIL-7/HGFβ-induced antitumor immunity enabled the host to resist tumor growth, BALB/c mice were injected s.c. with CT-26 colon cancer cells and treated with hrIL-7/HGFβ or PBS as described in **Figure 1C**. Twenty five days later, the tumors were surgically removed, and these mice were re-injected in the opposite flank with the same cancer cells, or with 66.1 murine breast cancer cells as specificity controls. We found that the growth of the re-injected colon cancer cells was significantly reduced in the hrIL-7/HGFβ-treated mice, as compared with PBS-treated mice (**Figure 6A**). In contrast, the growth of unrelated 66.1

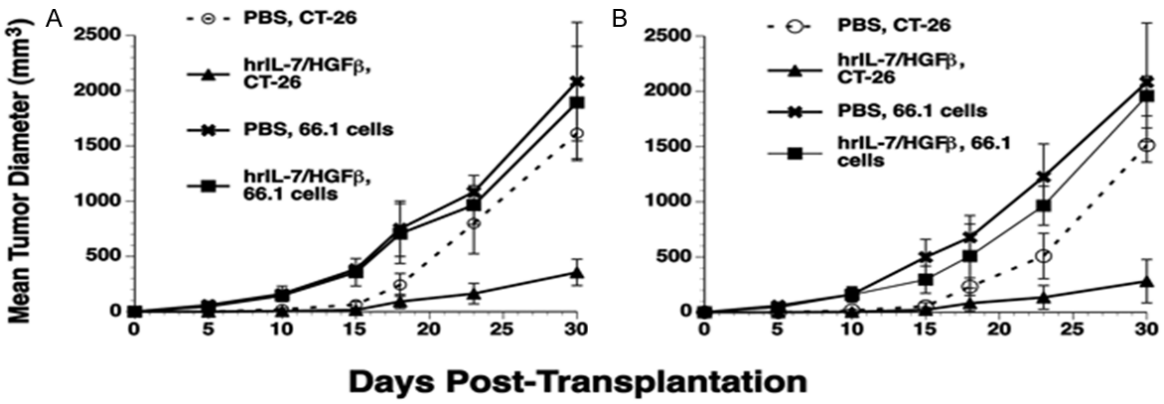

Figure 6. hrIL-7/HGFβ-induced tumor immunity enables the host to resist the same tumor growth. BALB/c mice were injected s.c. with CT-26 colon cancer cells and treated with hrIL-7/HGFβ or PBS as in **Figure 1C**. Twenty five days later, A. The tumors were surgically removed, and these mice were re-injected in the opposite flank with the same cancer cells (2×10^5), or with 66.1 murine breast cancer cells (8×10^5). B. Spleens were harvested from the hrIL-7/HGFβ or PBS-treated mice. A single-cell suspension of the splenocytes was adoptively transferred into naïve BALB/c mice that were then injected s.c. with CT-26 colon cancer cells (2×10^5) or 66.1 murine breast cancer cells (8×10^5). Tumors were measured twice weekly. The mean tumor volume (mm^3) ± S.D. at the indicated time points are shown. The data are representative of 2 independent experiments with 5 mice per group with nearly identical results.

murine breast cancer cells was not significantly different between the two groups (**Figure 6A**). These data suggest that hrIL-7/HGFβ treatment can induce systemic tumor-specific immunity, thereby enabling the host to resist same tumor cell re-challenge.

We also harvested spleen from the mice that had been injected with colon cancer cells and treated with hrIL-7/HGFβ or PBS as in **Figure 1C**. A single-cell suspension of the splenocytes was adoptively transferred into naïve mice that were then injected s.c. with CT-26 colon cancer cells. Again, 66.1 murine breast cancer cells were used as specificity controls. We found that local tumor growth from colon cancer cells, but not from unrelated breast cancer cells, was significantly reduced in the mice that had been injected with splenocytes from rIL-7/HGFβ-treated colon cancer-bearing mice (**Figure 6B**). Taken together, these results suggest that hrIL-7/HGFβ-induced tumor immunity can prevent recurrence of the same tumor or be adoptively transferred into naïve mice.

Discussion

We have shown that *in vivo* administration of hrIL-7/HGFβ inhibited the growth of local tumors or metastatic tumors in mouse models of colon cancer and melanoma. The antitumor mechanism is immunological for the following reasons: 1) hrIL-7/HGFβ did not directly act on the melanoma or colon cancer cells; 2) hrIL-7/HGFβ treatment increased the number of T cells and activated DCs in the tumors and lymph nodes; 3) the *in vivo* antitumor activity of hrIL-7/HGFβ was lost in immunodeficient mice; 4) transferring splenocytes from rIL-7/HGFβ-treated cancer-bearing mice into naïve mice enable these mice to resist same tumor cell challenge.

IL-7 is critical for the development, maintenance and regeneration of T cells [8-11]. IL-7 has been shown to have antitumor activity [12-18]. For example, tumor cell lines that were transfected with the IL-7 gene to produce IL-7 protein locally had reduced tumorigenicity *in vivo*, which was dependent on $CD4^+$ or $CD8^+$ T lymphocytes [12, 14, 16]. Injection of rIL-7 directly into tumors also slowed tumor growth [17, 18]. Consistent with these studies, we show here that *in vivo* administration of hrIL-7 inhibited tumor growth. However, when given in the same molar amount, hrIL-7/HGFβ had significantly higher antitumor effect than hrIL-7 that was used alone or mixed with hrHGFβ. This difference may be qualitative as well as quantitative.

It is well known that DCs play a critical role in the activation of T cells. DCs express both the IL-7 and HGF receptors [19-23]. Although it has been reported that both IL-7 and HGF can indi-

vidually affect DCs, they primarily influence the development of immature DCs from intrathymic precursors and peripheral blood monocytes [8, 20, 22-26]. Furthermore, IL-7 has been shown to down-regulate the expression of MHC II on DCs and to diminish the homeostatic proliferation of $CD4^+$ T cells [27]. HGF has been reported to favor the development of tolerogenic DCs from monocytes [23] and to suppress DC functions such as antigen-presentation [22]. Our data showed that the individual factors hrIL-7 and/or hrHGFβ did not significantly affect the number of DCs and their expression of CD80 and CD86 in the cancer-bearing mice. In contrast, hrIL-7/HGFβ not only increased the number of DCs, but also enhanced the expression of CD80 and CD86 by the DCs (**Figure 2B**), which may contribute the higher antitumor effect of the hybrid cytokine.

The mechanisms by which hrIL-7/HGFβ and the individual factors have different effect on DCs remain to be investigated. We have previously shown that rIL-7/HGFβ can cross-link the IL-7 and HGF receptors on co-expressing immune cells, such as T cells and B cells [3, 6]. Such cross-linking caused juxtacrine receptor interactions, downstream signal cross-talk, and novel functional readouts. It is possible that hrIL-7/HGFβ also cross-linked the IL-7 and HGF receptors on DC, resulting in novel functions including enhanced cell survival, activation and maturation of DCs.

Consistent with our previous reports, hrIL-7/HGFβ treatment also significantly increased the number of T cells [28, 29]. This could be caused directly by hrIL-7/HGFβ acting on T cells and indirectly by hrIL-7/HGFβ-induced increased number and activation of DCs. It remains to be determined which subsets of T cells are affected and whether hrIL-7/HGFβ directly affects the functions of T cells by cross-linking the IL-7 and HGF receptors on the cells.

Similar to our previous results by the use of murine form of cytokine, hrHGFβ alone or when complexed with hrIL-7, did not stimulate tumor cell growth *in vitro* although HGF/c-Met signaling has been shown to stimulate the proliferation of tumor cells *in vitro* [30]. Most likely, this is because although HGFβ binds to c-Met and is required for the mitogenic activity of HGF, it does not by itself stimulate cell growth [31, 32].

In summary, we have demonstrated the antitumor activity of hrIL-7/HGFβ in mouse models of melanoma and colon cancer. This hybrid cytokine, when used alone or in combination with other therapeutic agents, may prove to be very useful in the treatment of cancer patients.

Acknowledgements

This work was partly supported by grants from the Connecticut Biomedical Research Program (#2011-0145).

Disclosure of conflict of interest

L. Lai holds US patent No. 8,163,520B2 ("Nucleic Acids Encoding a Chemeric Polypeptide Comprising IL-7 and HGFbeta Chain and Methods of Use") and US patent No. 7,578,998 ("Chimeric Cytokine of IL-7 and beta-chain of HGF and Methods of Use"). Other authors declare that they have no competing interests.

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