A Definitive Role of RhoC in Metastasis of Orthotopic Lung Cancer in Mice

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ABSTRACT

Purpose: Lung cancer is a major cause of cancer death, and its incidence is increasing in the world. Conventional therapies remain less effective for metastases of lung cancer, leading to poor prognosis of this disorder. The present study investigates pathological roles of RhoC in metastasis of lung cancer using a clinically relevant mouse model of lung cancer.

Experimental Design: RhoA, RhoC, dominant-negative Rho (dnRho) or green fluorescent protein gene was retrovirally transduced to murine lung cancer cells. For in vivo study, these transduced cells were intrapulmonary inoculated in syngeneic mice, and subsequently, growth and metastasis were analyzed. Migration and invasion activities were further investigated by in vitro chemotaxis chamber assays. Expression levels and activities of certain matrix metalloproteinases (MMPs) were explored by reverse transcription-PCR and gelatin zymography.

Results: Metastasis of lung cancer in the animal model, as well as in vitro migration and invasion, were significantly enhanced or inhibited by overexpression of RhoC or dnRho, respectively, without affecting the growth of primary tumors. Expression levels of certain MMPs and the activity of RhoC were significantly enhanced or inhibited by overexpression of RhoC or dnRho, respectively.

Conclusion: RhoC plays a crucial role in metastasis of lung cancer. RhoC does not affect tumor growth but enhances the metastatic nature of lung cancer by not only stimulating cell motility but also up-regulating certain MMPs. Attenuation of RhoC activity may be a potential target in the development of a novel strategy for treating metastasis of lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer death and accounted for 28% of all cancer death in the United States (1–3). The 5-year survival rate for all lung cancer patients remains only 10–15% in the United States, and the incidence of lung cancer continues to increase in the world (1, 3). About 80% of lung cancers are non-small cell lung carcinoma (NSCLC). The current therapies, including chemotherapy and radiotherapy, are less effective for NSCLC; the response rates in NSCLC remain <50%, and complete regression is rare (4). On the other hand, small cell lung carcinoma has a more highly metastatic nature than NSCLC, leading to a 5-year survival rate of <8% (5).

Thus, the poor prognosis of lung cancer, including small cell lung carcinoma and NSCLC, indicates an urgent need for the development of innovative therapies for treating lung cancer (6, 7).

In general, prognosis of cancer patients, including cases of NSCLC and small cell lung carcinoma, deeply correlate with their clinical stages, which reflect the degree of metastasis to regional lymph nodes and distant organs, as well as the expansion of the primary tumor (1), e.g., lung cancer patients diagnosed with stage IV disease have a 2-year survival rate of <20% (1, 3). Thus, an inhibition of metastasis is a potential useful target in the development of a novel therapeutic strategy for treating cancer. From the biological viewpoint, tumor invasion and metastasis represent a complex process, in which numbers of molecules may be involved, and some crucial factors and mechanisms remain to be solved (8).

Authors of a recent study using DNA arrays implied that several genes involved in cellular matrix assembly or actin-based cytoskeletal reorganization might play an important role in the metastasis of melanoma cells (9). Furthermore, this study showed that RhoC might modulate the metastatic nature of melanoma (9). These findings encourage us to investigate the pathological role of RhoC in metastasis of lung cancer.

RhoC GTPase is a member of the Ras-homology family of small GTP-binding proteins and responsible for actin cytoskeletal reorganization during cellular motility (10, 11). Relatively small numbers of studies on RhoC and cancer have been reported to date, although molecular mechanisms of RhoC-dependent cytoskeletal organization have been extensively investigated from the biological viewpoint (10, 12). The involvement of RhoC has been implicated in only limited types of cancers, such as melanoma (9), inflammatory breast cancer...
C for the tissue inhibitors of metalloproteinase.

DMEM supplemented with 10% fetal bovine serum, 100 nM MMP-2, MMP-9, selection (Rockville, MD) and cultured in 5% CO2 at 37°C

MATERIALS AND METHODS

Cell Lines. The murine lung cancer cell line Lewis lung cancer (LLC) was purchased from American Type Culture Collection (Rockville, MD) and cultured in 5% CO2 at 37°C in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin.

Reverse Transcription-PCR. Total RNA was extracted from LLC cells using Sepazol RNA I super kit (Nacalai Tesque, Inc., Kyoto, Japan) according to the manufacturer’s protocol. For reverse transcription-PCR analysis, 1 μg of total RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen Corp., Carlsbad, CA), and then 1/100 of the cDNA was subjected to PCR amplification by 38 cycles of 94°C for 30 s, each annealing temperature for 1 min and 72°C for 1 min using the primer sets described previously (16–18). Annealing temperatures were 58°C for RhoA and RhoC genes; 55°C for MMP-2, MMP-9, and membrane-type (MT)-1 MMP genes; and 45°C for the tissue inhibitors of metalloproteinase (TIMP)-2 gene. The hypoxanthine-guanine phosphoribosyltransferase gene was amplified as an internal control with the same cycle and temperature as was used for each target gene using the following primer set: (a) sense 5’-CCTCGGATGATACATTA-AAGCATG-3’; and (b) antisense 5’-AAGGGCATATCCAA-CAACAA-3’. The amplified cDNA was electrophoresed on 2% agarose gel containing ethidium bromide. The reproducibility of the results was confirmed by three independent experiments.

Retroviral Vectors and Gene Transduction. The retroviral vector plasmids pMIG-RhoA, pMIG-RhoC, pMIG-N19RhoA (a kind gift from Dr. R. Hynes), and pMX-green fluorescent protein (GFP; a kind gift from Dr. T. Kitamura) were transfected to the 293T cell-derived ecotropic retroviral packaging cell line Phoenix (a kind gift from Dr. G. Nolan) to produce retroviral vectors that express RhoA, RhoC, dnRho and enhanced GFP, i.e., RV-RhoA, RV-RhoC, RV-dnRho, and RV-GFP, respectively (9, 19, 20). Supernatant containing each retroviral vector was collected at 48, 60, and 72 h after transfection and was used for three rounds of retroviral infection with LLC cells (21). Retroviral gene transduction efficiencies and transgene expressions were confirmed by flow cytometric analysis (FACStar; Becton Dickinson, Bedford, MA) of RV-GFP-infected cells and reverse transcription-PCR analysis of each transgene.

Animal Studies. Female C57BL/6 mice at 6–8 weeks old were purchased from Japan Slc, Inc. (Hamamatsu, Japan) and housed in cages in a temperature-controlled room on a 12-h light-dark cycle with free access to food and water. All animal studies were performed in accordance with the NIH guidelines as dictated by the Animal Care Facility at the Gifu University School of Medicine.

Mice received a percutaneous and intrapulmonary inoculation of the transected LLC cells as described previously with some modification (6, 22). Briefly, after a small incision had been made in the skin on the left chest wall, a 29-gauge needle attached to a 0.5-ml syringe was directly inserted into the left lung at a depth of 3 mm, whereas the animal was under ether anesthesia. Subsequently, 1 × 10⁵ retrovirally infected LLC cells mixed with Matrigel basement membrane matrix (Becton Dickinson) were injected into the left lung. The animals were sacrificed at 10, 14, and 21 days after tumor implantation; the sizes of primary tumors in the lung were measured, and the mediastinal lymph nodes were weighed. The volume of the primary tumor was calculated using the formula ½ × (long diameter) × (short diameter)².

Histopathology and Immunohistochemistry. Tissues were fixed in 10% formalin and embedded in paraffin, and 4-μm sections were stained with H&E to evaluate the morphology. For immunohistochemistry, 6-μm frozen sections were fixed in 4% paraformaldehyde and stained with rabbit anti-GFP antibody (Molecular Probes, Inc., Eugene, OR), Alexa Fluor 488-conjugated antirabbit IgG (Molecular Probes, Inc.), and Hoechst 33342 (Molecular Probes, Inc.).

In Vitro Cell Migration and Invasion Assays. Cell migration and invasion assays were performed using 8-μm pore size Transwell Biocoat Control inserts and Biocoat Matrigel invasion chambers (Becton Dickinson), respectively, according to the manufacturer’s protocol. In brief, 5 × 10⁴ retrovirally transduced LLC cells were seeded on a transwell containing numbers of 8-μm pores covered with or without a thin layer of Matrigel basement membrane matrix for invasion or migration assay, respectively, which was set on a 24-well plate. Cells on the top surface of the transwell were removed by scrubbing 24 h after incubation, and the remaining cells on the bottom surfaces of the membrane were fixed and stained using Diff-Quick staining kit (International Reagents Corp., Kobe, Japan). Such invaded and migrated cells were counted and compared among groups. Individual experiments were done in duplicate and repeated four times.

Gelatin Zymography. Gelatin zymography was performed using a Gelatinzymo electrophoresis kit (Yagai Research Center, Yamagata, Japan) according to the manufacturer’s protocol. In brief, 2.5 × 10⁴ cells were seeded onto 10-cm dishes and incubated for 24 h in the serum-containing media. The cells were then rinsed twice and incubated in serum-free media for an additional 24 h. After the collected supernatants were centrifuged to remove contaminated cells and debris, they were mixed with sample buffer and electrophoresed on a 10% SDS-polyacrylamide gel containing 2.5 mg/ml gelatin. The gel was washed and incubated in reaction buffer for an additional 36 h at room temperature. It was then stained with Coomassie Blue and subsequently immersed with destaining buffer for 3 h.

Statistic Analysis. Statistical significances between a control group (parental LLC cells) and each of the treatment groups (retrovirally transduced LLC cells) were determined using the unpaired Student t test. P < 0.05 was considered to have statistical significance.
RESULTS

Retroviral Gene Transduction and Expressions of Rho Family Genes. Reverse transcription-PCR analysis demonstrated that parental LLC cells endogenously expressed RhoA, RhoB, and RhoC at relatively low levels (Fig. 1A). A flow cytometric analysis demonstrated that a single infection with RV-GFP resulted in 56% gene transduction efficiency in LLC cells (Fig. 1B). Two or three rounds of retroviral infection further increased gene transduction efficiencies in LLC cells ≤74 or 86%, respectively. Importantly, expression levels of transgene were various among individual cells after three rounds of retroviral infection; this situation is similar to in vivo gene transduction but different from the use of artificial homogenous cloned cells. After three rounds of infection with each retroviral vector, expression of each target gene was significantly enhanced (Fig. 1C). Cells infected with RV-RhoA, RV-RhoC, and RV-dnRho expressed certain levels of GFP because pMIX plasmids comprise GFP cDNA downstream of the encephalomyocarditis virus internal ribosomal entry sequence (23). Increases in intensities of PCR bands corresponding to RhoA after RV-dnRho infections may be reasonable because the primer sets for RhoA also

Fig. 1 Retroviral gene transduction and expressions of Rho family genes. A, endogenous mRNA levels in Lewis lung cancer (LLC) cells by reverse transcription (RT)-PCR analysis. RT(+) and RT(−) indicate PCR analysis with or without reverse transcription, respectively. NT, no template as a negative control. Total RNA extracted from HepG2 cells or mouse lung tissue was used for a positive control of PCR assay. Hypoxanthine-guanine phosphoribosyltransferase gene was amplified as an internal control gene. B, flow cytometric (in top and center figures) and fluorescent microscopic (in the bottom figures) analyses after retroviral infections in LLC cells. The percentages of green fluorescent protein (GFP)-positive cells after one, two, or three rounds of infection with RV-GFP are shown in the top figure. The center figures show the intensities and numbers of GFP-positive cells that received three rounds of infection with RV-GFP and those of parental LLC cells without any retroviral infections. The LLC cells after three rounds of infection with RV-GFP were also observed under the phase contrast and fluorescent microscopy (the bottom figures). C, mRNA expression levels of transgenes after three rounds of retroviral infection in LLC cells.
have affinity to the sequence of dnRho (only substitution from Thr to Asn at codon 19 of the RhoA gene; Ref. 19).

Establishment of an Orthotopic Lung Cancer Model in Combination with Retroviral Gene Transduction. The primary tumor nodule in the lung parenchyma was macroscopically recognizable ~10 days after intrapulmonary implantation of parental LLC cells, and it rapidly grew and expanded between days 14 and 21 (Fig. 2, A and B). On the other hand, metastases of tumors to mediastinal lymph nodes were not macroscopically recognizable on day 10; however, metastatic foci were conspicuous on day 14, and metastases were prominent on day 21 (Fig. 2, A and C). It should be noted that there were not any differences in the growth of primary tumors, the degree of metastases to the mediastinal lymph nodes, or histological findings between RV-GFP-infected and parental LLC cells (Fig. 2A–D). Moreover, GFP immunohistochemistry demonstrated stable transgene expression in not only the primary tumor nodule but also metastases in the mediastinal lymph nodes on day 21 (Fig. 2E). Thus, retroviral gene transduction in combination with the orthotopic lung cancer model has advantages in stable transgene expression and in the lack of artificial effects relating to retroviral gene trans-
RhoC Enhanced Metastasis without Affecting Primary Tumor Growth in Vivo. We studied the effects of Rho family genes and the dominant-negative Rho (dnRho) gene for primary tumor growth and metastasis to the mediastinum lymph nodes using this new system (Figs. 3 and 4). Interestingly, there were no differences among groups in macroscopic sizes, volumes, or histological findings of the primary tumor during the 21 days of the experiment. On the other hand, metastases to mediastinal lymph nodes were drastically increased between 14 and 21 days after tumor implantation in the case of overexpression of RhoC. In contrast, overexpression of dnRho resulted in significantly smaller sizes and weights of the mediastinal lymph nodes on day 21 compared with parental and GFP-expressing LLC cells. Overexpression of RhoA or GFP did not change the weights of the lymph nodes. It should be noted that no differences were seen in histopathological findings of the metastatic tumor among groups, including groups of RhoC- and dnRho-expressing LLC cells (Fig. 3). Thus, RhoC was largely involved in the metastatic activity of lung cancer (LLC cells) without modulating the growth or morphological change of the primary tumor.

RhoC Enhanced Activities of Both Migration and Invasion of Lung Cancer in Vitro. To investigate the mechanism of RhoC-dependent metastasis, we carried out in vitro migration and invasion assays (Fig. 5). Overexpression of RhoC enhanced migration as well as invasion, although the effect for the latter was more prominent than that for the former. In contrast, overexpression of dnRho inhibited both migration and invasion, whereas overexpression of RhoA or GFP did not affect either. These findings indicate that RhoC may regulate the metastatic nature of lung cancer not only by stimulating cell motility (probably by modulation of the cytoskeletal structure that is well known) but also by directly enhancing the invasive activity, i.e., the degradation of extracellular matrix.

RhoC Enhanced the Expression and Activity of MMPs. It is well known that MMP family proteins play central roles in cancer invasion relating to enzymatic degradation of extracellular matrix. Therefore, we examined mRNA expression levels and activities of the representative MMPs to assess whether the invasive effect of RhoC may result from modulation of MMP activities (Fig. 6). Overexpression of RhoC resulted in significant increases in mRNA expressions of MMP-2, MMP-9, MT1-MMP, and TIMP-2. In contrast, overexpression of dnRho decreased mRNA expression levels of MMP-2, MMP-9, MT1-MMP, and/or TIMP-2 to some degree (prominently, those of MTP-9 and MT1-MMP) in comparison with those of control RV-GFP-infected or parental cells. Zymographic analysis further and strongly confirmed this finding; not only pro (inactive) forms of MMP-2 and MMP-9 but also the active form of MMP-2 were significantly up-regulated by overexpression of RhoC. In contrast, dnRho decreased proMMP-2 and proMMP-9, although the inhibitory effect for active MMP-2 could not be assessed in this experiment because of the absence of detectable levels of active MMP-2 in control parental LLC cells. All these results suggest that RhoC may up-regulate MMPs at the transcriptional and/or enzymatic processional levels, leading to enhancement of the invasive activity of the LLC cells.

DISCUSSION

This is the first study to reveal clearly and experimentally that specifically RhoC, but not RhoA, plays a crucial role in the metastasis of lung cancer. Previous studies implied involvement of the Rho family, including Rho (e.g., RhoA, B, and C), Rac, and Cdc42 proteins, in cancer (12), but the pathological role of each of the Rho family proteins was not clear, and the overall mechanisms in Rho-dependent oncogenic effects have not yet been fully clarified. Physiological functions of these family proteins have been largely investigated; they regulate a wide variety of cell function, especially cytoskeletal reorganization during cellular motility (10, 24). Importantly and interestingly, despite the high homology of the different Rho isoforms, RhoA, RhoB, and RhoC (25, 26), their physiological roles are distinct (24). Likewise, there is a possibility that a specific type of Rho isoform may be involved in the pathogenesis of lung cancer. In clinical studies, significant increases in RhoA in head and neck squamous cell (27), upper urinary tract (28), colon (29), breast (29), and lung (29) cancer and significant increases in RhoC in breast cancer (13, 14, 30) were shown, although other Rho isoforms were not investigated in these studies. Thus far, only four studies, to our knowledge, have compared the combined expression levels of RhoA, RhoB, and RhoC in cancer of human patients. Three of the studies demonstrated significantly higher levels of both RhoA and RhoC in ovarian carcinoma (15), bladder cancer (31), and breast tumors (32) than levels in nontumor tissues. It should be noted that only one of these four studies demonstrated a significant increase in RhoC only, and not in RhoA or RhoB, in ductal adenocarcinoma of the pancreas in human patients (16).

A majority of previous experimental studies on Rho and cancer used RhoA as the representative of the Rho family or the materials that might work on several Rho family proteins; based on such results, the pathological roles of the whole Rho family in cancer have been generalized and broadly speculated (19, 20). In addition, a variety of cancer cell lines has been used without any particular reason for their investigations, and based on the results, authors have roughly speculated about the function of the Rho family in a range of cancers. As a result, only one experimental study, to our knowledge, has investigated roles of the Rho family, specifically in lung cancer (33). However, the study used C3 exoenzyme that ADP-ribosylates Rho on Asn-41, i.e., that might work on both RhoA and RhoC, leading to indistinguishable assessments regarding predominant contribution of RhoA or RhoC (33). Thus, the pathological roles of RhoC in lung cancer have not yet been well defined from clinical or experimental studies; the present study for the first time presents convincing evidence that RhoC, but not RhoA, at least in the type of murine lung cancer cells we tested, modulates the metastatic nature of lung cancer.

Accumulating data concerning biological functions of the Rho family proteins have suggested that tumorigenesis, cell cycle impairment, inhibition of apoptosis, and stimulation of invasion and metastasis may be possibly the major pathological roles of the Rho family in cancer (12). Tumorigenesis was not our focus in the present study and may be explored elsewhere. It remains to be clarified whether RhoC may confer tumorigenesis on lung tissue, because early studies revealed involvements...
Fig. 3 The macroscopic and microscopic pictures of the primary and metastatic tumors in the mouse orthotopic lung cancer model. Lewis lung cancer cells ($1 \times 10^3$ cells) that were beforehand infected with each retroviral vector were intrapulmonary inoculated into a mouse. In A, the representative macroscopic pictures of the primary tumor (PT) and metastatic tumor in the mediastinal lymph nodes (LN) 21 days after the implantation of RV-RhoC-infected, RV-dominant-negative Rho (dnRho)-infected, and parental Lewis lung cancer cells were shown. B and C, the microscopic pictures of the PT (B) and LN (C). H&E staining. Original magnification: ×20, the top pictures and ×200, the bottom pictures. The mice that received RV-RhoA- or RV-green fluorescent protein-infected Lewis lung cancer cells showed similar findings to the parental control (data not shown).
of only RhoA and other Rho family proteins in malignant transformation (19, 20, 34). On the other hand, involvements of Rho family proteins in cell cycle-related molecules and/or mitogenic signal transduction pathways have been extensively studied from biological viewpoints (10, 12). In addition, the relationship between Rho family proteins and apoptosis has recently been implicated (10, 12). Because disregulations of cell growth and cell death are the characteristic and essential nature of cancer, it has been speculated that increases in Rho proteins may be involved in the proliferation of cancer cells and/or their resistance to death signals. In the present study, however, neither the growth nor death of tumor cells in the primary tumor was affected by gene transduction of dnRho or RhoC. In contrast, overexpression of RhoC remarkably stimulated not only the metastasis of lung cancer in this clinically relevant animal model but also the activities of both migration and invasion in the in vitro assays. Moreover, gene transduction of dnRho inhibited the metastasis in the animal model and the activities of migration and invasion. Thus, it is likely that a number of molecules, various signal transduction pathways, and diverse biological effects relating to RhoC may mostly contribute to the activities of migration and invasion of cancer cells, i.e., the metastasis in cancer diseases, but not to the enhancement of cancer cell growth or antiapoptotic effect in cancer cells.

It is reasonable to consider that RhoC is largely involved in migration, i.e., cell motility, of tumor cells, because RhoC regulates actin cytoskeletal organization, which is believed to provide the driving force for cell migration (10). On the other hand, it has been largely unclear how Rho family proteins exhibit the invasive activity, and the exact mechanism of RhoC-dependent invasion is almost unknown. The present study for the first time explored the relationship between RhoC and MMPs in cancer and clarified the novel mechanism; RhoC apparently up-regulated both expression levels and activities of MMPs, leading to enhancement of the invasive activity. We examined MMP-2, MMP-9, and MT1-MMP, as well as TIMP-2, which were the representative MMPs and their antagonist that were largely involved in invasion by a variety of

**Fig. 5** In vitro migration (A) and invasion (B) activities. Lewis lung cancer cells received three rounds of infection with each retroviral vector as described in Fig. 1. The numbers of these cells on the bottom surface of transwell containing 8-μm pores covered with or without the Matrigel were counted as migration and invasion activities, respectively. Data represent means and SDs (n = 15–19/group). *, P < 0.05; **, P < 0.01 (Student’s t test; each of treatment groups versus the parental control).
cancers (35), including lung cancer in human patients (35–37). In fact, a clinical study demonstrated increases in MT1-MMP mRNA expressions and activation of MMP-2 in lung cancer, including small cell lung carcinoma and NSCLC, and a high correlation of the degree of MMP-2 activation with that of MT1-MMP mRNA expression and lymph node metastases (37).

In the present study, gene transduction of RhoC significantly increased mRNA levels of MMP-2, MMP-9, MT1-MMP, and TIMP-2, and it activated MMP-2; gene transduction of dnRho apparently decreased mRNA levels of MMP-9 and MT1-MMP. Such RhoC-dependent transcriptional changes in MMP genes are new and interesting findings, and the detailed mechanisms, especially the transcriptional factors linking to RhoC, may be clarified in future studies. In this regard, the finding of an increase in mRNA levels of TIMP-2, an inhibitor for MT1-MMP and MMP-2, in this study should be noted. ProMMP-2 binds MT1-MMP using TIMP-2 as an adaptor by forming a trimolecular complex on the cell surface, and another MT1-MMP near the complex cleaves the propeptide bond of proMMP-2, leading to active MMP-2 (35). Although no study has investigated the mechanism of RhoC-dependent invasion, other recent studies have shown that RhoA and Rac1 could modulate the degradation and remodeling of the extracellular matrix by regulating the levels of either MMPs or their antagonists, TIMPs (38–40). In addition, Rac1 induced lamellipodia, and the MT1-MMP was localized there; Rac1 enhanced the activation of MMP-2 (35, 38, 41). The ruffled edge that forms the migration front is likely the site of the homo-oligomer formation of MT1-MMP that augments proMMP-2 activation (35, 41). Taken together, there is a possibility that RhoC may not only transcriptionally increase MMPs’ expressions but also be responsible for localization of such MT1-MMP complex to migration edge. In this regard, a recent study showing a relatively stable association of MT1-MMP with actin cytoskeleton should be noted; CD44, which associated with actin within cells, localized at the migration front and was identified as a linker that mediated the association of MT1-MMP with actin (42).

Finally, it is encouraging that therapeutic effects of dnRho gene transduction were statistically significant in this clinically relevant lung cancer model. Somewhat milder phenotype after dnRho gene transduction than that of RhoC may possibly result from a relatively low expression level of endogenous RhoC in LLC cells and/or effective but incomplete inhibition of RhoC activity by the present dnRho gene construct. Nevertheless, the present results importantly suggest that attenuation of RhoC activity may be one of the potential targets in the development of novel strategies for treating metastasis of lung cancer.

In conclusion, RhoC apparently confers a metastatic nature on lung cancer without affecting the growth of primary tumors. One of the definitive mechanisms of the RhoC-dependent metastasis is up-regulating expressions and activities of MMPs.

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REFERENCES

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