Abstract #: 281

M-ONC 402-a non anticoagulant low molecular weight heparin inhibits tumor metastasis

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Abstract

The major cause of death in cancer patients is due to metastases that are resistant to conventional therapy. Heparin, a complex glycosaminoglycan commonly used as an anticoagulant, has been reported to have anti-metastatic properties. In addition to inhibiting coagulation proteases, heparin is known to interact with a number of growth factors, cytokines, chemokines, and adhesion molecules known to be involved in tumor angiogenesis and metastasis. However, the ability to exploit the anti-metastatic properties of heparin has been limited by its anticoagulant activity. Here we describe M-ONC 402, a low molecular weight heparin derived from unfractionated heparin, engineered to substantially reduce the anticoagulant activity while retaining the activity against key pro-angiogenic and pro-metastatic molecules. In vivo anti-tumor efficacy was initially screened in the B16F10 murine melanoma experimental metastasis model. A single dose of M-ONC 402, administered subcutaneously prior to tumor inoculation, significantly reduced tumor colonization in the lung in a dose-dependent manner. Next, the anti-tumor efficacy of M-ONC 402 was further tested for the ability to inhibit spontaneous metastasis in an orthotopic murine breast cancer model utilizing syngeneic 4T1 breast carcinoma cells. This model mimics human stage IV breast carcinoma. Briefly, mice orthotopically implanted with 4T1 tumor cells were treated with either M-ONC 402, cisplatin, or the combination of M-ONC 402 and cisplatin. Primary tumors were removed between day 9 to day 15, and tumor metastases to the lung were quantified by lung weight, lung nodule counting, lung tumor size and volume quantification 30-35 days after tumor inoculation. M-ONC 402 in combination with cisplatin significantly inhibited 4T1 tumor cell metastasis to the lung compared to the saline control, M-ONC 402 or cisplatin monotherapy groups. Immunohistological analyses demonstrated a decrease in microvessel density in both primary tumors and lung metastases in M-ONC 402 treated groups, suggesting anti-angiogenesis to be one of the anti-tumor mechanisms of the compound. Tumor burden and treatment effect correlated with myeloid-derived suppressor cell population and plasma levels of G-CSF and MMP-9. These data, taken together, suggest the potential of M-ONC 402 in the treatment of cancers.

Introduction

Heparin

- Binds different proteins and enzymes that are essential for angiogenesis and metastasis: VEGF, FGFs, HB-EGF, HGF, PDGF, TGF-β, TNF-α, P-, L-selectins, heparanase, tissue factor
- Heparin or LMWH (low molecular weight heparin) was able to inhibit experimental metastasis in animal models
- The anti-tumor effect of heparins appears to be unrelated to the anti-coagulant activity.
- Retrospective analyses of clinical trials in which LMWHs were used to treat hyper-coagulability in cancer patients have suggested a survival benefit for the treated groups
- However, full exploration of anti-tumor effect of heparin or LMWH is limited by its anti-coagulant activity

M-ONCs

- Rationally engineered LMWHs generated at Momenta Pharmaceuticals, Inc
- M-ONC 402: lead compound with greatly reduced anti-coagulant activity
- M-ONC 202: compound with no detectable anti-coagulant activity

4T1 murine mammary carcinoma model

- Tumor growth and metastasis closely mimics Stage IV human breast cancer
- Metastasis through hematogenous route to lymph nodes, lung, liver, bone and brain.
- 100% animals develop and die of distal metastases if left untreated.

Methods and Results

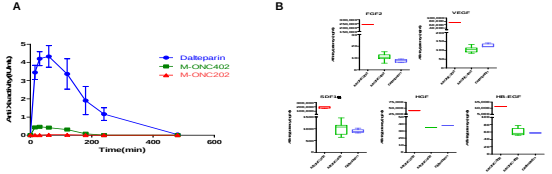


Figure 1. M-ONC 402 has greatly reduced anti-coagulant activity while retaining binding to many heparin binding proteins. (A) BALB/c mice were injected subcutaneously with 10mg/kg Dalteparin (anti-coagulant LMWH), M-ONC 402 or M-ONC 202. Animals were sacrificed at different time points, and plasma collected. The anti-Xa activity was determined with Chromogenix Coatest Heparin kit (Diapharma, West Chester, Ohio) using COAG-A-MATE MTXII (Organon Teknika Corporation, Durham, NC) according to manufactures' protocols. (B) The binding affinity of M-ONC 402, M-ONC 202 and Dalteparin to different heparin binding proteins was determined by Surface Plasmon Resonance.

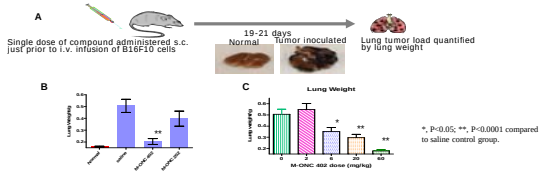
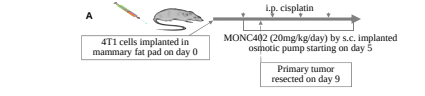


Figure 2. M-ONC 402 inhibits B16F10 tumor colonization to the lung in an experimental metastasis model. (A) Diagram of experimental protocol. (B) The anti-tumor efficacy of M-ONC 402. Groups of C57BL/6 mice (n=8-12) were treated with a single dose of saline or 10mg/kg M-ONC 402 or M-ONC 202 followed by iv injection of $2x10^5$ B16F10 cells within 5 minutes. The experiment was terminated on Day 21 and tumor colonization to the lung quantified by lung weight. M-ONC 402 significantly inhibited tumor colonization to the lung compared to saline control ($p<0.0001$). No significant difference observed when M-ONC 202 treated group is compared to saline control ($p>0.05$). (C) Dose response. C57BL/6 mice (n=12 per group) were treated with different doses of M-ONC 402 (0mg/kg, 2mg/kg, 6mg/kg, 20mg/kg and 60mg/kg) before iv tumor inoculation. M-ONC 402 inhibited B16F10 tumor colonization to the lung in a dose-dependent manner. *, P<0.05; **, P<0.0001 compared to saline control group.



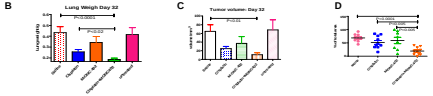


Figure 3. M-ONC 402 combined with cisplatin inhibits orthotopically inoculated 4T1 tumor metastases to the lung. (A) Diagram of experiment protocols. Groups of female BALB/c mice (n=16) were inoculated orthotopically with $5x10^4$ 4T1 cells in the 4th mammary fat pad on day 0. Weekly ip injection of saline or cisplatin (1.25mg/kg), and saline or M-ONC 402 treatment (20mg/kg/day) delivered by sc implanted osmotic pumps started on day 5. Primary tumors removed on day 9 by surgery. Experiment terminated on day 32, lung tissues were isolated and lung weight quantified (B), before fixation in buffered formalin. Tumor nodule numbers and diameters quantified under the dissecting microscope, tumor volumes were calculated according to the formula $\pi x(\text{average diameter}/2)^2 x$ nodule number as presented in (C). Fixed lungs were embedded in paraffin and % tumor (as % of total section areas) quantified under the microscope on H&E stained slides. Results are displayed in (D). *, P<0.05 compared to saline control, cisplatin, M-ONC 402 monotherapy groups. Experiment was repeated twice with similar results.

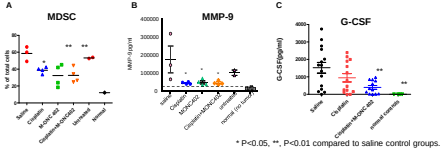


Figure 4. M-ONC 402 treatment normalizes myeloid derived suppressor cells (MDSC), plasma MMP-9 and G-CSF level in 4T1 tumor-bearing mice. (A, B) Groups of female BALB/c mice were inoculated orthotopically with $5x10^4$ 4T1 cells in the 4th mammary fat pad on day 0. Weekly ip injection of saline or cisplatin (1.25mg/kg), and saline or M-ONC 402 (20mg/kg) treatment by sc implanted osmotic pumps started on day 5. Primary tumors were removed on day 9 by surgery. Experiment terminated on day 32 and blood samples collected by cardiac puncture. 100ml of sodium citrate-treated pooled (by the cages) blood samples were treated with RBC lysis buffer, washed, and stained with different antibodies before analyzed with flow cytometry. (A) MDSC are defined by Forward and side scatter plot showing the expansion of these cells (more than 90% of these cells are CD11b+GR-1+) in 4T1 tumor-bearing mice. Quantification of these MDSC cells as % of total cells. *, P<0.05, **, P<0.01 when compared to saline control group. (B) Plasma samples were analyzed for MMP-9 concentration by Luminex system. *, P<0.05 compared to saline control group. (C) In a separate but similar experiment, plasma levels of G-CSF were determined by ELISA assay. **, P<0.01 compared to saline control group.

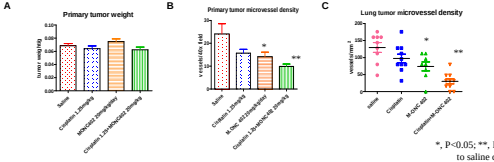


Figure 5. Decreased microvessel density in M-ONC 402-treated 4T1 primary tumors. Groups of female BALB/c mice (n=16) were inoculated orthotopically with $5x10^4$ 4T1 cells in the 4th mammary fat pad on day 0. Weekly ip injection of saline or cisplatin (1.25mg/kg), and saline or M-ONC 402 (20mg/kg) treatment delivered by s.c. implanted osmotic pumps started on day 5. Primary tumors removed on day 9 by surgery, tumor weight recorded (A). There was no significant difference between the groups 4 days after the start of the treatments (P>0.05). Primary tumors were fixed in buffered-formalin, embedded in paraffin and stained for CD31 by immunohistochemistry. (B) Quantification of microvessel density in primary tumor as numbers of vessels/40x field. *, P<0.05, **, P<0.001 when compared with saline control group. (C) Quantification of microvessel density in lung tumor as numbers per mm^2. *, P<0.05, **, P<0.001 when compared with saline control group.

Conclusions

- M-ONC 402 has lower anti-coagulant activity while maintained binding to key molecules
- M-ONC 402 inhibits tumor colonization to the lung in B16F10 experimental metastasis model
- M-ONC 402 inhibits spontaneous metastasis of orthotopically implanted murine 4T1 breast carcinoma
- M-ONC 402 inhibits microvessel density in 4T1 primary tumors
- M-ONC 402 treatment normalize MDSC, plasma MMP-9 and G-CSF levels in 4T1 model.