Abstract #: 2269

M402 – A novel heparan sulfate proteoglycan mimetic targeting tumor-host interactions

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Abstract

Heparan sulfate proteoglycans (HSPGs) play important roles in tumorigenesis by mediating tumor-stromal interactions through the presentation of growth factors, cytokines, and chemokines critical for tumor progression, survival and metastasis. M402 is a rationally engineered, non-cytotoxic HSPG mimetic, designed to disrupt tumor-host interactions. M402 binds and inhibits multiple factors including VEGF, FGF2, SDF-1α, and P-selectin. A single 10 mg/kg subcutaneous (s.c.) dose of M402 effectively reduced seeding of B16F10 murine melanoma cells to the lung in a syngeneic experimental metastasis model. Chronic administration of M402, alone or in combination with cisplatin or docetaxel, inhibited spontaneous metastasis of orthotopically implanted 4T1 murine mammary carcinoma in this model. M402 treatment also normalized circulating levels of $GR1^+$ immature myeloid cells and platelet counts in 4T1 metastatic tumor bearing mice. Fluorescently-labeled M402 exhibited selective accumulation in the primary tumor. Immunohistological analyses of primary tumor presented a decrease in microvessel density in M402-treated animals, suggesting anti-angiogenesis may be one of the mechanisms involved in vivo. Importantly, M402, as monotherapy or in combination with chemotherapeutics, also revealed significant survival benefits in this aggressive tumor model. These data demonstrate that targeting HSPG biology may provide a useful approach to attenuate multiple pathways involved in tumor progression and metastasis.

Introduction

Heparins

- Several growth factors (VEGF, FGFs, HB-EGF, HGF, PDGF, TGF-β), adhesion proteins (P-, L-selectins) and enzymes (heparanase) that play crucial roles in cancer progression and metastasis are heparin binding proteins.
- Both unfractionated heparin and several low molecular weight heparins (LMWH) are known to inhibit experimental metastasis in animal models.
- 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. Many small studies evaluating the effects of UFH and LMWH in cancer patients without thrombosis have also been conducted and have generally suggested a survival benefit. However, full exploration of anti-neoplastic effects of heparin or LMWH to date has been limited due to its anti-coagulant activity.

Compounds tested

- In order to fully explore the antitumor effect of LMWH, Momenta Pharmaceuticals, Inc generated a library of HSPG-mimetics rationally engineered to exhibit reduced anti-coagulant activity while maintaining anti-tumor efficacy. M402 is the lead compound selected following *in-vivo* screening of the above series using the B16F10 model of experimental metastasis. M-ONC 202 has been designated as control compound with no detectable anti-coagulant activity and anti-tumor efficacy.

4T1 murine mammary carcinoma model

- For efficacy, survival and biodistribution studies necessitated a need for more relevant orthotopic model of tumor metastasis like the 4T1 murine mammary model which closely mimics the tumor growth and metastasis observed in Stage IV human breast cancer. Metastasis in this model is through the hematogenous route to lymph nodes, lung, liver, bone and brain and 100% animals develop and die of distal metastases if left untreated.

Methods and Results

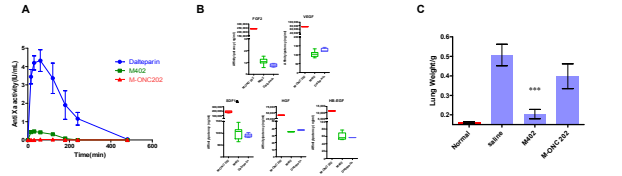

Figure 1. M402 has greatly reduced anti-coagulant activity while retaining binding to many heparin binding proteins and inhibited tumor colonization in murine melanoma experimental metastasis model. (A) BALB/c mice were injected subcutaneously with 10mg/kg Dalteparin (anti-coagulant LMWH), M402 or M-ONC 202. The anti-Xa activity in plasma collected at different time points was determined. (B) The binding affinity of M402, M-ONC 202 and Dalteparin to different heparin binding proteins was determined by Surface Plasmon Resonance. (C) Murine melanoma B16F10 experimental metastasis model. Groups of C57BL/6 mice (n=8-12) were treated with a single dose of saline or 10mg/kg M402 or M-ONC 202 followed by iv injection of $2x10^5$ B16F10 cells. The experiment was terminated on Day 21 and tumor colonization to the lung quantified by lung weight. ***, P<0.0001 compared to saline control group.

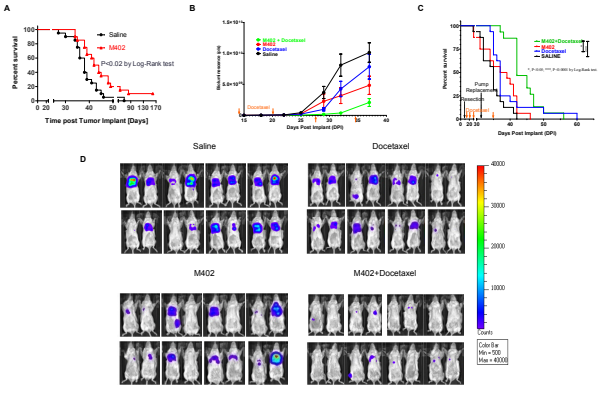

Figure 2. M402 monotherapy or in combination with Docetaxel shows survival benefits in elongation of lifespan studies. (A) Groups of female BALB/c mice (n=20) were inoculated orthotopically with $1x10^5$ 4T1 cells in the 4th mammary fat pad on day 0. M402 treatment was delivered by sc implanted osmotic pumps at 40mg/kg/day started on day 1. Primary tumors were removed on day 10 by surgery. Animals were monitored closely for survival. (B-D) Groups of female BALB/c mice (n=16) were inoculated orthotopically with $5x10^4$ 4T1-luc2-1A4 cells in the 4th mammary fat pad on day 0. M402 treatment was delivered by sc implanted osmotic pumps at 40mg/kg/day started on day 1. Primary tumors were removed on day 10 by surgery. Weekly ip injection of saline or Docetaxel (10mg/kg) was started on day 14. Animals were monitored twice weekly with bioluminescent imaging. (B) Whole body bioluminescence quantified as photons/second over time. (C) Kaplan-Meyer survival curve. (D)Bioluminescence imaging of all the experimental animals on day 29.

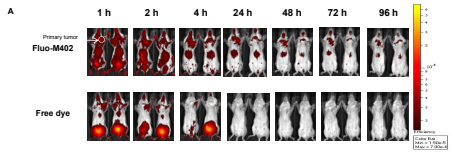

Figure 3. Fluo-M402 biodistribution in 4T1 OT Mammary Fat Pad Tumor Bearing Mice. 4T1-luc-1A4-tumor bearing mice (1st mammary fat pad, 3d after tumor inoculation) were injected with a single subcutaneous dose of either 10 mg/kg fluorescent-labeled M402 or free dye of approximately same intensity. Fluorescent signals persisted in the neck, brachial plexus, liver, injection site as well as the primary tumor through 96h in mice injected with Fluo-M402 (upper panels). In contrast, fluorescent signals in mice injected with free dye accumulated mostly in the bladder area within the first 4 hours, and were not detectable 24 hours after injection (lower panels).

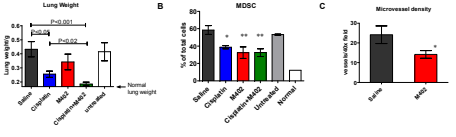

Figure 4. M402 inhibits tumor metastasis, circulating myeloid-derived suppresser cells (MDSCs) and microvessel density in primary tumors. (A) Groups of female BALB/c mice (n=16) were inoculated orthotopically with 4T1 cells in the 4th mammary fat pad on day 0. Weekly ip injection of saline or Cisplatin (1.25mg/kg), as well as saline or M402 (20mg/kg/day, delivered by sc implanted osmotic pumps) treatment was started on day 5. Primary tumors were removed on day 9 by surgery, weighed and fixed in buffered-formalin. The experiment was terminated on day 32, lung tissues were isolated and lung weight quantified (B) Blood samples were obtained by cardiac puncture. MDSCs were defined by gating on flow cytometry FSC and SSC plot which were shown to be >90% $CD11b^+GR-1^+$. *, p<0.05; **, P<0.01 compared with saline control group. (C) Fixed primary tumors were embedded in paraffin and stained for CD31 by immunohistochemistry. Quantification of microvessel density as numbers of vessels/40x field. *, P<0.05 when compared with saline control group.

Conclusions

- M402 is a HSPG mimetic with low anti-coagulant activity that retains binding to key factors crucial for tumor growth and metastasis.
- M402 inhibited tumor colonization to the lung in murine B16F10 melanoma experimental metastasis model.
- Chronic treatment with M402 in monotherapy or in combination with chemotherapeutic agents cisplatin or docetaxel inhibited spontaneous metastasis and prolonged survival in orthotopic murine 4T1 breast carcinoma model.
- Biodistribution studies indicated that M402 accumulated and persists in 4T1 primary tumors.
- M402 treatment normalizes MDSC levels, and inhibits microvessel density in 4T1 model suggesting possible interference with tumor-host interactions by M402.