Celecoxib Can Prevent Tumor Growth and Distant Metastasis in Postoperative Setting[1]

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ABSTRACT

Much evidence suggests that an inflammatory condition provides a microenvironment favorable for tumor growth. One of the main components in the healing wound is the induction of cyclooxygenase-2 (COX-2) and prostaglandins, and many solid tumors have been known to overexpress COX-2. The present study investigated the relationship between surgical wounds and tumor growth and the roles of COX-2 and inflammatory reaction in this microenvironment. We created surgical wounds in syngeneic mice for the implantation of SCC VII murine cancer cell line. Accelerated tumor growth and increased angiogenesis by surgical wounds were clearly observed in C3H/HeJ mice with SCC VII tumor. The COX-2 expression of peritumoral tissues and leukocyte infiltration partly explained the accelerated tumor growth, especially in the early phase after surgical wounding. Celecoxib had a significantly suppressive effect on tumor growth, angiogenesis, and metastasis in tumor-implanted mice with surgical wounds. This tumor-suppressive action of celecoxib did not show any noticeable side effects on the late wound healing and on the gastrointestinal tracts. Prophylactic use of the drug can be advocated in many clinical situations, such as residual tumors or contamination of surgical fields by tumor cells.

INTRODUCTION

In 1863, Rudolf Virchow suggested that cancer could originate from inflammation. He assumed that leukocytes in the tumor stroma, called "lymphoreticular infiltrate," reflected a connection between inflammation and cancer. Over the past several decades, our understanding of inflammation in cancer tissues based on clinical and experimental studies has supported Virchow's hypothesis. Clinically, tumors originating from a prior chronic inflammatory base have been observed in several tissues, such as the skin, mucous membrane, gallbladder, and urinary bladder (1–3). In fact, infections have a close relation to carcinogenesis in about 15% of human cancers (4).

Surgery is one of the standard modalities of cancer treatment. Surgeons often encounter contamination of surgical fields by tumor cells during operation or residual tumors in the fields after the completion of surgery. A surgical wound induces an inflammatory reaction. Leukocytes are recruited to the wounded site, and many cytokines, growth factors, and chemokines accumulate. Inflammatory cells and cytokines found in tissues around tumors are more likely to contribute to tumor growth, angiogenesis, and metastasis (5, 6). Experimental studies revealed that wounding had a growth-promoting effect on cancer cell lines implanted at surgical wounds (7, 8). All of these observations suggest that surgical wounds can provide a microenvironment favoring tumor growth. A thorough understanding of the connection between cancer and surgical wound would lead to the establishment of a proper treatment strategy for postoperative cancer

patients by appropriate control of inflammatory reaction. Among many cytokines or growth factors released in the wound, prostaglandin has been known as one of the key molecules modulating the wound-healing process. In addition, several studies revealed that the expression of cyclooxygenase-2 (COX-2), a prostaglandin-producing enzyme, was increased in many solid tumors (9–11). These findings lead to a possibility that COX-2 and prostaglandin might be the connection between inflammation and carcinogenesis or tumor promotion.

In the present study, we focused on COX-2 as a key molecule, which could increase in the healing wounds and provide a microenvironment favorable for the tumor growth. First, we tried to determine whether surgical wounds could affect tumor growth in a murine model and examined whether the accelerated tumor growth in the surgical wounds was related to COX-2 expression or other possible mechanisms. Second, celecoxib was evaluated for its roles in suppressing tumor growth, angiogenesis and metastasis in mice with surgical wounds.

MATERIALS AND METHODS

Cell Line. SCC VII, a murine squamous carcinoma cell line was maintained in RPMI 1640 (Life Technologies, Grand Island, NY), supplemented with 10% fetal bovine serum and antibiotic-antimycotics (100 units/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml amphotericin B), at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Surgically Wounded Murine Model, Tumor Implantation, and Treatment with Celecoxib. We developed a surgical wound model in mice, modified from a previously described method (8). Under general anesthesia with enflurane, the skin of the upper hind limb was incised about 2 cm and the s.c. loose areolar tissues were dissected. Muscles on the bed were then exposed and injured by scissoring and mangling, which led to a more severe wounding condition. The skin incision was continuously sutured with 4–0 silk. Murine cancer cell lines in a 100-μl suspension including equal amounts of cancer cells (10^3-10^5) were s.c. implanted into the site adjacent to the wound to prevent the injected cancer cells from spreading along the widely dissected plane. SCC VII tumor cells were transplanted into syngeneic mice of C3H/HeJ (male, 6–7 weeks old; Korea Biolink Co., Eumsung, Korea). In the other group of mice, a remote wound was made in the neck and upper back using the same method as described above and closed. The murine cancer cells were implanted into the upper hind limbs remote from the wounded site. Control mice had tumor transplantation on their upper hind limbs without surgical wounding. All experiments were performed with the authorization of the Animal Experiment Committee at the Clinical Research Institute of Seoul National University Hospital.

Celecoxib was administered in surgically wounded mice with tumor transplantation. Celecoxib was dissolved in corn oil (Sigma, St. Louis, MO) and administered twice a day at a dose of 20 mg/kg/day by oral gavage. Animals were divided into three groups: a group administered with celecoxib from 1 day before wounding; a group administered with celecoxib from the day after forming small tumor masses with a diameter of about 3–5 mm at the tumor-implanted sites (after tumor growth); and a control group that was not administered with celecoxib. Each group included 5–10 mice. The volume of the tumors (mm^3) was measured every other day using the standard formula: tumor volume = (largest diameter) $\times$ (shortest diameter) $\times$ (depth) $\times$ ($\pi/6$). All mice were sacrificed on the day that the tumor volume reached about 3,000 mm^3. Tumor and peritumoral tissues were separately obtained by fine dissec-

tion with using iris scissors under a surgical microscope. After the tissues were obtained, a part of them was fixed in 10% formalin, and the remaining tissues were immediately frozen in liquid nitrogen and stored at $-80°C$ until additional experiments. Lung tissues were also obtained from all mice and fixed in 10% formalin. Metastatic nodules in the lung were microscopically evaluated. The whole lung was sectioned at every 100 μm and stained with H&E.

In a separate experiment, C3H/HeJ mice were injured in their upper hind limbs using the same wounding technique as described above. SCC VII 10^5 cancer cells were implanted into the wounded fields and in the hind limbs of the nonwounded control mice. Five mice/group were sacrificed on days 1, 3, 6, 10, 14, and 21 after tumor transplantation. Tumors, peritumoral tissues, and the lungs were obtained in frozen and formalin-fixed conditions, and the number of leukocytes infiltrating into the tumor stroma was counted on high-powered fields with H&E staining.

Surveillance of Wound Healing with Celecoxib Treatment. We observed the adverse effects of celecoxib on the local wound healing and on the gastrointestinal (G-I) system. An excisional wound was made in C3H/HeJ mice in the upper hind limb, after shaving the hair. The wound was performed by excising a circular area of the skin about 5 mm in diameter. Tumor cells were not implanted in all wounded mice. Celecoxib was administered twice a day at a dose of 20 mg/kg/day by oral gavage from 1 day before wounding. Control mice had the same wounds but did not receive celecoxib. The body weights of mice were checked twice a week, and the wounds were photodocumented. Five mice/group were sacrificed on days 3, 7, 14, and 28 after injury. The wounded skins were obtained and stained with H&E. Local wound healing was histologically evaluated by the recovery rate of epithelialization at the excisional wound. The degree of re-epithelialization was calculated using a formula; percentage of re-epithelialization = (distance covered by the epithelium)/(distance between muscle edges) $\times$ 100 (12). Whole G-I tracts from all mice treated with celecoxib were washed and thoroughly examined under a microscope. Biopsies were performed in suspicious areas.

Immunohistochemistry. All tumor sections were stained, using the avidin-biotin immunoperoxidase method. Formalin-fixed tissues were embedded in paraffin and serially prepared as 5-μm sections. Slides were deparaffinized, hydrated, placed in citrate buffer (pH 6.0), and heated in a microwave for 20 min. The slides were washed and incubated with goat antimouse COX-2 (1:200), purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and rat antimouse CD 31 (1:200) purchased from Becton Dickinson (Heidelberg, Germany) at room temperature for 2 h. Secondary antibodies (1:200; Dako, Glostrup, Denmark) corresponding to primary antibodies were applied for 30 min at room temperature. Slides were counterstained with hematoxylin.

Microvessel Counting. Tumor angiogenesis was evaluated by counting the microvessel density according to the method described by Weidner *et al.* (13). The tumor sections were first carefully scanned at low magnification ($\times$40) to identify the area showing the most intense neovascularization, positively stained by CD 31. Individual microvessels in the spot were then counted in a single $\times$250 field, and the highest number of microvessels was identified.

Western Blot Analysis. Cell lines, tumor, and peritumoral tissues were homogenized in lysis buffer containing 150 mM NaCl, 100 mM Tris, 1% Tween 20, 50 mM diethyl dithiocarbamate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.001 μM aprotinin, and 1 μM pepstatin. The mixture was stirred for 1 h at 4°C and then centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was separated and determined by protein assay. Equal amounts of protein were applied to 12% SDS-polyacrylamide gel and separated by electrophoresis. The separated proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dachen, Germany) and blocked for 30 min at room temperature with Tris-buffered saline containing 0.2% Tween 20 and 5% nonfat dried skimmed milk at pH 7.5. After incubating with anti-COX-2 antibody (1:1,000) for 2 h at room temperature, the membranes were washed and incubated with secondary antigoat antibody (1:1,000) conjugated to horseradish peroxidase for 1 h at room temperature. COX-2 protein was visualized by developing in enhanced chemiluminescence substrate (Pierce Chemical Co., Rockford, IL) and then exposed to X-ray film (Photo Film Co., Tokyo, Japan).

Statistical Analysis. The data were expressed as the mean $\pm$ SE. Using the SPSS 10.0 for windows (SPSS Inc., Chicago, IL), we performed one-way ANOVA to compare leukocyte infiltration and microvessel count among the different animal groups, followed by Tukey's procedure. We used χ^2 test for categorical data such as the incidence rate of lung metastasis. We also performed Mann-Whitney test to compare the degree of re-epithelialization after surgical injury between two animal groups treated by celecoxib or not. Significance was accepted at $P < 0.05$.

RESULTS

Surgical Wounds Accelerate Tumor Growth. The growth of SCC VII tumor was promoted by wounding conditions surgically made in syngeneic mice (Fig. 1A). Tumor masses in wounded C3H/HeJ mice appeared at an earlier phase and showed more rapid growth than those in nonwounded mice after the implantation of 10^4 SCC VII cells. Interestingly, SCC VII tumor in the remote wound group grew more rapidly than that in the nonwounded control but less rapidly than that in the wound group.

Celecoxib Suppresses Tumor Growth in the Surgical Wound. After tumor implantation in surgical wounds, the growth of SCC VII tumor was significantly inhibited by celecoxib (Fig. 1B). The timing of drug administration affected the inhibition of tumor growth. Administration of celecoxib from 1 day before surgical wounding and tumor implantation (before wounding group) was more effective than that from the day after tumor masses were detected (after tumor growth group), in terms of inhibiting tumor growth in surgical wounds.
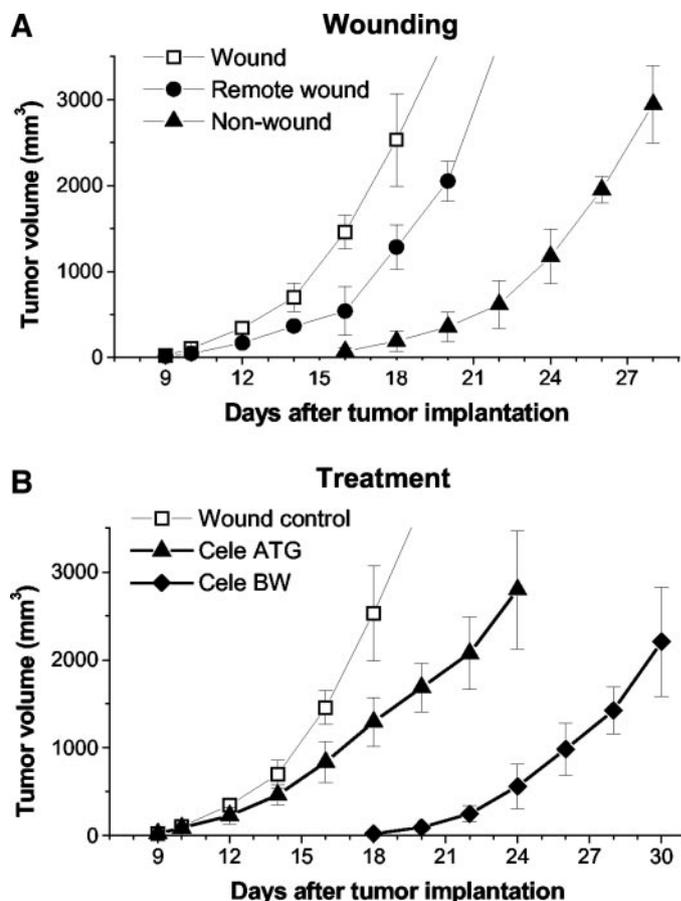

Fig. 1. *A*, acceleration of tumor growth in surgical wounds. Tumor volumes were measured after injection of SCC VII 10^4 tumor cells into the wounded and nonwounded upper hind limbs of C3H/HeJ mice. Remote wound was made in the neck and upper back of the mice, and tumor cells were implanted into the nonwounded upper hind limbs. The surgical wounds accelerated the growth of SCC VII tumor cells *in vivo*. *B*, inhibition of tumor growth in a surgical wound by celecoxib treatment. Celecoxib was used in the mice with implantation of SCC VII 10^4 tumor cells into the wounded sites. Celecoxib significantly inhibited tumor growth in a surgical wound, especially when administered from 1 day before wounding (*Cele BW*). The tumor growth was also suppressed in a group administered with celecoxib from the day after the tumor masses of measurable sizes were found at the tumor-implanted sites (*Cele ATG*).
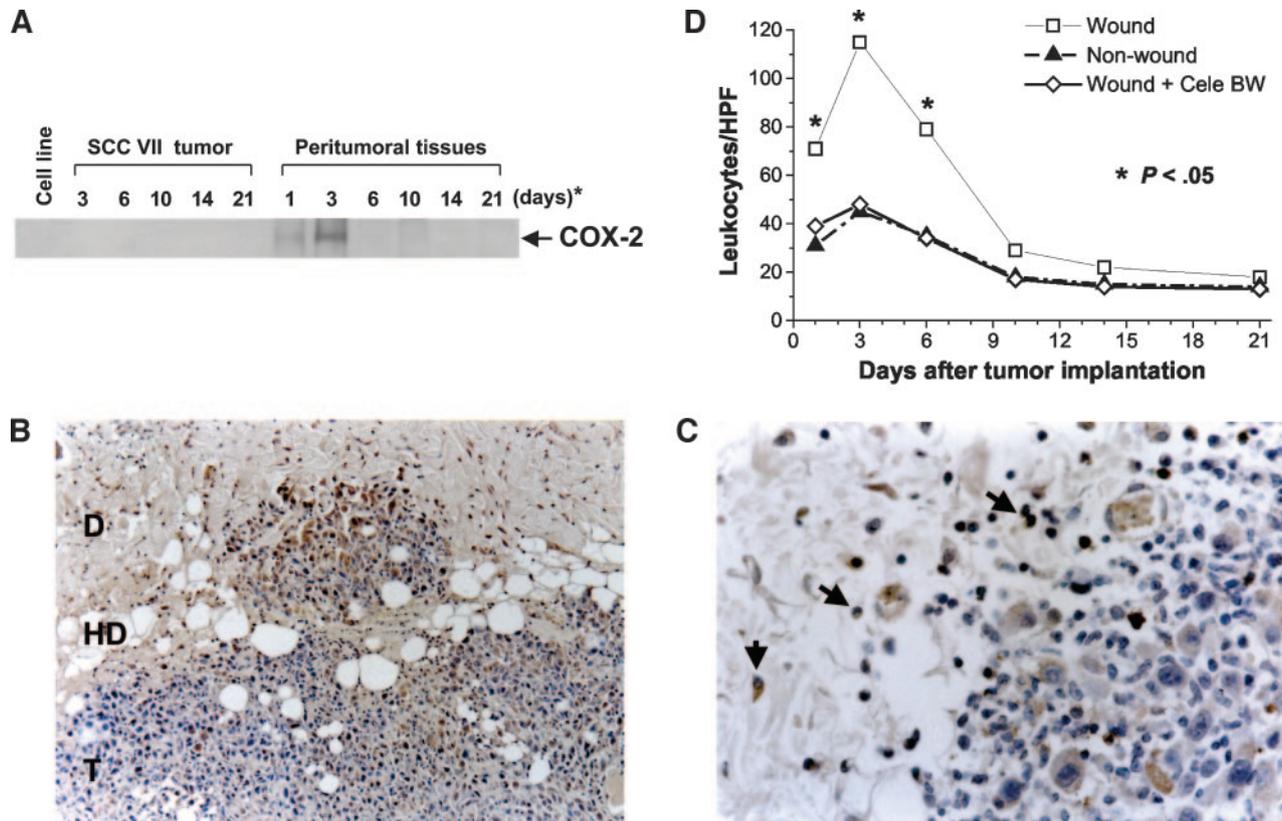
Fig. 2. *A*, COX-2 expression from tumor and peritumoral tissues *in vivo*. Tumor and peritumoral tissues were separately obtained from the wounded mice according to days after implantation of SCC VII tumor cells in a surgical wound. On Western blot analysis, SCC VII cell line *in vitro* and tumors *in vivo* did not show COX-2 expression. COX-2 protein was found only in the peritumoral tissues of the early phase (days 1 and 3). *, days after tumor implantation in a surgical wound. *B*, immunohistochemistry revealed COX-2-positive stains mainly in the peritumoral tissues and tumor margins of the specimens obtained at the day 3 after wounding and tumor implantation (original magnification, ×100). *C*, leukocytes and fibroblasts (*arrow*) of peritumoral tissues and tumor margins showed positive stains of COX-2 protein (original magnification, ×400). *D*, analysis of local inflammatory reaction according to surgical wounding or celecoxib treatment. The number of leukocytes infiltrating into the tumor stroma was counted on high-powered field (*HPF*). The accelerated tumor growth of the early phase was closely related to such an increased local inflammatory reaction after surgical injury. The level of leukocyte infiltration was reduced by celecoxib treatment to that of nonwounded condition. *T*, tumor; *HD*, hypodermis; *D*, dermis; *Cele BW*, celecoxib before wounding.

COX-2 Expression of Peritumoral Tissues and Increased Leukocyte Infiltration Partly Explain the Accelerated Tumor Growth of the Early Phase after Wounding. Interestingly, SCC VII tumor *per se* did not show increased expression of COX-2. Even though there was no evidence of COX-2 expression in SCC VII tumors, this cell line showed a more rapid growth in wounding conditions. To check the expression of COX-2 in the microenviromment of the tumors, tumor and peritumoral tissues were separately obtained on days 1, 3, 6, 10, 14, and 21 after tumor implantation in surgical wounds. COX-2 in total proteins extracted from cell lines, tumors, and peritumoral tissues was analyzed by Western blot, separately. COX-2 protein was expressed only in the peritumoral tissues of the early phase after wounding, with a peak at day 3 (Fig. 2*A*).

For confirming the active sites of COX-2 expression, we performed immunohistochemical staining for COX-2 on the tumor specimens of the wounded mice sacrificed time-serially. Immunohistochemistry revealed that the positive stainings of COX-2 protein were found in the leukocytes and fibroblasts of peritumoral tissues during the early phase of 1–6 days after surgical wounding and tumor implantation (Fig. 2, *B* and *C*). The SCC tumor was weakly stained only in the growing margins during the early phase. A part of leukocytes infiltrating the tumor stroma showed strong positive staining for COX-2.

After tumor implantation in surgical wounds, the number of leukocytes infiltrating into tumor stroma significantly increased in the early phase to day 6 after surgical injury on microscopic examination in comparison with that of the nonwounded control ($P < 0.05$; Fig.

2*D*). This inflammatory reaction was reduced by celecoxib treatment to the level of the nonwounded condition.

Celecoxib Suppresses Lung Metastasis and Angiogenesis of the Tumor. The effect of celecoxib on lung metastasis was evaluated in mice with SCC VII tumor (Fig. 3, *A–C*). On microscopic examination of serial sections, lung metastasis was found in about one-half of the mice with a tumor volume of more than 3,000 mm^3 and was not definitively correlated with the presence of wounding conditions ($P > 0.05$; Fig. 3*B*). However, celecoxib significantly decreased the rates of tumor metastasis to the lung regardless of the timing of drug administration in comparison with those of the celecoxib-nontreated groups ($P < 0.05$). From time-serial observations after SCC VII tumor implantation in the surgical wound, pulmonary metastases appeared at the late phase, which was suppressed by celecoxib treatment (Fig. 3*C*). Five mice were separately sacrificed on days 1, 3, 6, 10, 14, and 21 after tumor implantation. Lung metastasis was found in three of five mice with tumor in the wounded and nonwounded fields on day 21 after tumor implantation. Mice with rapid tumor growth at the wounded sites showed lung metastasis on day 14 (one of five mice), which is earlier than that of nonwounded group. Tumor volume of the mice with lung metastasis on day 14 after surgical wounding was about 2,500 mm^3 and that of all mice with lung metastasis on day 21 was more than 5,000 mm^3. The rate of lung metastasis was raised along with increase of tumor volume *in vivo*. However, mice treated with celecoxib had a volume range of 2,250–3,300 mm^3 (mean 2,780) at day 21, and all of these mice had no lung metastasis during follow-up to day 21.

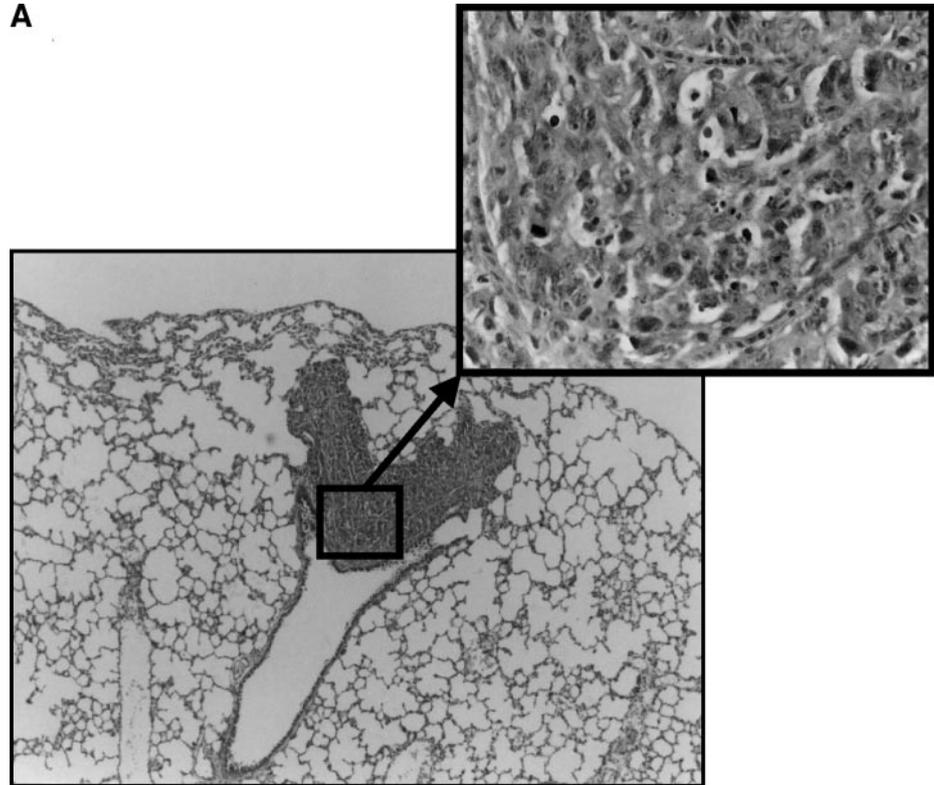Tumor angiogenesis was observed in mice sacrificed time-serially after

Fig. 3. Observation of lung metastasis and angiogenesis from *in vivo* growth of SCC VII tumor. *A*, photomicroscopic finding of lung metastasis of SCC VII tumor on H&E stain. *B*, analysis of lung metastasis *in vivo*. The pulmonary metastatic nodules were carefully observed on serial microscopic sections of whole lung specimens obtained from the mice with implantation of SCC VII tumor cells. Wounding condition did not significantly increase the lung metastasis of the implanted tumor cells in comparison with nonwounded condition ($P > 0.05$). However, celecoxib treatment significantly suppressed the lung metastasis of SCC VII tumor in comparison with celecoxib-nontreated groups ($P < 0.05$). *C*, time-serial observation of the lung metastasis. Five mice were separately sacrificed on days 1, 3, 6, 10, 14, and 21 after implantation of SCC VII 10^5 tumor cells. The lung metastasis was discovered in the relatively late phase and accompanied by an acceleration of tumor growth in a surgical wound. Celecoxib decreased the incidence rate of lung metastasis. *D*, analysis of microvessel density *in vivo* growth. Tumor angiogenesis was accelerated in wounding condition ($P < 0.05$) and significantly inhibited by celecoxib treatment ($P < 0.05$). *E*, celecoxib inhibited angiogenesis from the early through the late phase after tumor implantation. *Cele BW*, celecoxib before wounding; *Cele ATG*, celecoxib after tumor growth.
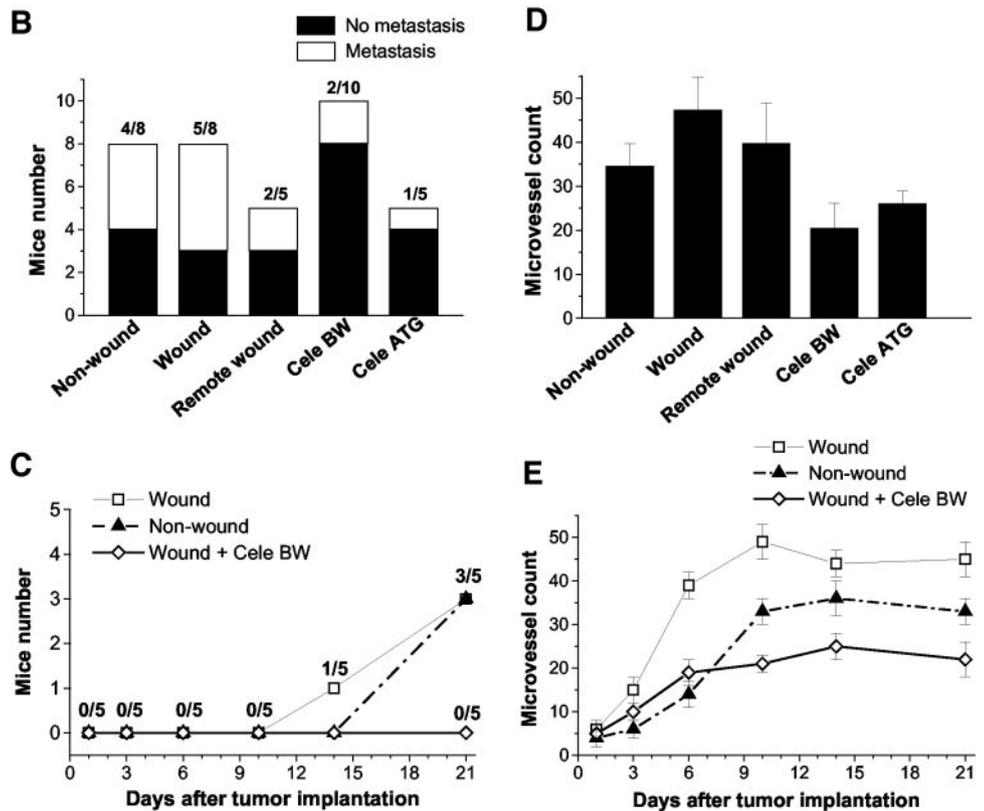
SCC VII tumor implantation in the surgical wound. Microvessel count in the wound group significantly increased from the day 6 after wounding to the last day of observation in comparison with that in the nonwounded control ($P < 0.05$; Fig. 3D). Celecoxib significantly suppressed neovascularization by tumor in surgical wounds ($P < 0.05$). Angiogenesis increased along with tumor growth at the early phase, and this was inhibited by celecoxib until the last day of observation (Fig. 4E).

Late Wound Healing Is Unaffected by Celecoxib Treatment. Macroscopic healing of the excisional wound in mice treated with celecoxib was similar to that of surgically wounded control mice not
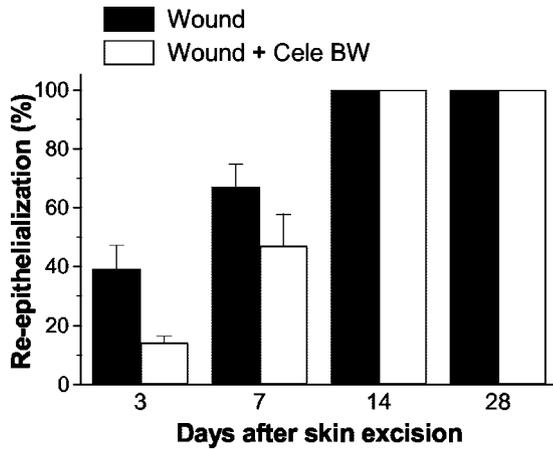
Fig. 4. Effect of celecoxib on wound healing. The degree of re-epithelialization after skin excision was analyzed. Early healing of the excisional wound until the day 7 after wounding was affected by celecoxib treatment ($P < 0.05$), but this was recovered in the later phase. *Cele BW,* celecoxib before wounding.

treated with celecoxib. Re-epithelialization observed on microscopic slides was suppressed during the early phase until the day 7 after surgical injury ($P < 0.05$) but then recovered in the all mice during the later phase, from day 14 (Fig. 4). No difference in body weights was observed in the celecoxib-treated and nontreated groups. Macroscopic and microscopic examinations of the G-I tracts revealed no definitive abnormalities in mice treated with celecoxib.

DISCUSSION

The acceleration of tumor growth by surgical wounding was clearly documented in our murine model. Tumor masses in wounded mice appeared at an earlier phase and showed a more rapid growth than those in nonwounded mice after implantation of SCC VII tumor cells. This finding supports that inflammation induced by surgical wounding provides a more favorable microenvironment for tumor growth. This result also implies that tumor cells remaining in surgical fields can have an enhanced opportunity for rapid growth and recurrence.

The next concern is what contributes to such a rapid growth of tumors in a surgical wound. Wound fluid produced in a surgical wound may affect tumor growth. Wound fluid, especially of the early phase, has many cytokines and growth factors, such as epidermal growth factor, basic fibroblast growth factor, transforming growth factor-β, platelet-derived growth factor, vascular endothelial growth factor, and insulin-like growth factor, which are provided from the blood, surrounding stromal cells or inflammatory leukocytes recruited to the wound site (7, 14, 15). An experimental study revealed that tumor growth was accelerated at nonwounded sites when tumor cells were implanted with growth factors, such as transforming growth factor-β and epidermal growth factor, or wound fluid of the early phase extracted from a surgical wound (8).

We focused upon COX-2, which is known to increase in wound healing and is closely related to tumorigenesis, angiogenesis, and metastasis (12, 16–19). In the present study, the level of COX-2 expression in tumor cells *per se* did not explain the enhanced growth of tumors in wounded animals. However, the expression of COX-2 in the peritumoral tissues of the surgical wound was increased in the early phase after surgical injury. This observation implies that tumor growth may be affected by the expression of COX-2 in peritumoral tissues to some degree. This early expression of COX-2 in peritumoral tissues was accompanied by leukocyte infiltration into the peritumoral tissues and tumor stroma. The level of leukocyte infiltration significantly increased mainly near the growing margins of the tumor during 1–6 days after

wounding. This phenomenon was also identified by immunohistochemistry showing positive staining for COX-2 on leukocytes and fibroblasts of the peritumoral tissues and tumor margins during the early phase after surgical wounding and tumor implantation. A surgical wound seemed to cause the recruitment or increase of these cells that produce many cytokines and growth factors including prostaglandins, COX-2 metabolites, which may partly contribute to the early emergence and rapid growth of the tumor, especially in the early phase after surgical wounding. We do not suggest that COX-2 expression in the surgical wound is a major contributor of such a rapid tumor growth. In fact, COX-2 expression of the peritumoral tissues was not so strong and lasted only within the early phase after wounding, which suggests that other COX-2-independent factors may be responsible for tumor growth in the surgical wound. Furthermore, continuation of rapid tumor growth and increased angiogenesis during the late phase after wounding cannot be explained only by COX-2 expression during the early phase. However, the emergence of the masses formed by the tumor cells seeded into the surgical wounds in this study was about 7 days earlier than that of nonwounded control, as seen from the tumor growth curve (Fig. 1A). Such an early emergence of tumor masses in the wounded groups seems to be more prominent than the tumor growth of late phase in comparison with that of nonwounded control. This finding suggests that the early events after surgical wounding provide a more important ground for such a rapid tumor growth. Although our observations of the early phase after wounding is not sufficient to explain all mechanisms of the enhanced tumor growth during the whole phases, these may be a part of contributors causing such an accelerated tumor growth in the surgical wound. However, other factors responsible for these events need to be elucidated by additional studies. Interestingly, the growth of SCC VII tumor in remote wound group was more rapid than that of the nonwounded control. Such an accelerated tumor growth at a site remote from the surgical wound may be due to the increased release of tumor-promoting growth factors from the wound, which should also be elucidated by additional studies.

In the present study, celecoxib significantly suppressed tumor growth, angiogenesis, and metastasis *in vivo*. The tumor growth was affected by wounding conditions and significantly suppressed by administration of celecoxib. The appearance of tumor masses was delayed by celecoxib treatment, especially when the drug was administered from 1 day before wounding and tumor implantation. Such an inhibitory effect of celecoxib may be caused by controlling the microenvironment favorable for tumor growth of the early phase. This is supported in part by the results that the level of leukocyte infiltration significantly increased in the tumor margins and peritumoral tissues during the early phase after wounding, which was reduced by celecoxib treatment to the level of the nonwounded condition. Because SCC VII tumor showed no COX-2 expression both *in vitro* and *in vivo*, the tumor suppressive effect of celecoxib may be not related to the expression level of COX-2 in tumor cells *per se*. These findings suggest that celecoxib may suppress tumor growth by a COX-2-independent manner or by modulating the microenvironment favorable for their growth in tumors not expressing COX-2 protein like our murine tumor model. In the present study, celecoxib had suppressive effects on tumor growth and angiogenesis continuously during the late phase as well as the early phase after wounding, which cannot be explained only by control of the microenvironmental factors, such as the COX-2 expression of the peritumoral tissues and increased leukocyte infiltration found mainly during the early phase. Recent *in vitro* studies revealed that drugs known as selective COX-2 inhibitors including celecoxib-induced apoptosis of cancer cells by COX-2-independent pathways (20–22). These observations may help to interpret our *in vivo* results using celecoxib. Therefore, celecoxib may suppress their growth and angiogenesis continuously in a direct man-

ner on the tumor cells, even not expressing COX-2 protein, implanted in the surgical wound. Besides the direct effect of celecoxib on tumor cells, the control of microenvironmental factors may be also an important part of its suppressive effect on tumor growth, especially during the early phase after wounding.

In addition, antiangiogenic and antimetastatic effects of celecoxib were identified in SCC VII tumor. From time-serial observation of SCC VII tumor, angiogenesis was inhibited continuously by celecoxib from the early to the late phase after tumor implantation. Lung metastasis was a relatively late-phase phenomenon, appearing after the tumor had grown to a considerable size. This implies that celecoxib may suppress tumor metastasis by delaying tumor growth and their antiangiogenic effect. Such a suppressive effect on tumor growth by celecoxib was most apparent in mice with the administration of celecoxib from 1 day before surgical wounding and tumor implantation. This suggests that the preventative effect of the drug can be maximized when the drug are administered before surgery is performed.

The present study also examined the side effects of celecoxib. Whole G-I tracts were intact on microscopic examination after administration of celecoxib to mice after surgical injury over 28 days. These mice also did not show the significant change of body weight during experiments. Wound healing was affected by celecoxib only in the early phase of days 3–7 and was not suppressed on days 14 and 28 after injury, on examining the degree of re-epithelialization after skin excision. These results lead us to conclude that celecoxib is a safe drug without significant side effects on the late wound healing and the G-I mucosa.

The present study can provide a useful clinical implication for chemoprevention. Many surgeons sometimes meet an inevitable spillage of tumor cells into surgical fields during operation or get information of potential residual tumor cells after completion of surgery. Our results support the prevention of tumor recurrence after surgery in these conditions by celecoxib. The clinical relevance of the drug has been made clear by the report that in patients with familial adenomatous polyposis, celecoxib treatment led to a significant reduction in the number of colorectal polyps (23). In fact, nonsteroidal anti-inflammatory drugs have been used for pain control in patients undergoing surgery, but their clinical relevance for cancer prevention has not been clearly evaluated in the field of surgical oncology. Although our results are still limited to preclinical murine models and clinical data are not enough to support the prophylactic application of the drug in post-surgical conditions like our models, the present study may help us to understand tumor growth in the surgical wounds and establish a proper treatment strategy for the post-surgical patients with cancer.

In conclusion, surgical wounding may accelerate tumor growth and increase the angiogenesis. The accelerated tumor growth of the early phase after surgical injury may be in part due to the tumor-favorable microenvironment provided by increased expression of COX-2 in peritumoral tissues and leukocyte infiltration. However, other COX-2-independent mechanisms may also have a role of such a rapid tumor growth in the surgical wound, which should be elucidated by additional studies. Celecoxib significantly suppressed tumor growth and angiogenesis accelerated by the surgical wound as well as lung metastasis. The treatment with celecoxib did not induce any side effects with respect to the late wound healing and the G-I mucosa. The prophylactic use of celecoxib can be advocated in several clinical situations, such as suspicious contamination of surgical fields with tumor cells or expectation of potential residual tumors.

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