209. 発がんを含めた炎症性腸疾患病態における plasmin

Heissig Beate

*東京大学 医科学研究所 幹細胞治療研究センター 幹細胞ダイナミクス解析分野

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緒 言

Despite therapeutic advances, the prognosis for patients with metastatic colorectal cancer (CRC) remains poor, with a median overall survival of 36 months.

Fibrinolytic factors are widely distributed in gastrointestinal tissues, contributing to processes of gastrointestinal tissue remodeling, and angiogenesis. The fibrinolytic factor plasminogen is converted into plasmin by tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Plasminogen activators have been suggested as biomarkers for patients with CRC.

Although best known for its ability to lyse blood clots, our group and others reported that fibrinolytic factors play a role in the pathogenesis of inflammatory diseases e.g. by regulating cytokine production. Indeed, inflammation is a well-established risk factor of both colitis-associated cancer (CAC) and sporadic CRC influencing all stages of CRC pathogenesis including initiation, progression, and metastasis.

Dysregulation of plasmin has been linked to cancer invasion/metastasis and chronic inflammatory diseases［1］. Evidence of antitumor activity was seen in a small study after a single intratumoral injection of the plasmin inhibitor aprotinin and an immunomodulatory agent in patients with CRC［2］. In this study, we studied the role of tPA/plasmin for CAC. We used the so-called AOM DSS model that has been reported to recapitulate the aberrant crypt foci-adenoma sequence that occurs in human CRC. The model is established by the administration of a combination of a single hit of axoxymethane (AOM), a potent carcinogen with exposure to the inflammatory agent DSS in mice［3］.

方 法

Mice. Pathogen-free 8- to 12-week old male WT C57BL6 mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). 6-8 week-old age and sex-matched C57/Bl6 mice were used for the experiments and Plg-/-, MMP9-/-, and tPA-/- mice on a C57BL6 genetic background were housed under specific pathogen-free conditions. Animal procedures were approved by the Institutional Animal Care and Use Committee of Juntendo University School of Medicine, Tokyo, Japan

1. Induction and analysis of tumors in mice (AOM model)

C57BL/6 or tPA and Plg gene-deficient mice and respective controls were injected intraperitoneally (i.p.) with 12.5 mg/kg Azoxymethane AOM (Wako) dissolved in physiological saline. Five days later, 2% dextran sulfate sodium (DSS) (ICN Biomedical molecular weight, 36,000–50,000 daltons; ICN Biomedicals Inc, Ohio, GA) was given in the drinking water over five days, followed by 16 days of regular water. The control group received only 50% ethanol. This cycle was repeated three times (five days of 2% DSS). Mice were sacrificed on day 84. Colons were removed, flushed with PBS, fixed in 4% paraformaldehyde at 4°C overnight and paraffin-embedded. Tumors counts were performed in a blinded fashion.

*現在の所属：順天堂大学 大学院医学研究科 免疫学診断講座

2. Drugs/reagents

In vivo: The plasmin inhibitor tranexamic acid TA; (kindly provided by Daiichi Sankyo Company, Limited) was added to drinking water at a concentration of 25mg/ml during phases of DSS administration or throughout the whole experimental periods. Trans-4-aminomethylcyclohexanecarbonyl-Tyr(O-Pic)-octylamide (YO-2) (kindly provided by Yoshio Okada, The Faculty of Pharmaceutical Science, Kobe Gakuin University) was administered by intraperitoneal injection, once per day at 4 mg/kg/day from days 0 to 28. The tranexamic acid moiety of YO-2 interacts with the active center of plasmin. All mice received the anti-inflammatory drug mezaladine (AKP, JAPAN) with drinking water at a concentration of 8mg/ml during phases of DSS administration.

3. Statistical analysis

Data are shown as the mean ± standard error of the mean (SEM). Student's t-tests were performed. Statistics were performed using the graph pad prism 5 software. P-values < 0.05 were considered statistically significant.

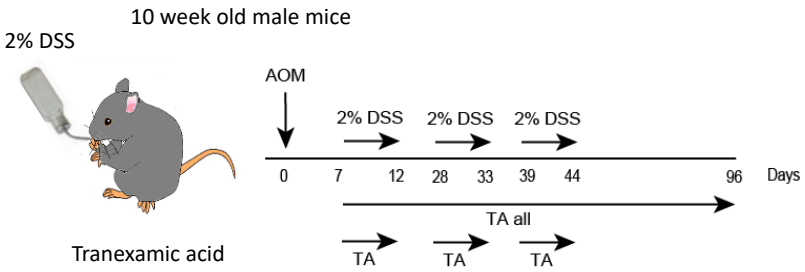
結果および考察

1. Inhibition of plasmin suppresses colitis-induced carcinogenesis in mice

We reported that inflammation-induced colitis did not develop in mice with pharmacological or genetic plasmin/ogen deficiency［4］. To document the possible involvement of the fibrinolytic factors tPA or plasminogen (Plg) for colon carcinogenesis, we studied its expression in C57BL6/J mice induced by injection with the clonotropic mutagen AOM followed by three rounds of oral administration of the luminal toxin dextran sodium sulfate (DSS) to trigger colitis-associated cancer (CAC) (Figure 1A). tPA expression was increased in colonic tissue of AOM DSS mice when compared to mice not treated with the AOM DSS (control) by day 84. Similarly, higher tPA plasma levels were found in plasma 84 days after the start of the AOM DSS treatment when compared to control animals (data not shown).

tPA converts Plg into the active enzyme plasmin. To determine whether plasmin contributes to the development of CAC, AOM DSS mice were cotreated with the plasmin inhibitor tranexamic acid (TA) following the treatment scheme provided in Figure 1A. TA-treated mice showed a reduced *tPA* mRNA expression by day 84 (data not shown). Similarly, TA treatment prevented circulating tPA increases in mice (data not shown). Co-treatment of the anti-inflammatory agent Mezaline with the plasmin inhibitors TA (see Figure 1B) or YO-2 (data not shown) during periods of DSS-induced inflammation nearly completely prevented tumor development AOM DSS mice. Tumor burden could not be further reduced when TA treatment was extended throughout the whole study (TAall vs. TA temp; Figure 1B). These data indicated that plasmin inhibition was most effective in the murine CAC model when given during the inflammatory phase.

A



B

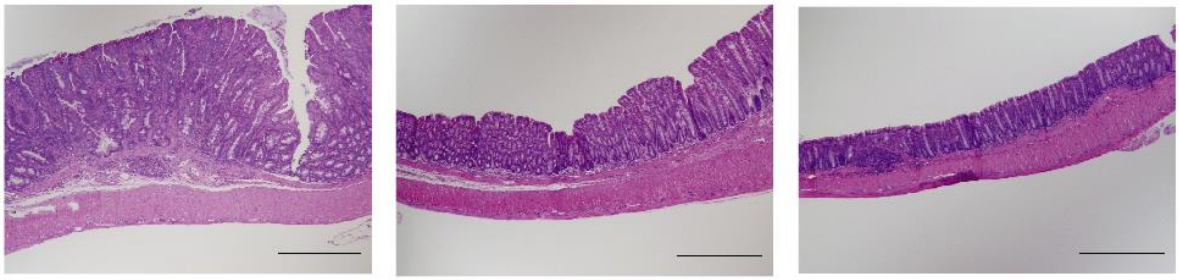


Figure 1. Plasmin inhibition prevents CAC.

(A) Treatment scheme of plasmin inhibitor TA administration using the AOM/DSS CAC model. TAall C57/BL6 mice were divided into the following treatment groups: PBS injection (Ctrl), plasmin inhibitor TA given continuously from day 7-84 (TAall) or only given during DSS administration (TAtemp).

(B) Representative H&E-stained colon sections are shown. Scale bars: 200 mm.

Comparing the expression in cell lysates of human CRC tumors and matched normal mucosa by Western blotting, a higher tPA expression was found in around 60% of colonic cell lysates from adenocarcinoma patients when compared to the matched normal tissues (data not shown). Using tissue arrays of human colon cancer tissues, we found immunoreactive tPA in 19 out of 24 tumor sections. Representative tPA staining of a patient with adenoma carcinoma is shown in Figure 2. tPA expression was found in both the tumor and the adjacent normal colonic tissues by immunostaining.

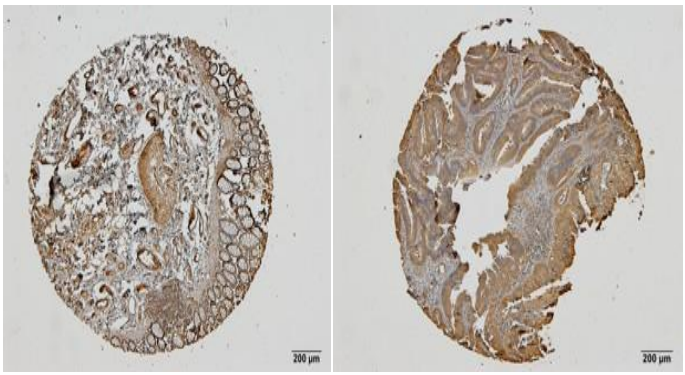


Figure 2. Representative immuno-histological sections stained for human tPA of tumor (right image) and adjacent non-tumor (left image) tissues using a tissue array for human colon cancer. Scale bar: 200 μm.

Next, tumor development was monitored in tPA and Plg gene deficient treated with AOM DSS as described. tPA and Plg gene deficient mice showed a significant reduction in the generation of colonic tumors when compared to control (data not shown). These data indicate that genetic deficiency of tPA and Plg and pharmacological blockade of plasmin suppressed tumor formation in a model of murine CAC. Together, these data indicate that plasmin is involved in the early steps of inflammation-induced colon carcinogenesis and controls CRC growth.

Although the protease matrix metalloproteinase-9 was highly expressed during CAC, MMP9 deficient mice still developed tumors in the CAC model (manuscript in preparation).

Mechanistically, we identified NF-kB as a critical target of tPA and plasmin during CAC (manuscript in preparation).

考　察

We reported that the fibrinolytic factors tPA and plasmin drive chronic inflammation of the gut［5］. tPA is involved in protease degradation and cytokine regulation, but its role in CAC remains unclear. In the current study, we provide evidence that tPA and plasmin enhance colon carcinogenesis by controlling NF-kB signaling and TNFa production. TNFa sustains tumor growth when chronically produced［6］.

Genetic and pharmacological inhibition of tPA and/or Plg/plasmin prevented CAC induction and disease progression in mouse models. We confirmed previous reports by others and show that tPA is expressed in tumor tissues of　CRC patients. Of interest, those studies indicated that high cytosolic tPA predicts shorter overall survival in resectable CRC patients［7］. Mechanistically, we identified NF-kB as a critical target of tPA and plasmin during CAC (manuscript in preparation) supporting findings by others showing that many CRC tumors and cell lines exhibit constitutive activation of NFkB as a response to pro-inflammatory cytokines［8，9］.In concert, fibrinolytic factors and NFkB control the expression of critical colon cancer-associated proteases including matrix metalloproteinases (data not shown) important for the malignant transformation (data not shown here).

Our study identifies fibrinolytic factors like tPA as a potential treatment target for inflammation-induced colon carcinogenesis.

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