Aberrant Expression of Connexin 26 Is Associated with Lung Metastasis of Colorectal Cancer

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Abstract

Purpose: Connexin 26 (Cx26) is one of the gap junction–forming family members classically considered to be tumor suppressors. However, recent studies show association of elevated expression of Cx26 with poor prognosis in several human malignancies. Furthermore, Cx26 has been observed to be indispensable to spontaneous metastasis of melanoma cells. Here, we assessed Cx26 expression in primary colorectal cancer (CRC) and the metastatic lesions to elucidate its role in metastasis.

Experimental Design: Cx26 expression was assessed in 25 adenomas, 167 CRCs, and normal mucosa, together with the metastatic lesions.

Results: Normal mucosa and adenomatous tissue expressed Cx26 mainly in the plasma membrane, whereas cancer cells mostly contained Cx26 in the cytoplasm. The incidence of aberrant Cx26 expression varied widely in CRC (mean, 49.5 $\pm$ 35.5%), and the expression levels were confirmed by Western blot and quantitative reverse transcription–PCR. Clinicopathologic survey revealed association of high expression with less differentiated histology and venous invasion ($P = 0.0053$ and $P = 0.0084$, respectively). Notably, high Cx26 expression was associated with shorter disease-free survival and shorter lung metastasis–free survival in 154 curatively resected CRC sets ($P = 0.041$ and $P = 0.028$, respectively). Survey of metastatic lesions revealed that lung metastasis, but not liver and lymph nodes metastases, expressed higher Cx26 than the CRC series or corresponding primary CRCs ($P < 0.0001$ and $P = 0.0001$, respectively).

Conclusions: These findings suggest that aberrant expression of Cx26 plays an essential role in lung metastasis. Thus, Cx26 is a promising therapeutic target, particularly for CRC patients who develop lung metastasis.

Gap junctions mediate intercellular communication and regulate cell proliferation and differentiation by allowing small molecules and inorganic ions, metabolites, to pass from one cell to the adjacent cell (1–3). A gap junction channel is composed of two membrane-integrated hemichannels supplied by two adjacent cells, and each hemichannel consists of a hexameric complex of connexin proteins; thus, one gap junction usually consists of 12 connexin subunits (4).

Neoplastic transformation is frequently associated with a loss of gap junction intercellular communication and a reduction in expression of connexins in various tumor types (5–7). A mechanistic study showed that abrogation of gap junction intercellular communication enhanced tumorigenicity of rat bladder squamous cell carcinoma cells (8). Conversely, forced expression of connexins in connexin-deficient cell lines resulted in inhibition of tumor growth and induction of apoptosis *in vitro* and prevented tumor formation *in vivo* (9–11). Thus, the multigene family of connexins, which includes at least 20 highly conserved members (1), may act as tumor suppressors by restoring cellular communication and reverting the phenotype of transformed cells.

Accumulating evidence, however, indicates that connexin 26 (Cx26), a connexin family member, is overexpressed in carcinomas of the pancreas, head and neck, colon, and prostate, as well as in keratinocyte-derived skin tumors (12–16). Interestingly, increased Cx26 expression was observed in invasive breast carcinomas and metastatic lymph nodes but not in the epithelial cells of benign lesions (17, 18); Cx26 expression was also detected at the invasive front of a malignant skin melanoma (19). Furthermore, recent reports showed that high Cx26 expression was associated with poor prognosis of lung squamous cell carcinoma and breast carcinoma (20, 21). Together these lines of evidence seem to be contrary to the

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conventional role of connexins as tumor suppressors and instead suggest that Cx26 may play a role in tumorigenesis.

In support of this possibility, a study using cDNA subtraction in two sublines of the B16 mouse melanoma cell line (F10 and BL6) indicated that the *Cx26* gene was responsible for spontaneous metastasis (19). The BL6 subline was originally obtained from the F10 subline through six rounds of *in vitro* selection. Nonetheless, only the BL6 subline that expresses high Cx26 levels was capable of inducing lung metastasis via spontaneous metastasis when implanted in the footpad, although both cell lines metastasize to the lung when injected into tail vein (via so called experimental metastasis). Moreover, introduction of *Cx26* cDNA into F10 conferred spontaneous lung metastatic ability (19).

To examine the precise function of Cx26 in promoting or suppressing tumorigenesis, we examined the levels of Cx26 expression in nonneoplastic and neoplastic tissue of the colorectum. We also examined Cx26 expression in surgically resected metastatic lesions to explore the role of Cx26 in metastasis of colorectal cancer (CRC).

Materials and Methods

Patients and tissue samples. Colorectal tissue samples were resected during surgery or during endoscopic polypectomy (1995-2001), and lung metastases were resected 10 months to 6 years later at the Department of Surgery, Osaka University and Osaka Medical Center for Cancer and Cardiovascular Diseases. CRC cases were selected without knowledge of prognosis after surgery and without knowledge of clinicopathologic features, except for tumor stage. The following set of colorectal samples was collected and examined for the expression of Cx26 by immunohistochemistry: adenomatous polyps (n = 25), CRCs and their normal counterparts (n = 167), and the metastatic lesions of lymph node (n = 56), liver (n = 14), and lung (n = 24). The samples were fixed in buffered formalin at 4°C overnight, processed through graded ethanol solutions, and embedded in paraffin. The use of the resected samples was approved by the ethics committee.

The study analyzed 106 male and 61 female CRC patients, with a mean age of 63.0 years (range, 35-84 years) at surgery. Primary tumors were distributed in the colon (n = 94) and rectum (n = 73) and classified as well-differentiated adenocarcinomas (n = 44), moderately differentiated adenocarcinomas (n = 113), and poorly differentiated carcinomas (n = 10). Seventy-seven patients had lymph node metastasis, whereas 90 patients were node-negative. Histology of adenomatous polyps indicated 22 low-grade dysplasia and 3 high-grade dysplasia.

Antibodies. Mouse anti-Cx26 monoclonal antibody 13-8100, obtained from Zymed Laboratories, Inc. (Invitrogen Corporation), recognizes the human Cx26 protein (molecular weight, 26.5 kDa). The rabbit anti-human actin antibody was purchased from Sigma-Aldrich.

H&E staining and immunohistochemistry. Tissue sections (4-μm thick) were deparaffinized in xylene, rehydrated, and stained with H&E solution for histologic diagnosis by the two pathologists. The tissue sections were autoclaved at 121°C for 20 min for antigen retrieval and processed for immunohistochemistry using the Vectastain ABC peroxidase kit (Vector Laboratories), as described previously (22, 23). The slides were incubated with appropriate primary antibodies overnight at 4°C, and the Cx26 antibody was used at a 1:500 dilution. Nonimmunized mouse IgG (Vector Laboratories) was used as a negative control and substitute for the primary antibody to exclude possible false-positive responses from the secondary antibody or from nonspecific binding of IgG.

Immunohistochemical assessment. All immunostained tissue sections were first evaluated by K.E., well trained in the pathology department, in a coded manner without knowledge of the clinical and pathologic background of patients. In each section, three high-power fields were randomly selected at three different occasions, and a total of nine fields and at least 2,000 cells were evaluated. To assess Cx26 expression, the percentage of clearly stained cells was calculated irrespective of intensity, as intensity was rather subjective and often not reproducible. Half of the specimens were then randomly selected and evaluated by H.Y. No discrepancy between the evaluations was detected.

Western blot analysis. Western blot analysis was done as described previously (24). Briefly, the protein samples (50 μg) were separated by 10% PAGE, followed by electroblotting onto a polyvinylidene difluoride membrane. The membrane was incubated with the primary antibodies at the appropriate concentrations (1:500 for Cx26 and 1:1000 for actin) for 1 h. The protein bands were detected using the Amersham enhanced chemiluminescence detection system (Amersham Biosciences Corp.). The differential expression of Cx26 in the membrane and the cytosolic fraction was assessed as described previously (25).

Quantitative real-time PCR for Cx26 mRNA. Total RNA was extracted using the TRIZOL reagent (Life Technologies, Inc.). cDNA was generated from 1 μg RNA with avian myeloblastosis virus reverse transcriptase (Promega). Quantitative real-time PCR was done using LightCycler (Idaho Technology, Inc.), as described previously (22). Quantification data from each sample were analyzed using the LightCycler analysis software. The transcription value of *Cx26* was determined by plotting against the standard curve constructed using MKN45 gastric cancer cells. The amount of each transcript was normalized against the expression of the housekeeping gene *β-actin* (24) from the same sample. The primer sequences were as follows: β-actin sense 5′-GAAAATCTGGCACCACACCTT-3′, β-actin antisense 5′-GTTGAAGGTAGTTTCGTGGAT-3′, Cx26 sense 5′-GCT GCA AGA ACG TGT GCT AC-3′, and Cx26 antisense 5′-TGG GTT TTG ATC TCC TCG AT-3′.

Statistical analysis. Statistical analysis was done using the StatView J-5.0 program (Abacus Concepts, Inc.). The Kaplan-Meier method was used to estimate tumor recurrence from CRC, and the log-rank test was used to determine the statistical significance. Associations between discrete variables were assessed using the χ^2 test. Mean values were compared using the Mann-Whitney test. All data were expressed as the mean ± SD. P values of <0.05 were accepted as statistically significant.

Results

Aberrant expression of Cx26 in multistage carcinogenesis of the colorectum. We first examined Cx26 expression by immunostaining CRC and normal colonic mucosa tissues. Sections were incubated with nonimmunized mouse IgG as a negative control (data not shown). In normal colonic mucosa, the Cx26 protein was detected mainly on the plasma membrane (Fig. 1A). High magnification revealed an intercellular staining of Cx26, and occasionally a few granules were noted in the cytoplasm of the normal epithelium (Fig. 1B). In adenomatous polyps, a similar expression pattern of Cx26 was noted (Fig. 1C). In contrast, the CRC tissues heterogeneously expressed the Cx26 protein mainly in the cytoplasm (Fig. 1D). Cytoplasmic accumulation of Cx26 was detected in 84.4% (141 of 167) of the CRCs. Membranous staining of Cx26 in CRC was present but focal and rather exceptional (16 of 167, 9.6%). In a carcinoma concurrently existing with adenomatous tissue (Fig. 1E), cytoplasmic Cx26 was detected in carcinoma cells (Fig. 1F), whereas membranous Cx26 localization was observed in the adenomatous tissue (Fig. 1G). The CRC tissues exhibited a wide range of cytoplasmic Cx26-positive cells, ranging from 0 to 100% (mean, 49.5 ± 35.5%; Fig. 2).

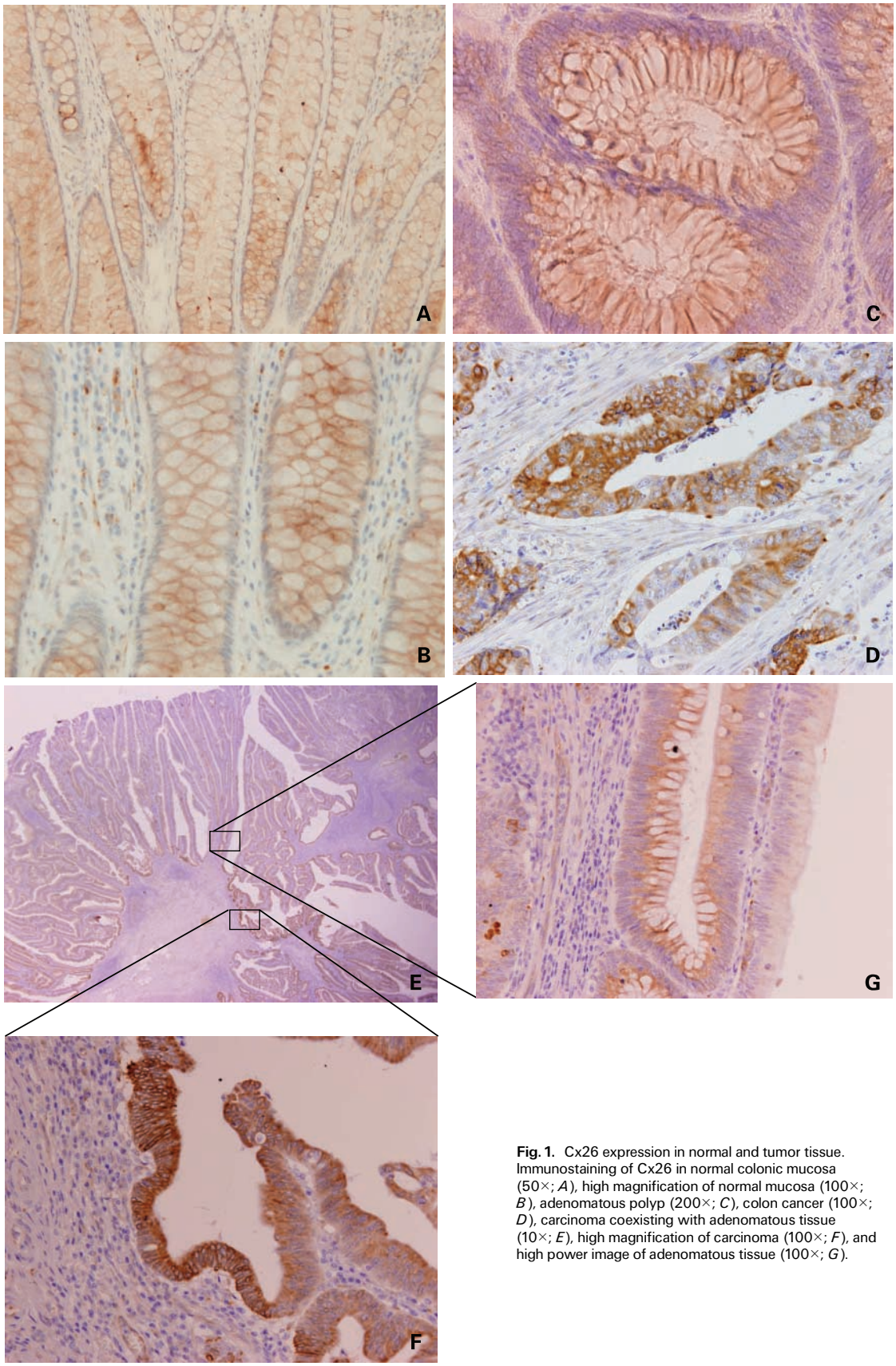

Fig. 1. Cx26 expression in normal and tumor tissue. Immunostaining of Cx26 in normal colonic mucosa (50×; *A*), high magnification of normal mucosa (100×; *B*), adenomatous polyp (200×; *C*), colon cancer (100×; *D*), carcinoma coexisting with adenomatous tissue (10×; *E*), high magnification of carcinoma (100×; *F*), and high power image of adenomatous tissue (100×; *G*).

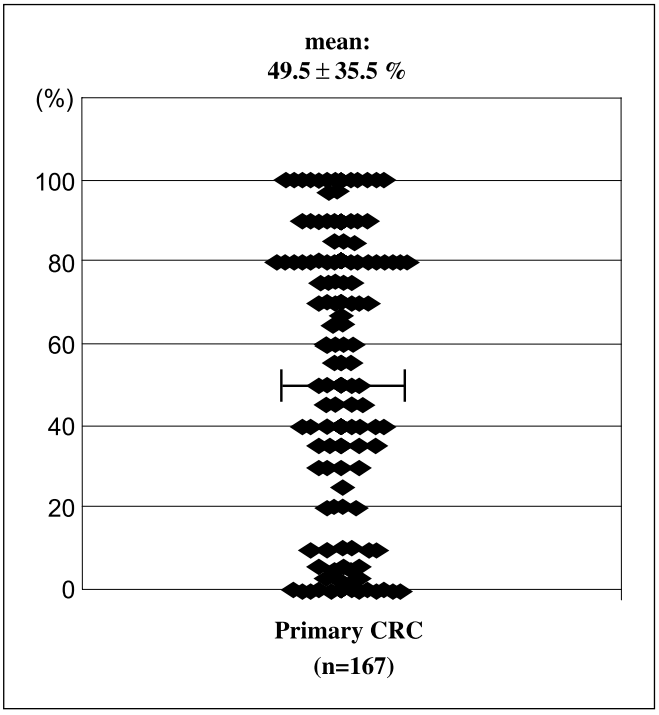

Fig. 2. Incidence of Cx26-positive cancer cells in CRC tissue. Cytoplasmic Cx26-positive cancer cells in CRC tissue were evaluated and plotted on the graph. Cx26 expression varied from 0 to 100%. Horizontal bar, mean value of 49.5 ± 35.5%.

Western blot analysis. Based on the mean value of Cx26 expression in CRC tissues at 49.5%, the cancer specimens were divided into two groups: those exhibiting high expression (n = 87, 52.1%), and those with low expression (n = 80, 47.9%). Tissue samples were randomly chosen from the low (n = 3) and high groups (n = 5), and Western blot analysis was done on the samples in parallel with the corresponding normal mucosa (Fig. 3A). The increased level of Cx26 protein in cancer tissues as detected by Western blot correlated well with the enhanced cytoplasmic accumulation of Cx26 in immunostaining. We also examined additional three paired samples on either end of the expression spectrum (tumor tissues with 10%, 80%, and 90% Cx26 expression) and observed an apparent correlation of Cx26 levels in cancer tissues between Western blot and immunohistochemical analysis (data not shown).

To confirm Cx26 cellular localization in tumor and normal tissue, we did Western blot analysis on fractionated cell lysates. Cx26 was present mainly in the membrane fraction in normal mucosa, whereas it was detected mostly in the cytosolic fraction in cancer tissues (Fig. 3B).

Cx26 RNA level in CRC tissues. Ten tumor tissues from both the low and high groups were subjected to quantitative reverse transcription–PCR analysis of *Cx26* RNA levels. We confirmed that the tumor samples expressing high levels of Cx26 protein generally expressed high levels of *Cx26* RNA, whereas samples with low Cx26 protein levels exhibited low levels of *Cx26* RNA (Fig. 3C).

Aberrant expression of Cx26 in CRC and clinicopathologic findings. The clinical pathologic survey indicated that high Cx26 expression was associated with a less differentiated histologic type and moderate to plenty venous invasion, as shown in Table 1 (P = 0.0053 and P = 0.0084, respectively). Consistent with this, we often observed diffused Cx26 expression in cancer cells invading the vein in the CRC tissue (Fig. 4).

Disease-free survival after curative surgery. Disease-free survival reflects the nature of cancer more specifically than does overall survival, because the latter does not distinguish patients with recurrent disease from those without recurrence (26). Moreover, disease recurrence is a practical concern after curative surgery. Therefore, we next analyzed the relationship between Cx26 expression and disease-free survival in 154 primary CRCs that were "curatively" resected by surgery (mean level of Cx26 expression, 50.3%). Notably, the group highly expressing Cx26 was associated with a significantly shorter disease-free survival compared with the group expressing low Cx26 levels (Fig. 5A; P = 0.041).

We then analyzed site-specific relapse-free survival according to the recurrent mode, i.e., recurrence in liver, lung, local site, and peritoneum, in patients stratified by Cx26 expression levels. Lung metastasis–free survival curves indicated that high Cx26 expression was linked to lung metastasis (P = 0.028; Fig. 5B), but no difference was observed in liver metastasis–free survival

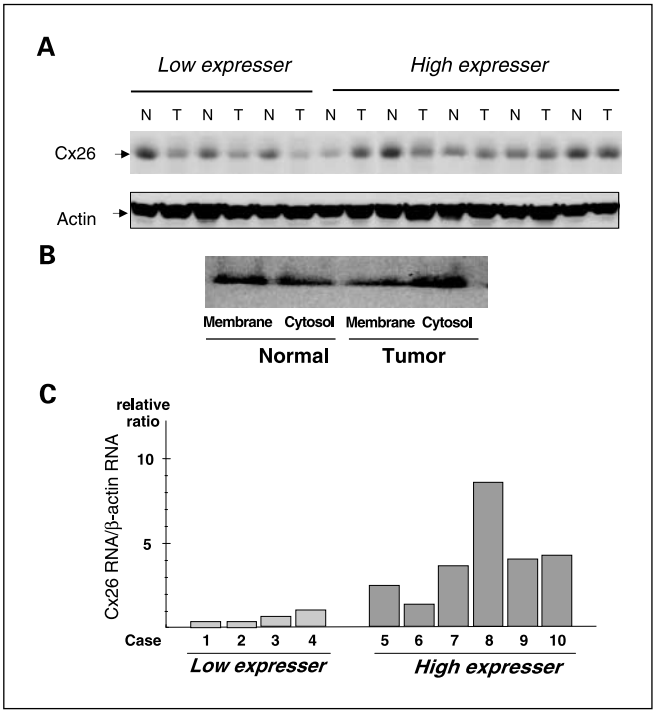

Fig. 3. Western blot analysis of Cx26 in normal and cancer tissue. *A,* CRC tissues and the corresponding normal mucosa were examined for Cx26 levels. Tissue samples were randomly chosen from the low group (n = 3) and high group (n = 5) and classified based on Cx26 expression by immunostaining. Levels of Cx26 in cancer tissues correlated well with the cytoplasmic accumulation of Cx26. N, normal mucosa; T, tumor. *B,* differential expression of Cx26 in the membrane and cytosol fractions. Fractionation revealed that Cx26 was localized mainly in membrane fraction in normal tissues and in the cytosol fraction in cancer tissues. The low Cx26 protein level detected in the cytosol of normal mucosa could be derived from intercellular Cx26 and also from the endoplasmic reticulum and Golgi apparatus. *C,* reverse transcription – PCR of *Cx26* RNA in 10 tumor tissues. *Cx26* RNA level was normalized against levels of the house keeping *β-actin* RNA that served as a control reference (24). The *Cx26* RNA levels in tumor tissues correlated well with Cx26 protein levels by immunohistochemistry. High expressers and low expressers, defined based on Cx26 expression by immunohistochemistry, were randomly chosen.

Table 1. Relationship between aberrant Cx26 expression and clinicopathologic variables

		Low (n = 80)	High (n = 87)	P
Gender	Male	51 (31%)	55 (33%)	N.S.
	Female	29 (17%)	32 (19%)	
Age		63.4 ± 10.6	62.3 ± 11.0	N.S.
Tumor size		46.1 ± 22.4	47.6 ± 21.7	N.S.
Site	Colon	47 (28%)	47 (28%)	N.S.
	Rectum	33 (20%)	40 (24%)	
Lymph node metastasis	Negative	48 (29%)	42 (25%)	N.S.
	Positive	32 (19%)	45 (27%)	
Depth of invasion	~mp	25 (15%)	23 (14%)	N.S.
	ss~	55 (33%)	64 (38%)	
Differentiation	Well	29 (17%)	15 (9%)	0.0053
	Moderately/poorly	51 (31%)	72 (43%)	
Venous invasion*	0/1+	77 (46%)	73 (44%)	0.0084
	2+/3+	3 (2%)	14 (8%)	
Stage	~II	45 (26%)	37 (22%)	N.S.
	III~	35 (21%)	50 (29%)	

Abbreviations: mp, muscularis propria; ss, subserosa; N.S., not significant.
*Venous invasion: classified according to the pathology reports: 0, none; 1+, slight; 2+, moderate; 3+, plenty.

or with other sites (data not shown). In multivariate analysis with clinicopathologic variables, such as lymph node metastasis, depth of invasion, differentiation grade, venous invasion, and lymphatic invasion, Cx26 expression was not a significant predictive factor of disease recurrence, although we detected a predictive association with lymph node metastasis and depth of invasion (P = 0.019 and P = 0.016, respectively).

Expression of Cx26 in metastatic lesions. We then examined Cx26 expression in metastatic lesions in lymph nodes, livers, and lungs, together with the corresponding primary CRCs (Fig. 6). Compared with the metastatic lesions of lymph nodes and liver, lung metastatic tissue broadly expressed abundant Cx26 expression (Fig. 6A). The mean value of Cx26 expression in lung metastatic tissue, but not in other tissues, was significantly higher than that of the mean value of Cx26 expression in CRCs (P < 0.0001; Fig. 6B). Furthermore, compared with the paired primary CRC lesion, lung metastasis showed a significantly higher incidence of Cx26 expression (P = 0.0001; Fig. 6C).

Discussion

Gap junctions and the connexin subunits are shown to be down-regulated in various cancers (5–7), but accumulating evidence indicates that the specific connexin Cx26 is increased in various carcinomas (12–21). Our preliminary immunohistochemical studies confirmed these studies and indicated that Cx26 expression was induced not only in CRCs but also in a considerable fraction of other gastrointestinal malignancies, including gastric cancer (62.5%, five of eight), squamous esophageal cancer (75%, six of eight), and pancreatic cancer (87.5%, seven of eight; data not shown).

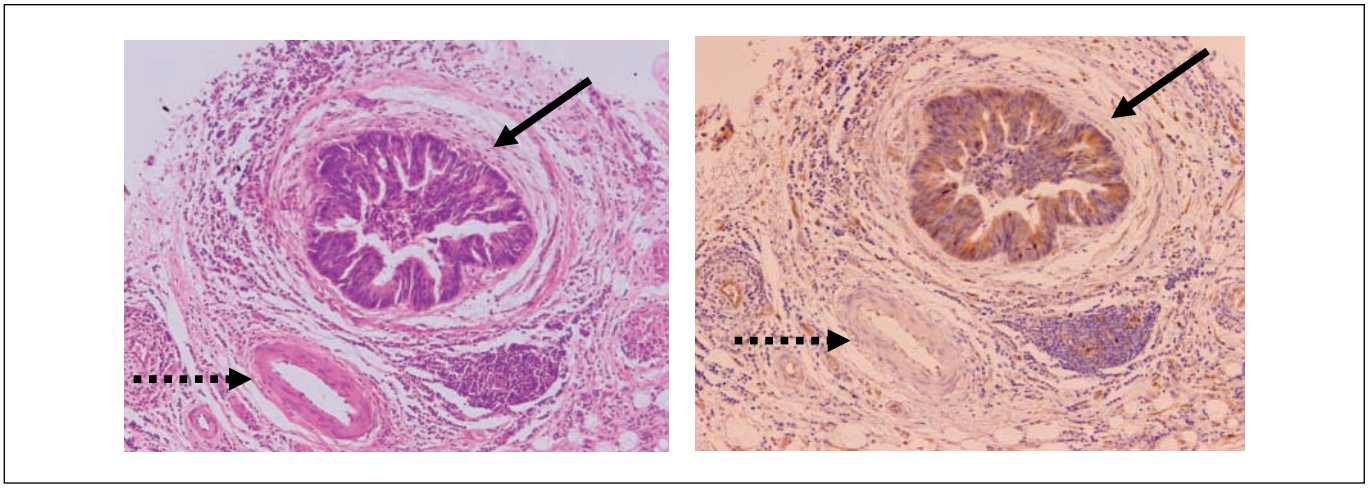

Fig. 4. Cx26 expression in cancer cells undergoing vascular invasion. Left, H&E staining. Numerous cancer cells invading the venous lumen were detected (*arrow*). The arterial lumen is observed below and to the left of the vein (*dotted arrow*). Right, immunostaining of Cx26. The cancer cells undergoing vascular invasion exhibited diffuse Cx26 expression. Magnification, 200×.

To carefully assess the relevance of Cx26 expression in malignant cells, we selected CRC, the multistage carcinogenesis model of normal colon to adenoma to cancer tissue (27), to reveal how alterations in Cx26 expression may accompany malignant transformation. As a result, we found that normal epithelium and adenomatous tissue in colon polyps expressed the Cx26 protein on the plasma membrane, whereas cancer tissue mainly displayed cytoplasmic Cx26 accumulation. In a parallel study, Kanczuga-Koda and colleagues analyzed Cx26 expression during colorectal carcinogenesis and found intercellular Cx26 staining in normal epithelium and adenoma and cytoplasmic Cx26 staining in carcinomas, consistent with our results (14). Moreover, abundant Cx26 expression was shown to be associated with poor prognosis of lung squamous cell carcinoma and breast carcinoma (20, 21). Taken together, these findings underscore the previously observed complexity of Cx26 function in human malignancies and its previously suggested cellular activity as a tumor suppressor gene (15, 17, 19).

A recent study suggested Cx26 as a critical facilitator in the spontaneous lung metastasis observed in a subcutaneous tumor derived from a mouse melanoma cells (19). To ascertain the role of Cx26 in disease recurrence and metastasis of CRC, we analyzed both primary CRC tissues and metastatic lesions, such as lung, liver, and lymph node. Although lymph node metastasis was previously examined in lung and breast carcinoma (18, 20), distant metastases have not been examined for Cx26 expression. Notably, univariate analysis indicated that aberrant expression of Cx26 in primary CRCs was linked to disease relapse of CRC. In particular, our data on lung metastasis–free survival curves would account for much of the Cx26-related lung metastasis. Although multivariate analysis indicated that Cx26 expression was not an independent predictor for disease recurrence, Cx26 may not be a universal prognostic factor but rather specifically associates with lung metastasis. Consistent with these findings, we found that lung metastatic lesions, but not liver and lymph node metastases, broadly expressed Cx26 at a very high incidence. In Japan, the liver and lung are the two major organ sites of metastases from CRC, and the ratio of liver metastasis to lung metastasis is 3:2 (28). In the current study, the prevalence of lung metastasis was more than that of liver metastasis; nevertheless, it is noteworthy that the lung metastases exclusively expressed abundant Cx26 expression.

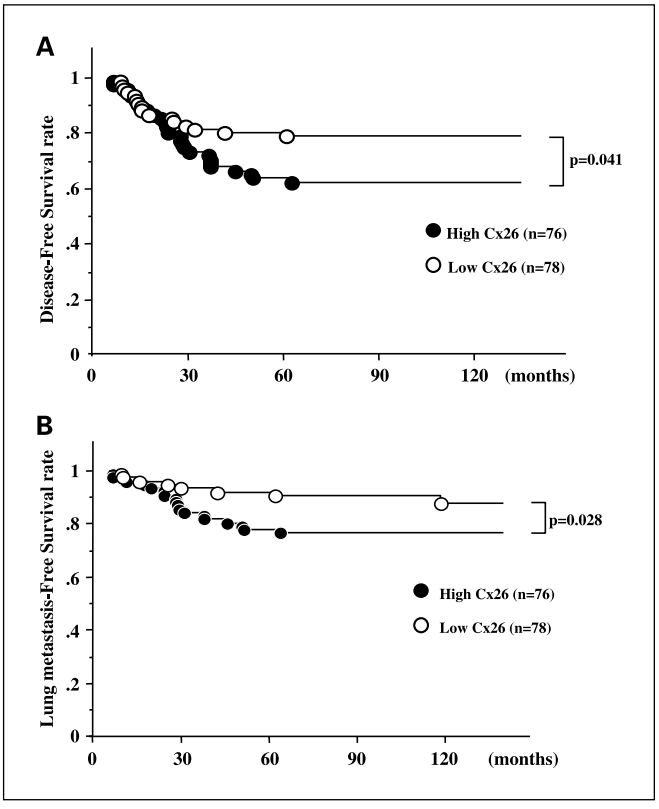

Fig. 5. Disease-free survival after curative surgery. *A,* the high Cx26 group was associated with significantly shorter disease-free survival than the low Cx26 group ($P = 0.041$). *B,* lung metastasis – free survival curves indicated that the high Cx26 expressing group was associated with lung metastasis ($P = 0.028$).

Investigation of metastatic foci provides critical insights as to the contribution of Cx26 in development of metastases. Even when the number of Cx26-positive cells is relatively low in a CRC, the tumor population of metastatic lesions is predicted to be largely Cx26-positive if Cx26 is indispensable to form distant metastasis. This prediction is based on the theory proposed by Fidler, which states that highly metastatic tumor cell variants preexist in the parental tumor population and induce metastasis to other organs (29). To address this possibility, we compared Cx26 expression in primary CRC samples compared with corresponding lung metastatic tissue and found that lung metastases expressed significantly higher levels of Cx26 than did primary CRCs (Fig. 6C). These findings strongly suggest that Cx26 may play an essential role in the establishment of lung metastasis from primary CRC.

In normal cells, we detected Cx26 localization mainly on the plasma membrane and in the intercellular space, as reported previously (14). A few granules in the cytoplasm were also occasionally noted, likely reflecting the presence of connexin polypeptides in the endoplasmic reticulum and Golgi apparatus where connexin synthesis occurs (14, 30). In contrast, we observed cytoplasmic accumulation of Cx26 via immunohistochemical analysis of CRC samples. This indicates that Cx26 translocated from the plasma membrane to the cytoplasm in tumor cells, as previously observed (14, 17, 18, 31, 32). Interestingly, the translocation occurred even at an early cancer stage, i.e., carcinoma with adenomatous tissue (Fig. 1D-F). Although the precise function of cytoplasmic Cx26 is not yet clear, one possibility is that cytoplasmic accumulation of Cx26 may be required to exert their roles in the plasma membrane, contributing to gap junction formation as needed.

That many studies have shown that introduction of the Cx26 gene into tumor cells usually causes growth inhibition *in vitro* and *in vivo* (10, 11) is not necessarily inconsistent with our findings that Cx26 may facilitate lung metastasis. Indeed, inhibitory effects of tumor growth via gap junction formation may suppress initial tumor formation (8–11); however, once malignant formation is completed, growth arrest of cancer cells may be rather essential to their extravasation at a later metastasis stage (33). Furthermore, gap junctions may have distinct functional roles in cell growth and cell invasion, as a gap junction inhibitor decreased the invasion of prostate cancer cells (15). In a lung metastasis model using melanoma cells, Cx26 was indispensable for dye transmission from melanoma cells to the vascular endothelial cells (19), suggesting that transmission of certain micromolecules derived from cancer

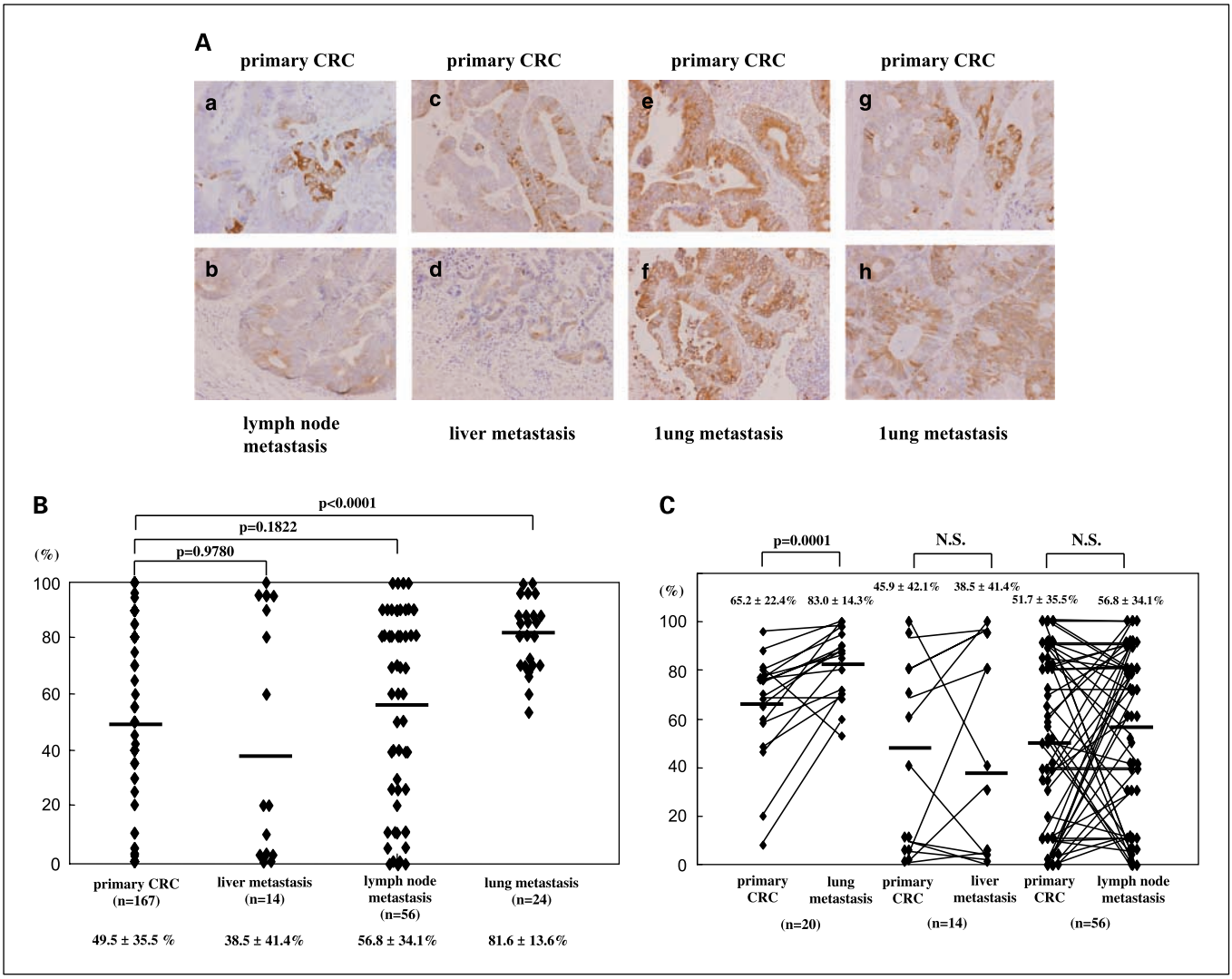

Fig. 6. *A,* immunostaining of paired primary CRC and metastatic lesions: (*a*) primary CRC and (*b*) the corresponding lymph node metastasis; (*c*) primary CRC and (*d*) the liver metastasis; (*e*) CRC and (*f*) lung metastasis (both expressed abundant Cx26); and (*g*) CRC and (*h*) lung metastasis. Lung metastatic tissue expressed more abundant Cx26 than the primary CRC. Magnification, 100×. *B,* Cx26 expression in CRC and metastatic lesions. Lung metastatic tissue exhibited a significantly higher incidence of Cx26 compared with CRC tissue ($P < 0.0001$). *C,* comparison of Cx26 expression in paired primary CRC and metastatic tissue. Lung metastatic tissue displayed significantly higher Cx26 expression compared with the corresponding primary CRC ($n = 20$, $P = 0.0001$).

cells might contribute to endothelial retraction and facilitate vascular invasion. Alternatively, several studies showed that Cx26 directly down-regulated expression of the fibroblast growth factor receptor-3 and Bcl-2 genes in a gap junction–independent manner (34, 35). Therefore, we suggest the possibility that Cx26 might have distinct metastasis-promoting function(s) independent of gap junction intercellular communication. Supporting this model, a recent study reported a correlation between Cx26 expression and insulin-like growth factor I receptor, whose overexpression induces active proliferation and inhibition of apoptosis of colon cancer cells (36). Therefore, one possibility is that the insulin-like growth factor system may be involved in the regulation of Cx26 and may contribute to Cx26-mediated disease progression.

Cx26 may likely facilitate the venous invasion of cancer cells (Table 1; Fig. 4). Gap junction formation might also facilitate the attachment of cancer cells to the vascular endothelial cells at the distant organ, also known as the "spiked tire hypothesis" (37). Among hematogenous metastases, however, why Cx26 was highly expressed in metastasized tumor cells in the lung but not in the liver is not clear and likely due to organ-specific factors. Recent gene profiling analyses using cDNA microarrays identified various candidate genes essential for liver metastasis from CRC (38, 39). In contrast, the gene profile of lung metastasis completely differed from that of liver metastasis in the same CRC population.[6] This is consistent with the seed and soil theory, suggesting that different organs provide growth conditions optimized for specific cancers (40) or contain organ-specific combinations of certain chemokines and receptors (41).

Our data also provide important clinical implications for Cx26 as a potential preventive target against lung metastasis. The selective Cx26 inhibitor oleamide derivatives MI-18 and

[6] Our unpublished data.

MI-22, both of which inhibited Cx26-mediated gap junction intercellular communication and drastically suppressed spontaneous lung metastasis of melanoma cells, are already feasible as clinically important prototypes (30, 42). Particularly, MI-22 is now commercially available from WAKO, can be easily dissolved in oily solvents, and thus is likely to be more useful for clinical treatment. Lung metastasis is one of the two major problems after surgery for local recurrence of rectal cancer as well as local re-recurrence (43). The incidence of lung metastasis after abdominal sacral resection is much higher than that after surgery of the primary CRC. Therefore, it is possible that the Cx26 inhibitor may be useful as a preventive agent.

In conclusion, our study found that Cx26 was aberrantly expressed in a considerable fraction of CRC and that high Cx26 levels was associated particular with lung metastasis.

Acknowledgments

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