Increased platelet-derived growth factor (PDGF) release after laparotomy stimulates systemic tumor growth in mice


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Abstract

Background: Our laboratory has demonstrated that tumors grow larger and are more easily established following laparotomy than after carbon dioxide (CO2) pneumoperitoneum or anesthesia alone. We have also shown that tumor cells incubated with serum from laparotomized mice proliferated significantly faster in vitro than those incubated with plasma from mice that underwent laparoscopy or anesthesia alone. We hypothesized that differing levels of a plasma-soluble growth factor(s) postoperatively causes tumors to proliferate faster after laparotomy. This study's purpose was to isolate and characterize the plasma growth factor(s) responsible for the increased growth of systemic tumors after laparotomy.

Methods: Female Balb/C mice (n = 100) were randomized to two groups: anesthesia control (AC) or midline sham laparotomy (4 cm) (Open). Plasma was collected on Post-operative day 4. For the tumor proliferation assay, C-26 colon cancer cells were incubated in media with either 10% AC or Open "raw" plasma (not passed through column), or AC or Open plasma that had been passed through the column. For elution of heparin-binding proteins, plasma from each group was passed through a heparin-sepharose column. Elution of bound proteins was accomplished with a 0.1-2 M NaCl gradient. Each fraction was examined for protein content. For the anti-platelet-derived growth factor (PDGF) neutralizing antibody study, C-26 cells were incubated with one of four plasma preparations: AC or Open plasma alone, or AC or Open plasma incubated with anti-PDGF antibody. For both studies, tumor proliferation was determined after 2 days with an MTS/PMS assay. Results from each group were compared and differences determined using analysis of variance (ANOVA) and Tukey-Kramer tests.

Results: On heparin chromatography, a single elution peak consistent with PDGF was present in both AC and Open plasma and was 1.5 times greater in the Open plasma. The first tumor proliferation assay showed that tumor cells incubated with Open plasma proliferated 2.5 times faster than those with AC plasma (p < 0.0001). Passage of AC plasma through the column did not alter its mitogenic activity, but Open plasma thus treated demonstrated significantly decreased mitogenic activity. The second tumor proliferation assay showed that anti-PDGF antibody had no effect on the mitogenic activity of the AC plasma but decreased the mitogenic activity of the Open plasma to the AC plasma level.

Conclusions: Laparotomy is associated with higher levels of a heparin-binding plasma factor, consistent with PDGF. The enhanced mitogenic activity of the OP plasma was neutralized with anti-PDGF antibody. Increased plasma levels of PDGF after laparotomy may be responsible for accelerated tumor growth following laparotomy in mice.

Key words: Plasma soluble factor — Tumor — Laparotomy — PDGF — Platelet-derived growth factor

Using three different tumor cell lines, our laboratory has demonstrated that tumors grow larger and are more easily established following sham laparotomy than after carbon dioxide (CO2) pneumoperitoneum [1, 9]. We further showed that the differences persist in the setting of a bowel resection [3]. The exact mechanism underlying differences in tumor growth following laparotomy vs laparoscopy is unclear. Significantly better-preserved cell-mediated immune function has also been observed in our laboratory after pneumoperitoneum, accounted for some of the differences in tumor growth [2]. Our laboratory performed a study in nude mice that demonstrated that better-preserved postoperative immune function, as noted after pneumoperitoneum, accounted for some of the differences in postoperative tumor growth between the open and closed groups. The results of this study also suggested the existence of factors that stimulate tumor growth after surgery [3, 6].

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We hypothesized that following laparotomy, systemic release of a serum soluble factor(s) stimulates tumors to proliferate more rapidly. To test this hypothesis, we compared the proliferation rates of tumor cells separately incubated with plasma from mice undergoing either laparotomy or CO₂ insufflation. We found that tumor cells incubated with plasma from laparotomized mice proliferated significantly faster in vitro than those incubated with plasma from mice who had undergone laparoscopy. The effect was greatest with plasma collected 4 days after surgery and was lost by postoperative day (POD) day 7 [5]. We concluded that a plasma soluble factor (or factors) present in elevated levels following laparotomy causes increased tumor growth postoperatively. Previous attempts to characterize that factor by heat lability or molecular size have been unsuccessful. The aim of this study was to identify and characterize that factor using heparin-sepharose chromatography.

Methods
The study protocol was submitted to and approved by the Columbia University Institutional Animal Care and Use Committee.

Tumor cell preparation
The murine colon-26 adenocarcinoma (C-26) cell line was utilized for this study. The C-26 tumor line is syngeneic to the Balb/C mouse strain, and the tumor is grown as a monolayer in tissue culture flasks (Fisher Scientific, Pittsburgh, PA, USA) in RPMI 1640 medium supplemented with 2 mM L-glutamine (Fisher Scientific), 150 U/ml penicillin, 150 mg/ml streptomycin, and 0.25 mg/ml amphotericin B (Gibco BRL; Life Technologies Inc, Carlsbad, CA, USA). For each of the experiments in this study, the cells were washed once, trypsinized (2.5%), and then resuspended in the culture media at the desired cell concentration. After we determined the viability of the cells using the trypan blue exclusion test, a total of 20,000 cells were placed in each culture well. Viability always exceeded 95%.

Surgical interventions and collection of plasma samples
One hundred 5- to 6-week-old female Balb/C mice (Charles River Laboratories, Wilmington, MA, USA) were used in this study. On the day of and prior to the intervention, the mice were restrained, shaved and, then randomly assigned to one of two groups: anesthesia control (AC) or sham laparotomy (Open). Immediately before the procedure, all animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Anesthesia control mice underwent no procedure and were returned to their cages after 20 min. Laparotomy group animals underwent a midline incision from xiphoid to pubis (4 cm), which was clipped closed after 20 min.

Blood was collected from all mice on POD 4 via cardiac puncture using heparinized syringes. Each of the blood samples was placed in a serum separator tube (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged for 10 min at 500 g. Plasma from each group of animals was pooled, filtered through a 0.22-mm filter (MSI, Westboro, MA, USA), and frozen at -80 °C.

Tumor proliferation assay
Twenty thousand C-26 colon adenocarcinoma cells in RPMI 1640 supplemented with antibiotics were incubated with either 10% AC or Open plasma that had not been passed through the heparin-sepharose column or 10% AC or Open plasma that had been passed through the column (non-heparin-binding plasma fraction).

Preparation of non-heparin-binding plasma fraction
First, 1 ml of plasma from each group passed separately through a pre-washed heparin-sepharose column (Amersham Pharmacia Biotech). The non-heparin-binding fraction of plasma from each group was then “washed out” by passing 4 l of RPMI 1640 supplemented with antibiotics, yielding 5-ml preparations containing 20% plasma in RPMI 1640 media. Then, 100 μl of each preparation was added to wells containing 20,000 cells in a volume of 100 μl of RPMI 1640 media. The final preparation then contained 20,000 C-26 tumor cells in 200 μl of RPMI 1640 with 10% non-heparin-binding fractions of AC or Open plasma.

Tumor proliferation determination
Seven wells were created for each of four plasma preparations on a 96-well plate; each well contained 20,000 C-26 colon adenocarcinoma cells in a total volume of 200 μl. A background well (no cells) was also created for each plasma preparation. A 96-well plate containing tumor cells and background preparations were incubated for 48 h at 38 °C in 10% CO₂. Tumor proliferation was then analyzed using an MTS/PMS assay. In this assay, viable tumor cells convert tetrazolium salt into a more optically dense formazan product. The number of viable cells in each well is directly proportional to the optical density at 490 nm, minus the corresponding background, after a brief incubation with MTS/PMS.

Neutralizing anti-PDGF antibody study
A goat polyclonal anti-platelet-derived growth factor (PDGF) IgG antibody known to neutralize all three isoforms of rodent PDGF (AA, BB, AB) was used (Upstate Biotechnology, Lake Placid, NY, USA). A total of 20,000 C-26 colon adenocarcinoma cells were incubated with one of the following four plasma preparations: (a) 10% AC plasma, (b) 10% Open plasma, (c) 10% AC plasma preincubated with neutralizing anti-PDGF antibody, or (d) 10% Open plasma preincubated with neutralizing anti-PDGF antibody. For neutralizing anti-PDGF antibody preparations, a suspension of 20% plasma and 40 μg/ml of antibody in RPMI 1640 media was preincubated for 1 h at 38 °C in 10% CO₂. Seven wells were then created for each preparation on a 96-well plate; each well contained 100 μl of the preincubated plasma and 100 μl of the suspension of 20,000 cells. Thus, each well contained 20,000 cells and 10% neutralized or control plasma. A background well (no cells) was also created for each plasma preparation. The entire plate was incubated for 48 h at 38 °C in 10% CO₂ and assessed by MTS/PMS assay.

Statistical analysis
Data were expressed as the mean ± standard deviation (SD) (optical density at 490 nm). Statistical significance was established using the one-way analysis of variance (ANOVA) test followed by the Tukey-Kramer multiple intergroup comparison test. Probabilities of <0.05 were considered significant.