obtained from Riken Cell Bank (Tsukuba, Japan). The stock cultures were grown at 37 °C under 5 % CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 5 % fetal bovine serum (FBS), 10 μg/ml insulin, 50 U/ml penicillin, 50 U/ml streptomycin, and 4 mM glutamine. The pressure-loading apparatus was set up as previously described [19]. The cell culture plates were placed in an acrylic flask from which the upper panel had been removed, and the flask was then resealed with a rubber seal and tightly clamped. The rubber seal was pierced by a needle connected to tubing attached to a 3-way rotary valve, a sphygmomanometer, and a pressure valve. Compressed helium was pumped into the flask to raise the internal pressure; none of the prepacked room air was released during this process. The flask was kept at 37 °C throughout pressurization.

Cells were cultured in 96-well multiwell plates (200 cells per well) in DMEM containing 5 % FBS. The cells were allowed to attach to their substrates for 24 h, and then the culture medium was changed to DMEM containing 0.1 % FBS for the growth experiments. Four and 48 h after each treatment, the numbers of cells were determined as follows. At the end of the incubation period, the rate of reduction of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salts] to formazan was assessed using the CellTiter 96 AQ non-radioactive cell proliferation assay kit (Promega Co., Madison, WI, USA) and the absorbance of formazan at 490 nm was measured.

DNA synthesis was assessed with an immunocytochemical assay kit that uses monoclonal anti-bromodeoxyuridine (BrdU) antibody to detect the incorporation of BrdU into the cellular DNA (RPN 210; Amersham Corp., Tokyo, Japan) [19]. Bound antibody was detected using a peroxidase-conjugated antibody to mouse IgG_{2a} and a peroxidase substrate, yielding a soluble green reaction product proportional to the amount of BrdU incorporated.

The number of cells with membrane barrier dysfunction was determined using the cationic fluorescence dye propidium iodide (PI, Molecular Probes, Inc., Eugene, OR, USA).

MAPK Inhibition and Determination of MAPK-Associated Phosphorylation

The effect of inhibition of MAPK on pressure-induced cell proliferation was assessed by adding the selective MEK1 inhibitor PD98059 (Calbiochem, San Diego, CA, USA) [29] to the culture medium. The agent was added just before the cells were pressure-loaded.

MAPK activity was assessed using a p42/44 MAPK enzyme assay system (Amersham International plc, Buckinghamshire, UK), which measures incorporation of ^{32}[P]-ATP into a synthetic peptide substrate that is highly selective for p44/42 MAPK. MAPK activity was assessed by counting the radioactivity with a scintillation counter. The peptide used in this assay is based on the Thr^{669} phosphorylation site of the epidermal growth factor (EGF) receptor and has been modified to contain only one phosphorylation site.

The activity of the MAP kinases extracellular signal-regulated kinase (ERK) was assessed by western blot analysis using a p44/42 MAPK assay kit (New England Biolabs Inc., Beverly, MA, USA). The activity of the c-Jun NH_2-terminal kinase (JNK) was assessed by western blot analysis using a SAPK/JNK assay kit (New England Biolabs Inc., Beverly, MA, USA). The activity of the p38 MAP kinases was assessed by western blot analysis using a p38 MAP kinase assay kit (New England Biolabs Inc., Beverly, MA). The RGM-1 cells (1×10^6) in the flasks were exposed to a pressure of 80 mmHg for 48 h, washed with PBS, and lysed in ice-cold cell lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid [EDTA], 1 mM EGTA, 1 % Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin) with 1 mM phenylmethylsulfonyl fluoride (PMSF). The lysates were clarified by microcentrifugation and each MAPK activity was assessed according to the manufacturer's protocol. Briefly, p44/42 and p38 MAPK were immunoprecipitated using Sepharose bead conjugated phospho-specific anti-p44/42 MAPK monoclonal antibody or agarose hydrazide beads conjugated phospho-p38 MAP Kinase (Thr180/Tyr182) monoclonal antibody for 4 h. The resulting immunoprecipitants were incubated for 30 min with Elk-1 or ATF-2 fusion protein in the presence of 200 μM adenosine triphosphate (ATP) and kinase buffer (25 mM Tris pH 7.5, 5 mM β-glycerophosphate, 2 mM dithiothreitol [DTT], 0.1 mM Na_3VO_4, and 10 mM $MgCl_2$) in order to allow the immunoprecipitated active MAPK to phosphorylate each substrate. For assessing JNK activity, the lysates were incubated with GST-c-Jun fusion bound to glutathione agarose beads in the presence of 200 μM ATP. The phosphorylation reactions were terminated by mixing the lysate samples with 25 μl each of 3× sodium dodecyl sulfate (SDS) sample buffer (187.5 mM Tris–HCl pH 6.8), boiled for 5 min, and subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE). The proteins were transferred to nitrocellulose membranes and probed with the appropriate antibodies. The western blots were developed as follows: the membranes were blocked with a blocking buffer (1× Tris-buffered saline [TBS] with 0.1 % Tween-20 and 5 % w/v nonfat dry milk) and incubated with primary antibody (phospho-specific anti-Elk-1 antibody, phospho-c-Jun [Ser63] antibody, phospho-ATF-2 [Thr71] antibody [1:1,000]) overnight and then with horseradish

peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1:2,000) or HRP-conjugated anti-biotin antibody (1:2,000) for 1 h. Finally, the membranes were incubated with 10 mM 1× LumiGLO (20× LumiGLO, 20× peroxide, and water) for 1 min and the bands visualized by chemiluminescence.

PCR Amplification of IREGs

Total RNA was isolated from RGM-1 cells using RNAzol (Biotcx, Houston, TX, USA). The cells were lysed by the addition of 1.0 ml of RNAzol to each dish, and the RNA was isolated and extracted according to the manufacturer's protocol. Briefly, the RNA was extracted with chloroform, precipitated with isopropanol, and then washed with 70 % ethanol. The concentration of the extracted RNA was calculated by measuring the optical density at 260 nm, and the 260/280 ratio was >1.9 for all RNA preparations. The RNA quality was assessed based on the integrity of the 28S and 18S bands and the absence of degradation during agarose gel electrophoresis.

Aliquots of RNA (5 μg) were reverse-transcribed using 1st Strand cDNA Synthesis Kit (Stratagene, La Jolla, CA). In brief, 5 μg of RNA in 38 μl of diethyl pyrocarbonate-treated water was mixed with 0.3 μg of oligo (dT), heated at 65 °C for 5 min, and then cooled slowly to room temperature. The following reagents were then added to the tubes: 5 μl of 10× concentrated synthesis buffer (final concentrations, 10 mM Tris–HCl pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$), 1 μl of RNase block (40 U/μl), 2 μl of 100 mM dNTPs, and 1 μl of Moloney murine leukemia virus reverse transcriptase (50 U/μl). The reaction mixture was incubated for 1 h at 37 °C and the reaction was then terminated by incubating the tubes at 90 °C for 5 min and placing them on ice for 10 min. The tubes were stored at −80 °C pending PCR. PCR was performed using the Takara Taq kit (recombinant Taq DNA polymerase; Takara Biochemicals, Tokyo, Japan) with rat-specific primers prepared on a DNA synthesizer (Sawady Technology, Tokyo, Japan). The sequences of the primers designed to amplify the cDNA sequences of rat *c-fos*, *c-myc*, and *c-jun* were as follows:

c-fos	Sense	5′-GGTCATCGGGGATCTTGC-3′	
	Antisense	5′-GGGCTCTCCTGTCAAC-3′	511 bp
c-myc	Sense	5′-AGGTCCGAGTCAGGGTCATC-3′	
	Antisense	5′-AACTGTTCTCGCCGTTTCCT-3′	455 bp
c-jun	Sense	5′-CGACCTTCTACGACGATGCC-3′	
	Antisense	5′-TCGGTGTAGTGGTGATGTGC-3′	258 bp

The predicted lengths of the cDNA amplification products were 511 bp for *c-fos*, 455 bp for *c-myc*, and 258 bp for *c-jun*. The PCR was initiated by adding 2 μl of reverse transcriptase products to a PCR master mix containing 10× PCR reaction buffer diluted to final concentrations of 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of recombinant Taq DNA polymerase, 50 pM each of the primers, and 200 μM dNTPs. The tubes were placed in a Programmed Tempcontrol system (Applied Biosystems, Tokyo, Japan) that was programmed as follows: incubation for 3 min at 94 °C for initial denaturation, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min for *c-fos*, 25 cycles of denaturation at 94 °C for 0.5 min, annealing at 52 °C for 1 min, and extension at 74 °C for 1 min for *c-myc*, or 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min (annealing), and extension at 72 °C for 3 min for *c-jun*, followed by a final extension at 72 °C for 7 min for all products. The PCR products were size-fractionated by agarose gel electrophoresis, stained with ethidium bromide, and then visualized with an ultraviolet transilluminator.

Immunofluorescence Staining for c-Myc and c-Fos Proteins

The distributions of the c-Fos and c-Myc proteins in the cultured cells were determined by immunofluorescence staining using specific monoclonal antibodies. Cells were cultured in 8-well Lab Tek tissue culture chambers, washed three times with PBS (pH 7.4), and fixed with 4 % paraformaldehyde for 30 min. The fixed cells were washed three more times with PBS and then incubated for 5 min in PBS containing 0.1 % Triton X 100. The permeabilized cells were rinsed with PBS and incubated at 4 °C for 60 min with PBS containing 1 % BSA, a mouse monoclonal antibody (IgG_{2a}) directed against c-Fos (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and a rabbit polyclonal antibody (IgG) directed against c-Myc (Santa Cruz Biotechnology, Inc.). After hybridization, the cells were washed three times with PBS containing 1 % BSA and incubated at room temperature for 30 min in the dark with an FITC-conjugated rabbit anti-mouse IgG antibody (20 μg/ml, Chemicon International, Inc., Temecula, CA, USA) in PBS containing 1 % BSA. The cells were then washed again, mounted on the chamber slide, and covered with a non-fluorescent cover slip. Confocal imaging was performed with an MRC 600 confocal microscope (Bio Rad, Watford, UK) mounted on an inverted-type fluorescence microscope (Diaphot, TMD-2S, Nikon, Tokyo, Japan) with a 63× oil immersion lens. A multiple-line argon-ion laser beam (25 mW) was used for excitation at 488 nm.

DNA-Binding Activity of AP-1

The DNA-binding activity of AP-1 was determined by electrophoretic mobility shift assay (EMSA) using a FITC-labeled consensus AP-1 oligonucleotide as the probe. Subconfluent RGM-1 cells (1×10^6) were exposed to a pressure of 80 mmHg for 1–2 h. The proteins were then extracted as follows. The cells were washed with PBS, collected by scraping with a cell scraper, and homogenized at 4 °C in Buffer A (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM PMSF, 1 mM DTT, and 1 μg/ml each of antipapain, chymostatin, leupeptin, and peptin A). The suspension was then centrifuged at 11,000 rpm for 2 min and the supernatant removed. The precipitate was homogenized again in Buffer A and centrifuged at 11,000 rpm for 2 min and the supernatant removed. This precipitate was then homogenized for 40 min at 4 °C in Buffer C (20 mM HEPES pH 7.9, 10 mM KCl, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM PMSF, 1 mM DTT, and 1 μg/ml each of antipapain, chymostatin, leupeptin, and peptin A), and the suspension was centrifuged at 15,000 rpm for 10 min and the precipitate removed. The protein concentrations of the extracts were measured, and the extracts were stored at −70 °C until the gel shift assays with FITC-labeled AP-1 oligonucleotide (5′-CGCTTGATGAGT-CAGCCGGAA-3′) were performed. In brief, 20 μg of extracted protein was incubated with AP-1 oligonucleotide (1 ng) for 45 min at room temperature, mixed with 50 % sucrose (5 μl), and subjected to electrophoresis at 160 V on nondenaturing 5 % polyacrylamide gels for 1 h. The fluorescence intensities of the bands in the gels were quantified using a fluorescence laser scanning system equipped with a computer-assisted image analyzer (FluorImager 575; Molecular Dynamics, Sunnyvale, CA, USA).

Statistical Analysis

All results were expressed as means ± standard errors of the mean (SEM), and differences between groups were evaluated by one-way analysis of variance (ANOVA) and Fisher's post hoc test. Statistical significance was set at $P < 0.05$ for all tests.

Results

Pressure-Induced Cell Proliferation and Inhibition Studies

RGM-1 cells exhibited proliferation in response to exposure to high transmural pressure without shear stress. Exposure of the cells to high transmural pressures in the range of 40–120 mmHg resulted in significantly increased cell numbers after treatment for 48 h but not 24 h (Fig. 1). The greatest cell number was observed after treatment at a pressure of 80 mmHg and was about 1.8 times (182 ± 9 %, $P < 0.05$) than that of the control (0 mmHg). Light microscopy and trypan blue exclusion assays at the end of the experiments showed that the cells were intact, and the number of detached cells was negligible even after exposure to pressures up to 160 mmHg (data not shown). Examination of BrdU incorporation into the cellular DNA revealed a pressure-induced increase in DNA synthesis even 24 h after the beginning of the high transmural pressure treatment (Fig. 2); therefore, the enhanced DNA synthesis preceded the increased cell numbers.

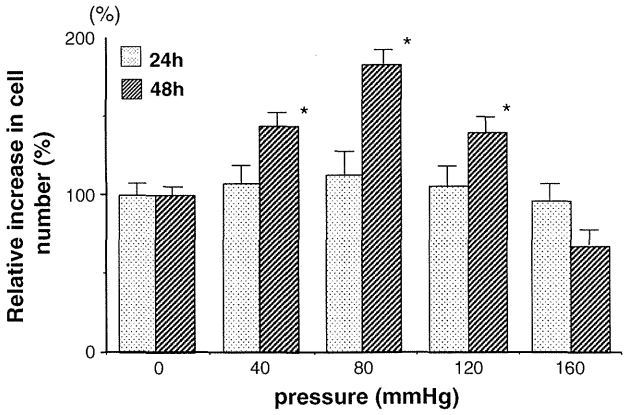

Fig. 1 Effect on cell proliferation of exposure of RGM-1 cells to high transmural pressures. RGM-1 cells in medium containing 0.1 % FBS were subjected to high transmural pressures, and the cell numbers were counted after exposure for 24 and 48 h. Values are expressed as the mean ± standard error of the mean (SEM) ($n = 6$) % increase relative to the 0 mmHg control value. *$P < 0.05$ versus without additional pressure (0 mmHg)

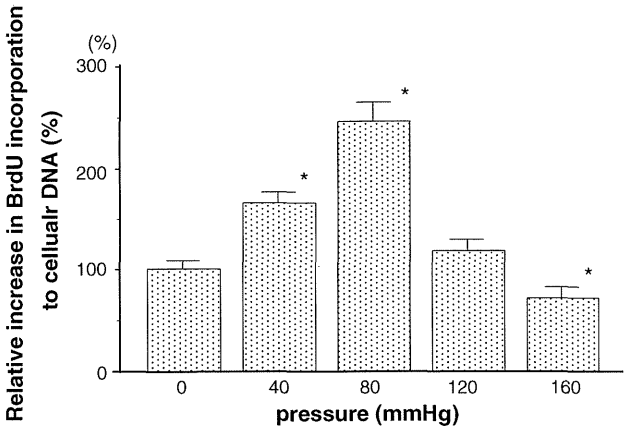

Fig. 2 Effect on DNA synthesis of exposure of RGM-1 cells to high transmural pressures. RGM-1 cells in medium containing 0.1 % FCS were subjected to high transmural pressures, and DNA synthesis was determined after exposure for 24 h. Values are expressed as the mean ± SEM ($n = 6$) % increase relative to the 0 mmHg control value. *$P < 0.05$ versus without additional pressure (0 mmHg)

Activation of MAPK by Pressure Loading of Cells

MAPK enzyme activity was evaluated by measuring the incorporation of ^{32}P-ATP into a synthetic peptide substrate highly selective for p44/p42 MAP kinase (Fig. 3). The MAPK assay showed that the radioactivity count from phosphorylation of the Thr^{669} phosphorylation site of the EGF receptor was significantly increased by exposing the cells to a transmural pressure of 80 mmHg for 6 h.

Western blot analysis was also used to evaluate MAPK-associated phosphorylation of the Elk-1, c-Jun, AFT-2 substrate after subjecting the cells to a transmural pressure of 80 mmHg. Figure 4 shows the pressure-induced increase in phosphorylation corresponding to activation of the MAP kinases (Fig. 4). By normalizing each of the band densities for the control (0 h) using ImageJ densitometry, we observed that although the ERK-associated phosphorylation increased to 9.4-fold at 3 h and 7.0-fold at 6 h after loading of transmural pressure at 80 mmHg, no significant enhancement of JNK-associated phosphorylation (0.9-fold at 3 h and 0.8-fold at 6 h) or p38-associated phosphorylation (0.6-fold at 3 h and 0.8-fold at 6 h) was detected after transmural pressure loading (Fig. 4).

Then we used PD98059, a MEK1 inhibitor, prior to subjecting cells to a transmural pressure of 80 mmHg for 48 h, in order to specifically attenuate ERK-dependent cell proliferation (Fig. 5). PD98059 (10 μM) significantly inhibited the induction of cell proliferation due to exposure to transmural pressure. Treatment with PD98059 or vehicle alone without transmural pressure for 48 h did not suppress cell proliferation (vehicle, 106 ± 2.03 % and PD98059, 98.7 ± 2.46 % of control).

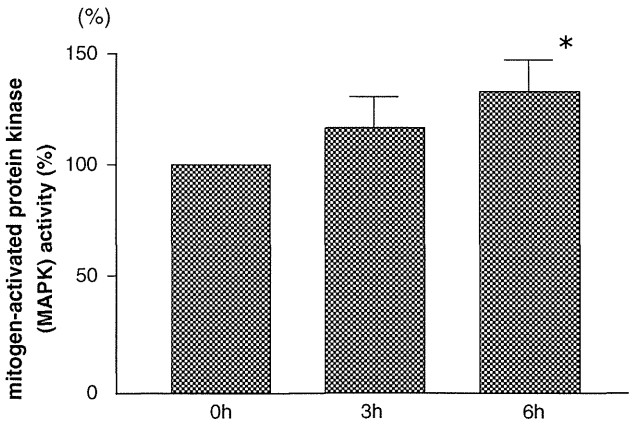

Fig. 3 Effect on MAPK activation in RGM-1 cells of exposure to high transmural pressure (80 mmHg). MAPK enzymatic activity levels in cells exposed to pressure for 0, 3, or 6 h were assessed by measuring the incorporation of ^{32}[P]-ATP into a synthetic peptide substrate selective for p44/42 MAP kinase. Values are expressed as the mean ± SEM (of 6 experiments) % increase relative to the 0 mmHg control value. $^{*}P < 0.05$ versus controls (0 h)

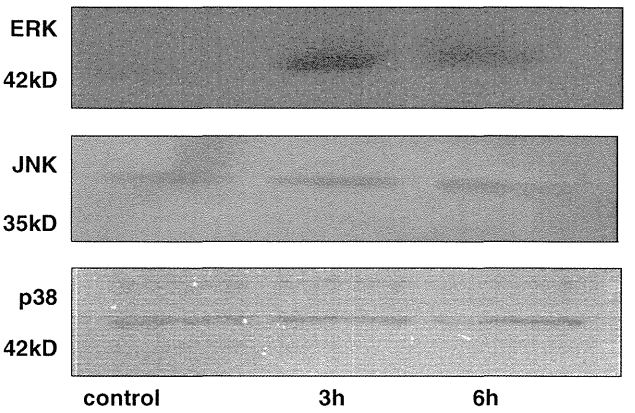

Fig. 4 Western blot analysis of MAPK-associated phosphorylation of the Elk-1, c-Jun and ATF-2 substrate in RGM-1 cells exposed to a transmural pressure of 80 mmHg for 0, 3, or 6 h. The pressure-induced increase in the phosphorylation of each substrate, corresponding to the activation of MAP kinases (ERK, JNK and p38), was determined. Representative results from four different experiments are shown

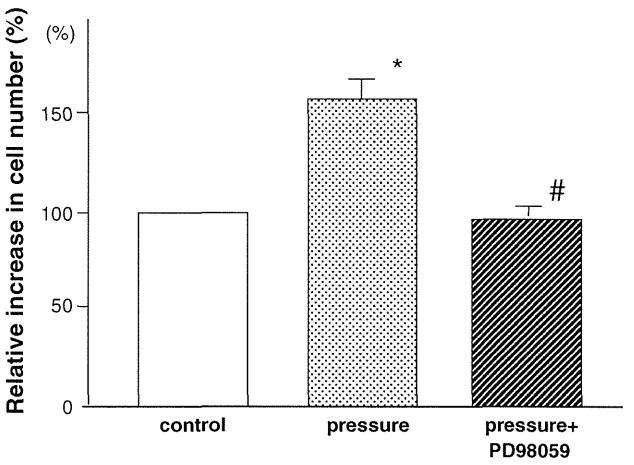

Fig. 5 Effect of a MAPK inhibitor (PD98059) on the pressure-induced proliferation of RGM-1 cells. PD98059 (10 μM) was added to the culture medium immediately prior to pressure loading. The cell numbers were then determined after exposure to a transmural pressure of 80 mmHg for 48 h. Values are expressed as the mean ± SEM ($n = 6$) % increase relative to the 0 mmHg control value. $^{*}P < 0.05$ versus without additional pressure (0 mmHg). $^{\#}P < 0.05$ versus pressure alone

mRNA Expression of IREGs

RT-PCR showed that RGM-1 cells expressed *c-fos*, *c-myc*, and *c-jun* mRNA, producing band sizes of 511, 455, and 258 bp, respectively (Fig. 6). Exposing the cells to a transmural pressure of 80 mmHg for 3 h markedly increased the level of *c-fos* mRNA to 8.6-fold of that in the control cells (0 mm Hg). The expression level of *c-fos* mRNA remained 1.5-fold higher in the pressure-treated cells than in the control cells after treatment for 6 h. The

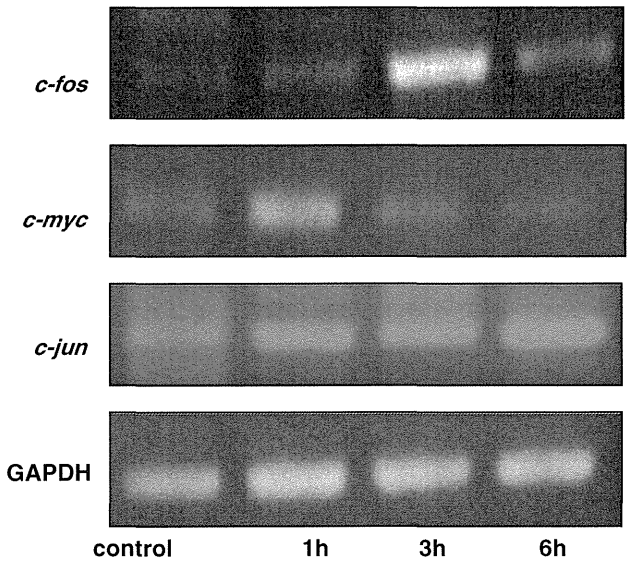

Fig. 6 Expression of *c-fos*, *c-myc*, and *c-jun* mRNA in RGM-1 cells exposed to a transmural pressure of 80 mmHg for 0, 1, 3, or 6 h, as determined by RT-PCR. Expression of *c-fos*, *c-myc*, and *c-jun* mRNA in RGM-1 cells is shown by bands at 511, 455, and 258 bp, respectively. Representative results from four experiments are shown

level of *c-myc* mRNA in the RGM-1 cells was markedly increased by 2.2-fold after 1 h of exposure to a transmural pressure of 80 mmHg, although the levels had fallen again after 3 and 6 h. No significant increase in the expression of *c-jun* mRNA (0.8-fold at 1 h, 0.9-fold at 3 h, and 0.9-fold at 6 h) was observed after pressure loading of these cells.

Immunofluorescence Staining for c-Fos and c-Myc Proteins

Increased expression of c-Fos and c-Myc proteins was detected by immunofluorescence staining and laser confocal microscopy (Fig. 7). In control cells not subjected to increased pressure, FITC-labeled anti-c-Fos and c-Myc antibodies were predominantly observed in the cytosol. Three hours after the cells were subjected to a transmural pressure of 80 mmHg, strong induction of the c-Fos and c-Myc proteins was evident not only in the cytosol but also in the nuclei of the cells. Although the total expression of c-Fos and c-Myc appeared to be increased by a transmural pressure of 120 mmHg, as compared to 80 mmHg, the nuclear localization of c-Fos and c-Myc was higher at 80 mmHg than 120 mmHg, suggesting that nuclear localization of c-Fos and c-Myc may be able to detect enhanced cell proliferation and DNA synthesis. At the 3-h time point, nuclear staining of c-Myc was higher than that of c-Fos, possibly in line with the earlier response of *c-myc* assessed by RT-PCR in Fig. 6.

Activation of Nuclear Factor AP-1

Members of the c-Fos and c-Jun protein family dimerize to form AP-1 transcription factor complexes that regulate the expression of other genes. To determine whether pressure loading increased AP-1 binding activity in RGM-1 cells,

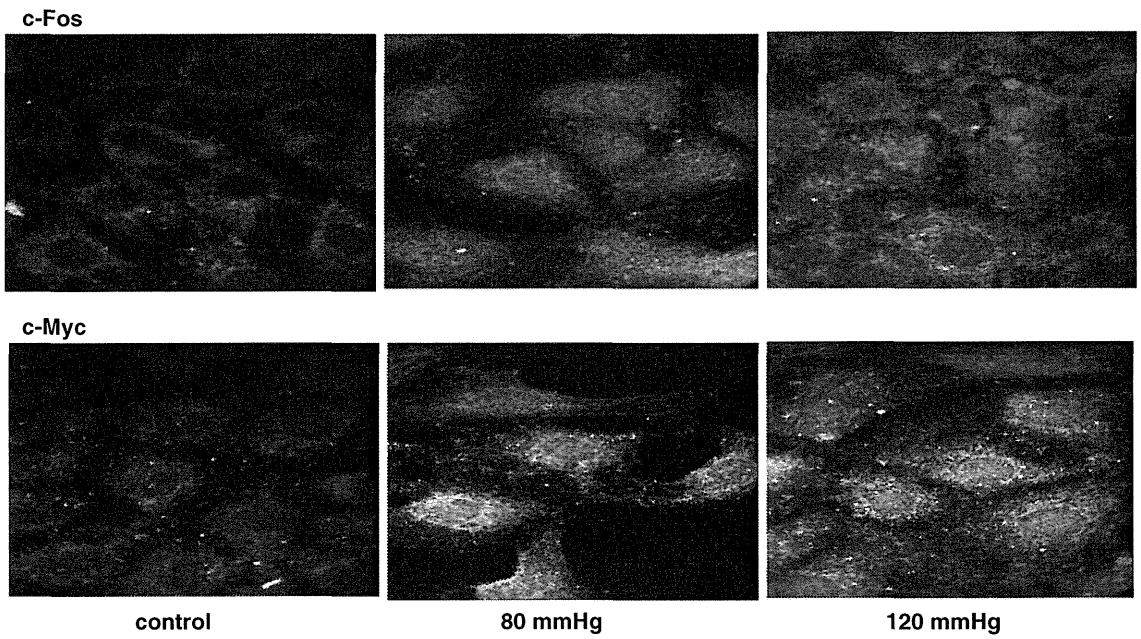

Fig. 7 Induction of c-Fos and c-Myc protein expression in RGM-1 cells exposed to high transmural pressures, as detected by immunofluorescence staining and laser confocal microscopy. Three hours after exposure to a transmural pressure of 80 or 120 mmHg, strong induction of the c-Fos and c-Myc proteins was observed not only in the cytosol but also in the nuclei of the cells. Representative results from three experiments are shown

we performed an electrophoretic mobility gel shift assay using an oligonucleotide probe containing an AP-1-binding site. Figure 8 shows activation of AP-1 in RGM-1 cells exposed to a transmural pressure of 80 mmHg; in contrast, control extracts contained little activated AP-1 (Fig. 8, left lane). Increased amounts of activated AP-1 were evident 1 and 3 h after the cells were subjected to a transmural pressure of 80 mmHg (middle and right lanes). The degrees of AP-1 activation after 1 and 2 h appeared to be almost equal.

Discussion

In the present study, we demonstrated that exposure to transmural pressure induced gastric epithelial cells to proliferate and that this proliferation might be due to activation of exclusively ERK, but not JNK and p38, and IERGs such as *c-fos* and *c-myc*. Our results indicate that transmural pressure may be involved in the remodeling of gastric wall. Exposure to pressures >40 mmHg significantly induced proliferation of the RGM-1 cells. Intragastric pressures have been reported to reach over 60 cmH_2O (44 mmHg) in the proximal gastric pouch during fundic waves and to over 100 cmH_2O (74 mmHg) in the distal stomach during antral peristaltic waves [17].

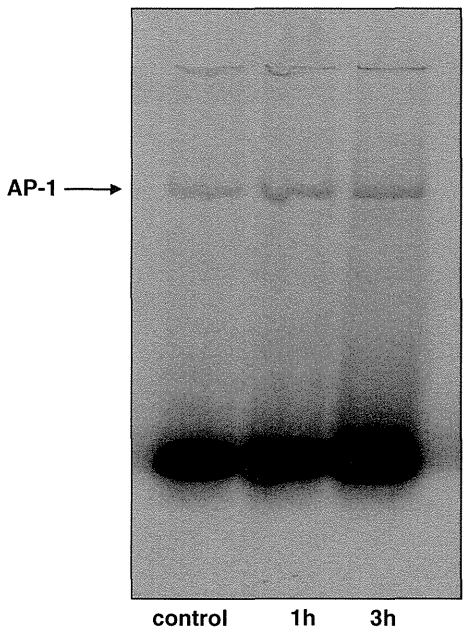

Fig. 8 AP-1-DNA binding activity as determined by electrophoretic mobility shift assay (EMSA) using a FITC-labeled consensus AP-1 oligonucleotide as the probe. This fluorograph from an RGM-1 cell gel-shift study demonstrates AP-1-DNA binding activity in cells exposed to a transmural pressure of 80 mmHg. An extract of RGM-1 cells not exposed to additional pressure (0 h) was used as a control. An increase in the amount of activated AP-1 is evident 1 and 3 h after exposure of the cells to a transmural pressure of 80 mmHg (*lanes 1h* and *3h*)

Our results indicate that gastric epithelial cell growth can be promoted by physiological levels of intragastric pressure produced by gastric peristaltic waves. Although continually high transmural pressures could be encountered in IHPS due to the narrowed antropyloric canal, resulting in the extraordinary levels of mucosal hypertrophy [10], the present results are not applicable to physiological conditions, in which the gastric mucosa is subjected only intermittently, rather than continuously, to high transmural pressures. The growth of gastric epithelial cells under periodic pressure loading, mimicking physiological conditions, should therefore be investigated as well. For example, in FD, especially PDS based on the Rome III classification [11, 12], dysmotility of the gastroduodenum is a major pathogenetic factor. The weakened antral peristalsis or disturbed fundic accommodation may lead to the development of intragastric pressure after food intake is intermittently enhanced, possibly to supraphysiological levels, leading to high transmural pressure overloading to the gastric wall.

We found that exposure to a pressure of 160 mmHg inhibited cell proliferation, suggesting that epithelial cell growth might be attenuated by supraphysiological transluminal pressures. Exposure to a pressure of 120 mmHg was associated with an increase in the number of gastric cells after 48 h; however, no increase in DNA synthetic activity was noted after 24 h. These results are consistent with early activation of immediate early genes or AP-1 after 3–6 h, and we suspect that the bulk of the DNA synthesis may have occurred before 24 h had elapsed.

The MAPKs are part of a well-characterized protein kinase system that has been shown to mediate cellular responses to physical forces, including stretching and osmotic stress [30, 31]. Activation of the MAPK signal transduction pathway seems to be especially important in the healing of injured gastrointestinal mucosa. Pai et al. recently reported that activation of an EGF-receptor-ERK signal-transduction pathway may play an important role in the healing of experimentally-produced gastric ulcers [32, 33]. Recent in vitro studies have also shown that injury to intestinal epithelial cells activates the Raf-1, ERK1, ERK2, and JNK1 [34]. In our present study, western blot analysis showed that ERK kinase was activated in RGM-1 cells 3 h after the onset of pressure loading and remained activated for up to 6 h. In contrast, JNK and p38 kinases were not significantly activated by pressure loading. In contrast, MacKenna et al. [21] reported that while ERK and JNK1 were rapidly activated in rat cardiac cells by 4 % static biaxial stretch in an apparently integrin-mediated manner, p38 was not activated by this 4 % static biaxial stretch. These results showed that the MAPK family kinases do not necessarily act in the same fashion in response to physical-force overload in line with the present results.

Products of the *c-fos*, *c-myc*, and *c-jun* genes are thought to be important in cell proliferation, and regulation of their expression levels is therefore crucial. In our previous study using intestinal epithelial cells (IEC-6 cells), we demonstrated that transmural pressure loading promoted the expression of *c-fos* and *c-myc*, but not *c-jun*, perhaps as a result of MAPK activation [35]. Recently, gastric myenteric cellular c-Fos expression has been shown to be enhanced in response to stresses such as the administration of a thyrotropin-releasing hormone agonist, suggesting a possible contribution of gastric transmural pressure overload. It has been shown that feeding a liquid diet to fasting rats induces the expression of c-Fos in the brainstem [36], an area associated with the responses to gastric distension [37], possibly linked to the genesis of accommodation. Dimaline et al. [14] reported induction of c-Fos expression in the gastric myenteric neurons in response to physiological stretching of the stomach wall. They therefore speculated that supraphysiological stretching of the stomach wall might also induce expression of c-Jun and c-Myc. Activation of JNK leads to phosphorylation of c-Jun, a component of the transcription factor AP-1 that in turn is an important mediator of cell growth regulation by trophic peptides [38]. Activation of AP-1-mediated transcription and cell growth regulation commonly involves rapid and transient induction of mRNA and protein expression of c-Fos and c-Jun [38, 39]. AP-1 was reported to form complexes with DNA when cultured vascular endothelial cells were subjected to shear stress [40]. In the present study, we demonstrated that AP-1 formed complexes with DNA when gastric epithelial cells were subjected to transmural pressure. As a hypothesis, transmural pressure might enhance ERK-associated phosphorylation by MEK1. Activated ERK then phosphorylates c-Myc, and phosphorylated c-Myc translocates into the nucleus and enhances *c-fos* transcription. c-Fos might then bind to DNA as transcription factor AP-1, resulting in enhancement of cell proliferation.

An understanding of the control of the responses to pressure loading in gastric epithelial cells may provide novel insights into the regulation of gastric epithelial remodeling under the conditions of long-term deregulation of gastric motor function [12] that may be encountered in patients with (IHPS) [10] or severe gastric motor dysfunction including gastroparesis.

In conclusion, gastric mucosal proliferation was enhanced by transmural pressure loading through the AP-1 mediated induction of early genes such as *c-fos* and *c-myc* and early activation of ERK.

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Conflict of interest None.

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A Questionnaire-Based Survey on Screening for Gastric and Colorectal Cancer by Physicians in East Asian Countries in 2010

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Key Words

Colorectal cancer · Gastric cancer · Screening · East Asian countries · Questionnaire-based survey

Abstract

Background: The incidence of gastric cancer (GC) is high, and colorectal cancer (CRC) has significantly increased in Asian countries. ***Aim:*** To examine the current screening for GC and CRC within East Asia by means of a questionnaire survey. ***Methods:*** Representative members of the Committee of the International Gastrointestinal Consensus Symposium provided a questionnaire to physicians in six East Asian countries. ***Results:*** A total of 449 physicians participated in this survey. In all countries, more than 70% of physicians started GC screening between 40 and 59 years. The most popular method to screen for GC was endoscopy (92.7%), but combination methods such as *Helicobacter pylori* (HP) antibody, barium X-ray, and tumor marker with endoscopy differed by country. For HP-positive individuals, most physicians screened every year by endoscopy, and for individuals post-HP eradication, about half of physicians (56.3%) thought there was a need to follow-up with GC screening. Among all physicians, the most common age to start CRC screening was in the 40s (39.8%) and 50s (40.9%). Based on the American Cancer Society Recommendations, a fecal occult blood test every year was the most popular method for CRC screening overall. However, among each country, this test was most popular in only Japan (76.9%) and Indonesia. In other countries, sigmoidoscopy every 5 years and total colonoscopy every 10 years were the most popular methods. ***Conclusion:*** There are similarities and differences in the screening of GC and CRC among East Asian countries.

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Introduction

Gastric cancer (GC) is the fourth most common cancer in the world and the second leading cause of cancer-related death [1, 2]. Asian countries, including China, Japan, and Korea, show the highest incidence of GC in the world [1]. Since 1960, mass screening for GC using photofluorography has been done in Japan, and survival rates have significantly improved due to early detection of cancer [3]. Recently, other opportunistic screening methods such as endoscopy, serum pepsinogen testing, and *Helicobacter pylori* (HP) antibody testing have been used for GC screening. However, the efficacy of these methods is not clear. In addition, as new endoscopic techniques such as chromoendoscopy, narrow band imaging, confocal endoscopy, and autofluorescence have been developed, further studies on GC screening are needed.

Colorectal cancer (CRC) is one of the most common cancers worldwide, accounting for more than 1 million new cancer cases annually and approximately half a million deaths each year. The incidence of CRC in Asia is increasing [4]. Although various screening examinations, such as fecal occult blood testing, sigmoidoscopy, and colonoscopy can be used, little is known about how or when physicians recommend screening and whether patients follow different recommendations. Although Asia-Pacific consensus recommendations for CRC screening have been published [4], guidelines and recommendations for the screening and prevention of CRC in Asia have not been established. Screening and surveillance of CRC is effective for diagnosing early stages of CRC [5], but compliance rates are low in many countries worldwide [6–10].

In this survey, we intended to define the current situation concerning screening strategies for GC and CRC in East Asian countries.

Subjects and Methods

Subjects

Gastroenterologists, gastroenterological surgeons, and general physicians in China, Indonesia, Japan, Korea, Philippines, and Thailand were asked to fill in a questionnaire about screening for GC and CRC. All subjects work in an urban environment.

Methods

A representative person from each country was selected from the Committee of the International Gastrointestinal Consensus Symposium, which is the international part of the Japanese Gastroenterological Association. The representative members provided a questionnaire to physicians in each country, starting at the beginning of July 2010 and collected responses until the end of December 2010. The questionnaire asked the following questions about GC and CRC: (1) GC: age of starting screening examinations, the most common age when screening examinations are done, and the preferred screening examination. In addition, physicians were asked about the management of HP infection in terms of the follow-up period for patient who did and did not undergo HP eradication. (2) CRC: age of starting screening examinations, the most common age when screening examinations are done, and the preferred examination for screening. The questionnaire for CRC screening criteria was based on American Cancer Society Recommendations [11] (table 1). Details of the questionnaire are shown in the Appendix.

Table 1. CRC screening criteria by American Cancer Society recommendation

1	Fecal occult blood test every year
2	Fecal DNA test every year
3	Sigmoidoscopy every 5 years
4	Total colonoscopy every 10 years
5	Barium enema every 5 years
6	CT colonography every 5 years

Table 2. Number of physicians who participated in the survey by speciality

	Gastro-enterologists	Gastro-enterological surgeons	General physicians	Total
China	26	12	0	38
Indonesia	34	0	10	44
Japan	216	11	21	248
Korea	44	0	7	51
Philippines	21	0	7	28
Thailand	18	14	8	40
Total	359	37	53	449

Results

Characteristics of Respondents

A total of 449 physicians were enrolled in this survey. Among all countries, the number of physicians participating in this survey was largest in Japan and smallest in the Philippines (table 2).

GC: Age of Starting Screening Examinations

Among all physicians, the most common age to start GC screening was in the 40s (54.7% of physicians; fig. 1a).

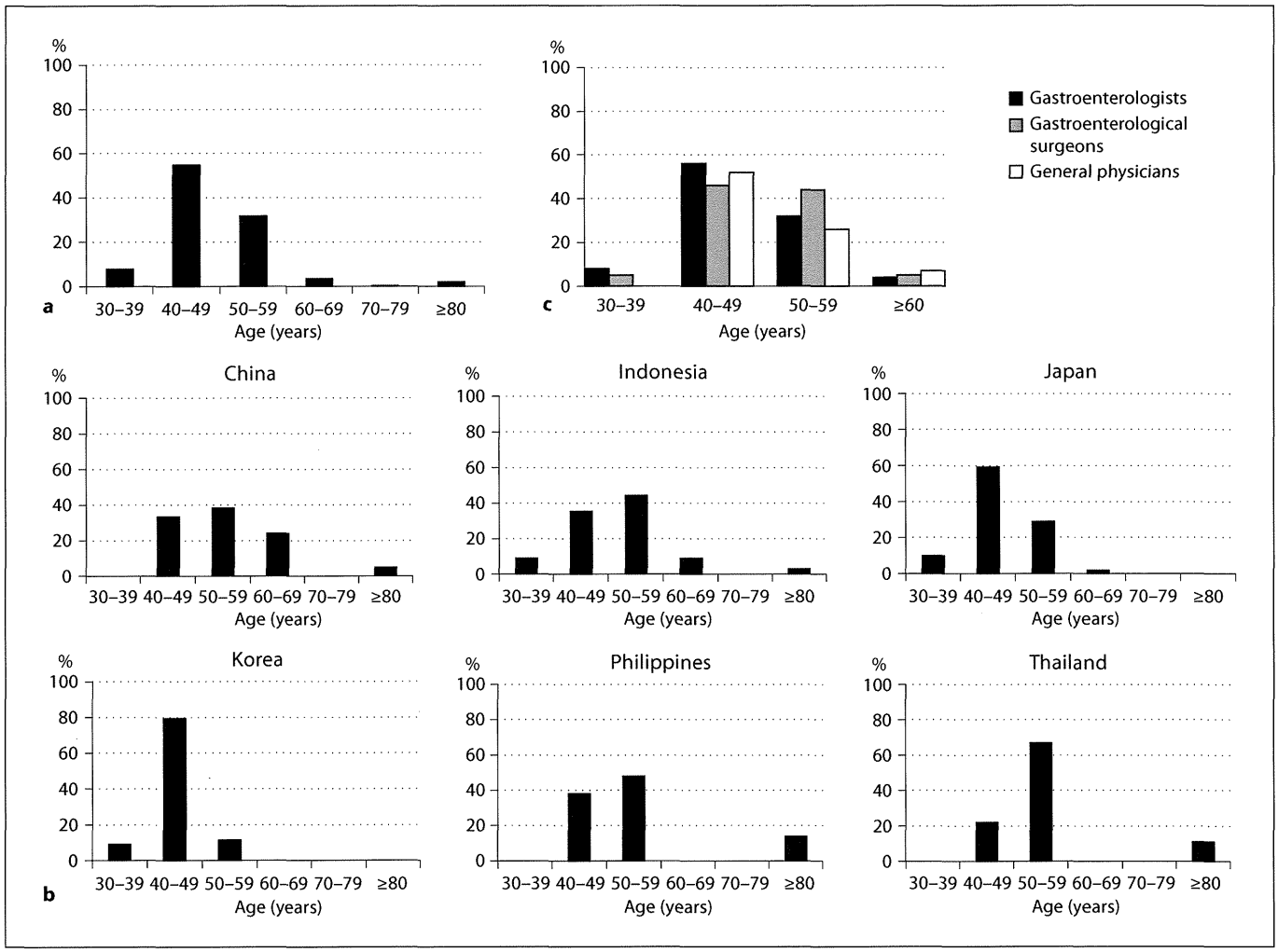


Fig. 1. Starting age of GC screening. **a** Among all physicians. **b** Screening by country. **c** Differences among gastroenterologists, gastroenterological surgeons, and general physicians for starting age of GC screening.

Among the countries surveyed, Korea had the largest ratio of physicians who started GC screening in the 40s (fig. 1b). In terms of physician type, 56% of gastroenterologists start GC screening in the 40s; 46 and 44% of gastroenterological surgeons start in the 40s and 50s, respectively, and 55% of general physicians start in the 40s (fig. 1c).

GC: Preferred Screening Examination

The most common screening method for GC was endoscopy both among all physicians (92.7%) and in each country (China, 100%; Indonesia, 100%; Japan, 89.8%; Korea, 100%; Philippines, 95.2%; Thailand, 83.3%). There were no differences among gastroenterologists, gastroenterological surgeons, and general physicians in terms of the preference for endoscopy as the best GC screening method (92.7, 92.9, and 92.0%, respectively). However, there were several different suggestions about combination screening methods (fig. 2a). Though endoscopy was included in most combination methods, HP antibody, barium X-ray, and tumor marker surveys were also popular as part of combination screening methods for GC (fig. 2b).

Screening Methods for HP-Positive Patients

Data on the duration of follow-up and the methods of screening HP-positive patients were provided by 359 gastroenterologists. Results showed that 65% of gastroenterologists follow up every year (fig. 3a) and 77% of them use endoscopy (fig. 3b).

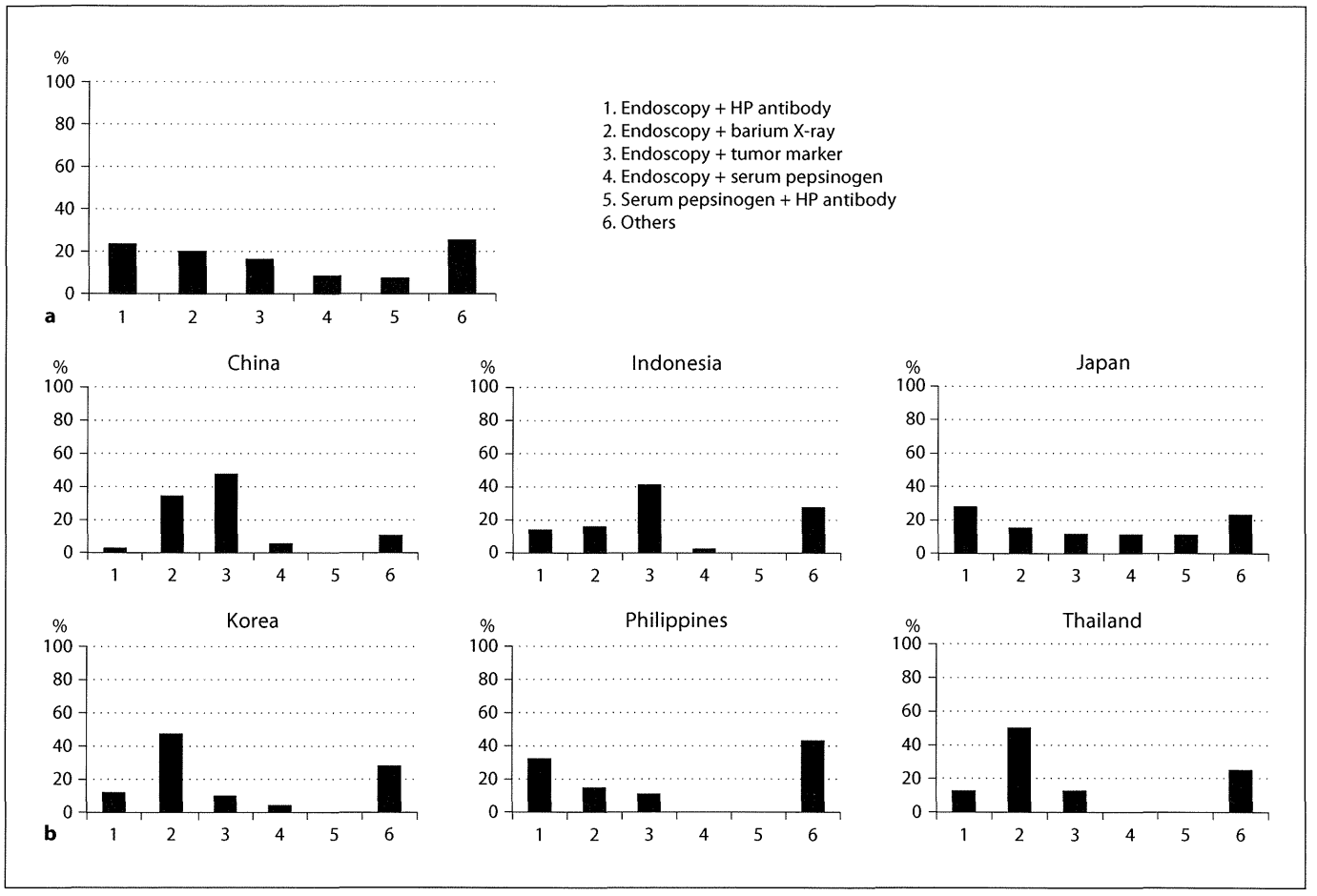


Fig. 2. Combination of GC screening methods used by all physicians (**a**) and by country (**b**).

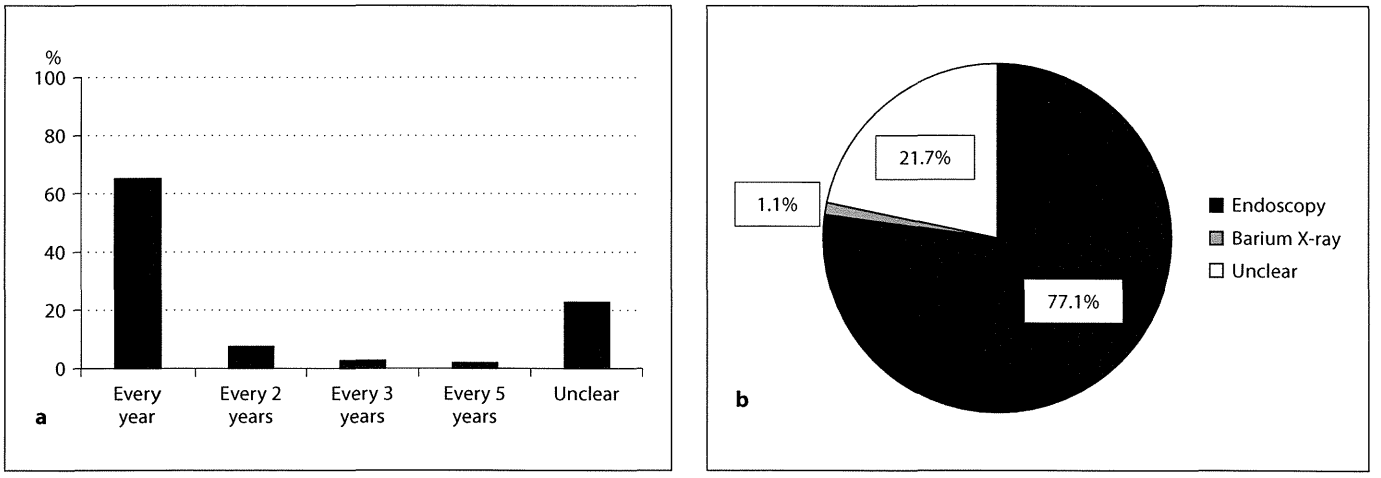
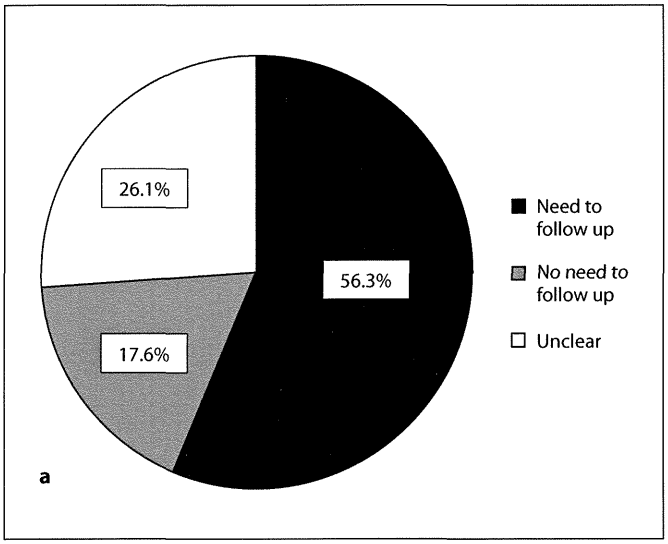
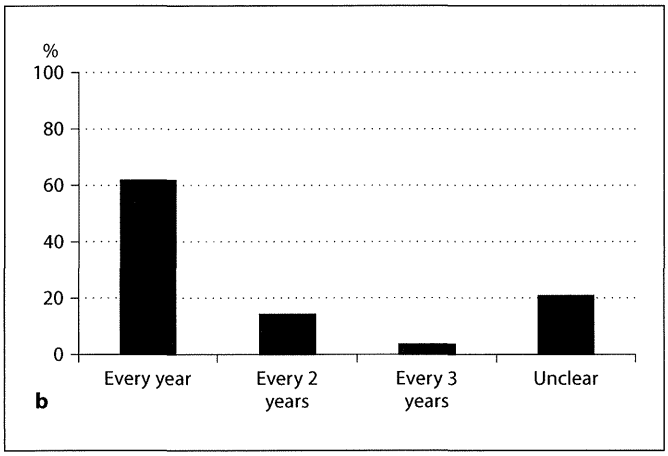


Fig. 3. a Interval to follow-up of HP-positive patients among gastroenterologists. **b** Methods used by gastroenterologist to follow up HP-positive patients.





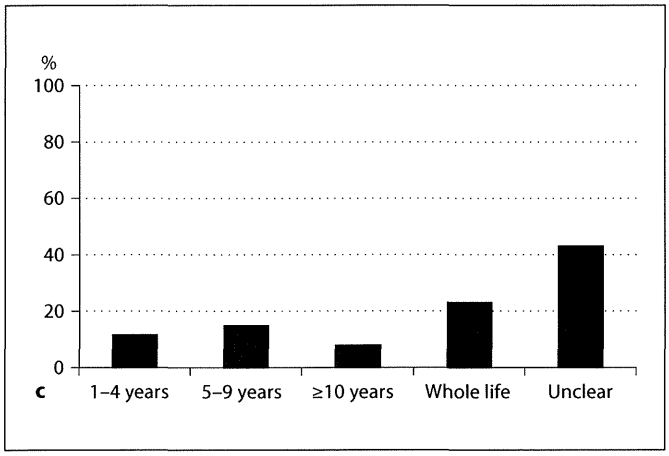


Fig. 4. a Need to follow up after HP eradication among gastroenterologists. **b** Interval of follow-up examinations for patients post-HP eradication among gastroenterologists. **c** Length of follow-up period of patients post-HP eradication among gastroenterologists.

Screening after HP Eradication

Among the 359 gastroenterologists who provided data on HP eradication, 202 (56.3%) thought that follow-up GC screening after HP eradication was necessary (fig. 4a). Among these 202 gastroenterologists, more than 60% perform GC screening every year (fig. 4b). The duration of follow-up varied (fig. 4c). Whole-life follow-up was the most popular response (22.9%) regarding the follow-up period; however, 42.5% answered 'unclear'.

CRC: Age of Starting Screening Examinations

Among all physicians, the most common ages to start CRC screening were in the 40s (39.8%) and 50s (40.9%), and more than 90% of physicians start CRC screening before 60 years (fig. 5a). Among the countries surveyed, Japan tended to start earlier compared with other countries (fig. 5b). There were no significant differences between gastroenterologists and gastroenterological surgeons in terms of starting age of screening (fig. 5c).

CRC: Preferred Screening Examination among Gastroenterologists

Among all gastroenterologists, fecal occult blood test every year was the most popular method (52.5%) for CRC screening (fig. 6a). However, this result was largely driven by responses of Japanese gastroenterologists, who represented the largest number of gastroenterologists (n = 216). Sigmoidoscopy every 5 years and total colonoscopy every 10 years were common responses from gastroenterologists in other countries (fig. 6b).

Discussion

Although a decline in the number of GC patients has been observed in Japan, China, and Korea, GC still represents a tremendous burden in each country. According to GLOBOCAN 2008, GC is the third most frequently diagnosed cancer and the second leading cause of cancer deaths in Japan, with an estimated 102,040 new cases and 50,156 cancer deaths in 2008. The overall estimated age-adjusted incidence rate in 2008 was 31.1 per 100,000 people [12]. In China, there is no systematic national vital statistics, but a retrospective sampling survey from 2004 to 2005 found that the mortality rate from GC ranked third in overall cancer mortality [13]. GC is the second most frequently diagnosed cancer in China and the third leading cause of cancer death, with an estimated 464,439 new cases and 352,315 cancer deaths in 2008. The overall estimated age-adjusted incidence rate in 2008 was 29.9

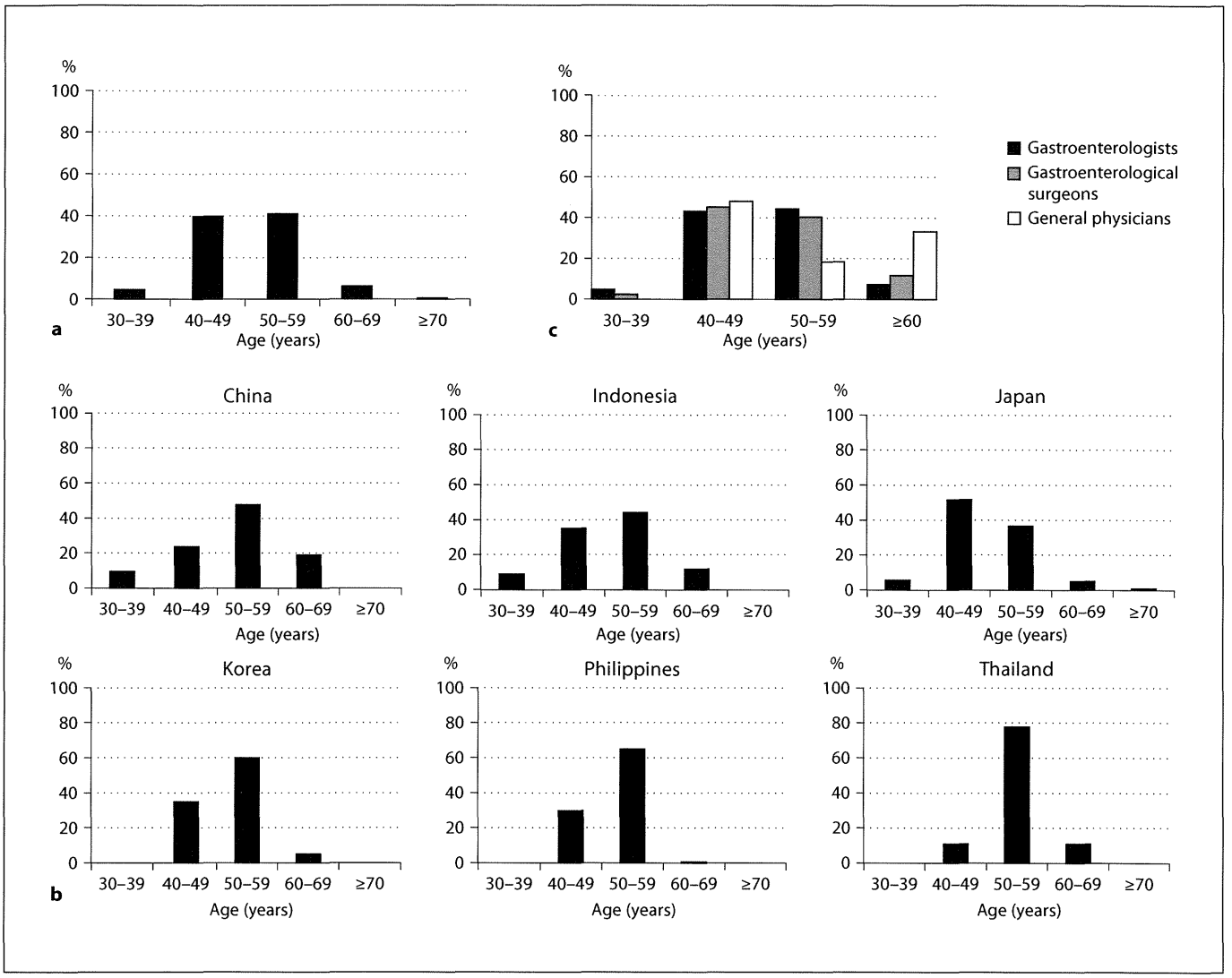


Fig. 5. Starting age of CRC screening. **a** Starting age of CRC screening among all physicians. **b** Starting age of CRC screening by country. **c** Differences among gastroenterologists, gastroenterological surgeons, and general physicians for starting age of CRC screening.

per 100,000 people. In Korea, GC mortality has been consistently decreasing; however, it is still a clinically important disease due to its high incidence and mortality [14]. In 2008, an estimated 28,078 new cases were diagnosed, accounting for 15.7% of new cancer cases. The incidence of disease was 43.8 per 100,000 in 2008. In Thailand, the estimated age-standardized incidence rate in 2002 of GC shows some variation with higher rates in Bangkok, which were 4.9 per 100,000 for males and 3.7 per 100,000 for females [15]. The estimated age-standardized incidence rate in 2007 of stomach cancer in the Philippines was 7.9 per 100,000 for males and 5.4 per 100,000 for females [16]. The incidence of GC in Indonesia is exceedingly rare and it has been reported to relate with the relative lack of infection with HP [17]. These statistical data include that although the number of GC patients is decreasing in Asian countries, it is still the second most common cause of death worldwide [1, 18] as well as in many Asian countries, such as China, Japan, and Korea. However, a screening program for GC has not been established in Asian countries.

According to the Asia-Pacific Working Group on GC, the optimum age for initiation of screening should be 40–45 years [19]. In particular, in high-risk regions, such as

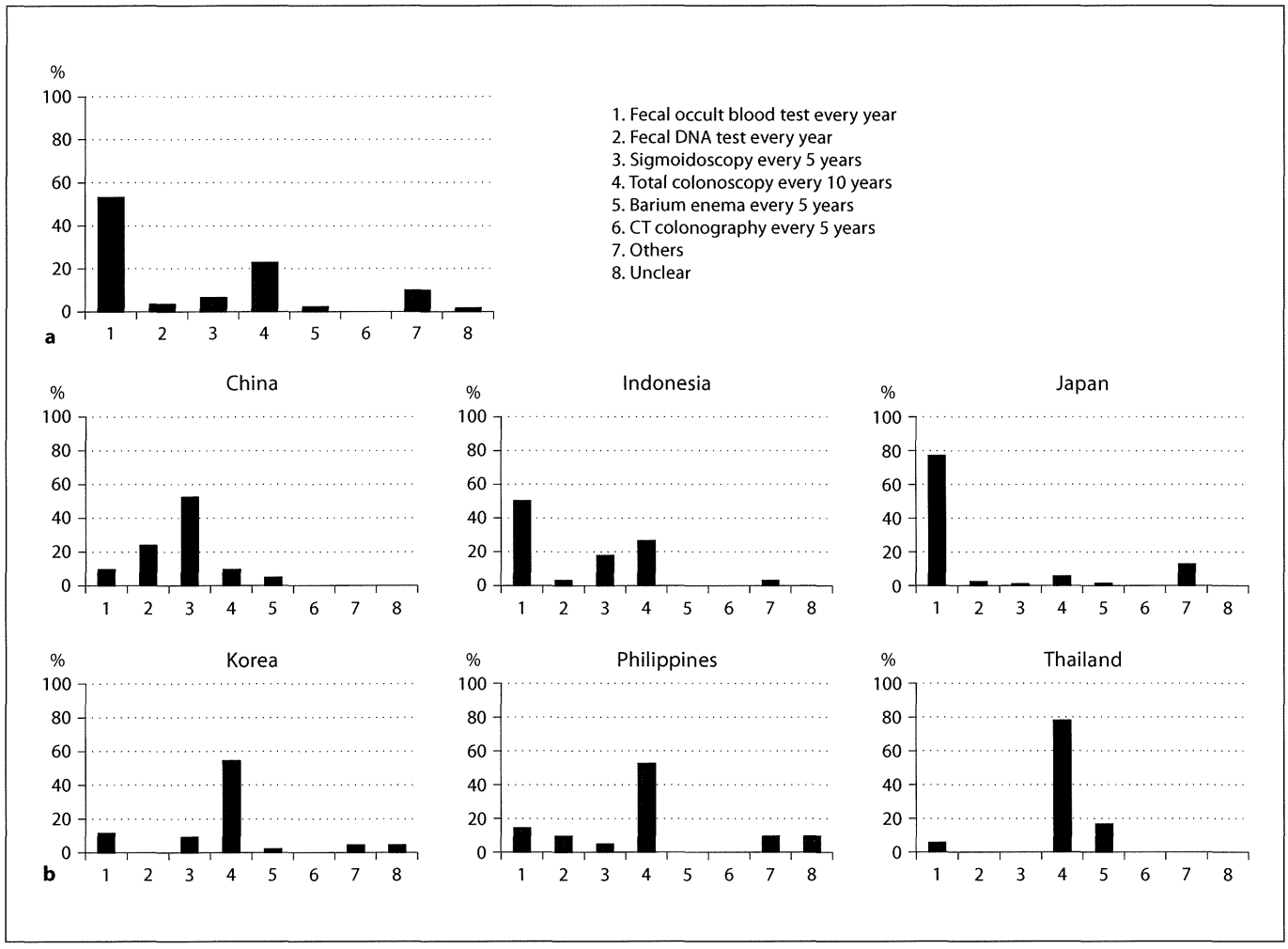


Fig. 6. Methods for CRC screening by all physicians (**a**) and by country (**b**).

Japan and Korea, the working group recommends screening all individuals older than 40 years. In this survey, most physicians agreed about starting GC screening at 40 years.

Endoscopy was the most popular screening method for GC among physicians in this survey. In general, endoscopy is increasingly used for GC screening because of a high detection rate of superficial flat and non-ulcerative lesions that conventional barium examination cannot detect [20]. However, the sensitivity of GC detection by endoscopy compared with that of barium X-ray (photofluorography) is not clear. Although endoscopic screening is expected to be an alternative strategy to photofluorography, no studies have evaluated whether endoscopic screening reduces GC mortality. As new endoscopic techniques such as chromoendoscopy, narrow band imaging, confocal endoscopy, and autofluorescence are developed, the sensitivity and detection of GC are expected to increase. Although most physicians in this survey recommend endoscopy, further study is necessary to evaluate the efficacy of endoscopy for GC screening using up-to-date screening tools.

Photofluorography has been used for GC screening in Japan since 1960 [21]. Though there is no randomized, controlled study of GC screening using photofluorography, several case-control and cohort studies dealing with photofluorography have been published [3, 22–26]. These studies indicate that GC screening with photofluorography resulted in a reduction in mortality from GC. However, data from prospective studies that define death from

GC as an endpoint are inconsistent. In the current survey, photofluorography was the second most common method used in combination with endoscopy to screen for GC. Use of this method was higher in Japan compared with other countries (data not shown).

Measurement of serum pepsinogen (PG) levels is a popular serological screening test for GC, particularly in Japan. There are two types of serum PG – PGI and PGII. PGII remains constant, but PGI concentrations decrease with loss of fundic gland mucosa. Therefore, a low PGI concentration or a low PGI/II ratio indicates atrophic gastritis, which represents a preneoplastic gastric lesion. A PGI/II ratio of >3.0 has a sensitivity of 93% and a specificity of 88% for diagnosis of a normal fundic gland in Japanese patients [27]. In a Japanese study, subjects with atrophic gastritis diagnosed by pepsinogen concentrations had a significantly higher risk of developing GC than those with normal pepsinogen concentrations who were negative for the HP antibody [28]. However, the serum pepsinogen test detects the presence of atrophic gastritis and is therefore more applicable to detect intestinal cancer only. In this survey, the serum pepsinogen test was used in combination with other methods by a small number of physicians.

HP is a well-known carcinogen for GC, and the Asia-Pacific *H. pylori* Consensus Group concluded that eradication of HP is a good strategy in selected societies where the risk of GC is high [29]. There is one randomized controlled study from China that showed that eradication of HP reduced the incidence of GC [30]. In that cohort, individuals who had not developed gastric mucosal atrophy and intestinal metaplasia had a lower incidence of GC after HP eradication, but those who already had gastric mucosal atrophy and intestinal metaplasia showed no such difference after HP eradication. There is no randomized controlled study concerning the duration of follow-up among HP-positive individuals. It has been reported that the doubling time of early GC is approximately 16.6 months [31]. Based on that study, results of the current survey showing that most physicians insist on annual GC screening in HP-positive individuals seem appropriate. In terms of follow-up after HP eradication, GC has been seen to develop in some patients even after HP eradication [32–34]. From these studies, the rate of GC development is about 0.3% per year as long as 10 years after HP eradication. Based on these results, it has been suggested that follow-up GC screening for patients who have been treated for HP infection should be continued for more than 10 years [33]. However, in this survey, 17.6% of gastroenterologists indicated they thought there was no need for follow-up of patients after HP eradication. Though there does not appear to be a consensus regarding follow-up after HP eradication, it seems prudent to continue GC screening even after HP eradication for at least several years. Further studies are needed to clarify the length of follow-up required.

The usefulness of screening for the GC risk method using a combined assay for serum anti-HP IgG antibody and serum PG levels, called the 'ABC method', has been reported. Subjects are classified into one of four risk groups based on the results of two serologic tests: group A (HP(–) PG(–): infection-free subjects), group B (HP(+) PG(–): chronic atrophic gastritis-free or mild), group C (HP(+) PG(+): chronic atrophic gastritis), and group D (HP(–) PG(+): severe chronic atrophic gastritis with extensive intestinal metaplasia). The ABC method allows stratification of risk for development of GC, as chronic gastritis progressed, a gradual and significant increase in the incidence of GC and hazard ratio was noted [28, 35–38].

The Asia-Pacific Working Group on CRC has reported that CRC is one of the most common cancers in Asia in both males and females, and that the incidence of CRC in Asian Pacific countries is similar to that of Western countries. Although the death rate of CRC is declining in Western countries, mortality associated with CRC is rising in Asian countries [4]. According to the database of GLOBOCAN [39], the incidence of CRC for males is especially high in Japan and Korea, that is 41.7 and 46.9 per 100,000. In China, the Philippines, Indonesia, and Thailand, the incidence of CRC for males shows almost same numbers, that is 16.3, 10.0, 19.1, and 13.2 per 100,000. For females, the tendency of CRC incidence shows the same as males, that is high group as Japan and Korea, 22.8 and 25.6 per 100,000, and low group as China, the Philippines, Indonesia, and Thailand, 12.2, 7.3, 15.6, 13.4 per 100,000. Screening and surveillance for CRC is useful for early disease detection [5], but compliance with screening recommendations is low in many countries [6–10].

Based on the recommendations of Asia-Pacific Consensus Group, CRC screening should be started at the age of 50 years [4]. Most national guidelines also recommend starting CRC screening at this age because the risk of CRC begins to increase at age 50 [40, 41]. In Asian countries, the risk of finding advanced CRC significantly increases by 1 to >3% at the age of 50 years compared with younger subjects [42–45]. This survey showed that most physicians, except those in Japan, start screening for CRC at 50 years. Japanese physicians tend to start screening

for CRC earlier. Overall, all physicians in this survey started screening for CRC between 40 and 50 years.

Fecal occult blood tests (FOBTs) are based on the fundamentals of detecting blood in stool that may originate from a bleeding CRC or large adenoma. Because of their ease of use, FOBTs have been used to screen for CRC worldwide. However, FOBTs cannot detect precursor lesions. In addition, because CRCs usually bleed intermittently, repeat testing may be required to detect CRC. Two primary FOBTs are available: guiac-FOBT and immunochemical FOBT (iFOBT). Though dietary intake of red meat leads to false-positive findings and vitamin C intake to false-negative findings in guiac-FOBT, iFOBT is specific for human hemoglobin. The sensitivity of iFOBT in detecting CRC is 66–82% and the specificity is 95–97% [46, 47]. Previous reports showed that FOBT screening reduced mortality resulting from CRC [48–53], and several countries emphasize this procedure for general population screening. With the use of FOBT, the number of patients with stage I or II disease has significantly increased, while the number of patients with stage IV diseases has decreased [53]. In this survey, physicians from Japan and Indonesia tended to choose FOBT for CRC screening. However, there is no evidence from randomized, controlled trials that CRC-related mortality is reduced over a 10-year period of iFOBT screening. One large study included 94,000 persons who were randomized to one round of iFOBT or no screening. Colon cancer mortality did not differ significantly between groups over an 8-year follow-up period: CRC mortality was 90 per 100,000 in the screening group versus 83 per 100,000 in the control group [54]. Based on this study, which shows no benefit of iFOBT on mortality, physicians in Japan and Indonesia may want to consider other methods of CRC screening.

Flexible sigmoidoscopy is an endoscopic procedure that shows up to 40–60 cm distal of the colon. When an adenoma of any size is detected, a full colonoscopy is recommended, because the risk of advanced adenomas or cancer in the proximal colon is increased [55]. The problem with flexible sigmoidoscopy is that the quality of the examination is not always as good as it should be, as the insertion depth is sometimes difficult to determine. In addition, sigmoidoscopy should be performed by trained endoscopists with acceptable adenoma detection rates [56]. In one study, sigmoidoscopy had a higher detection rate for advanced adenomas and cancer compared with FOBT [57]. In addition, individuals without adenomas in the distal colon, as shown by sigmoidoscopy, frequently do not receive a follow-up colonoscopy. The percentage of asymptomatic individuals with isolated advanced proximal adenomas or cancer who undergo a colonoscopy is 1.3–5% [58, 59]. Atkin et al. [60] reported that sigmoidoscopy screening reduces the mortality from CRC. They compared a control group (113,195 persons) and an intervention group (57,237 persons) and showed that advanced adenomas or cancer were detected in 5% of subjects in the intervention group. In their study, sigmoidoscopy led to a 23% reduction of CRC incidence and 31% reduction in CRC-related mortality [60]. However, other studies showed a higher percentage of missing transverse and right-sided lesions (24% [61], 20% [62]) even when sigmoidoscopy was combined with FOBT. In this survey, only China reported a higher rate of sigmoidoscopy for screening for CRC compared with other screening methods. Sigmoidoscopy might be better than FOBT for CRC screening, but results from ongoing large, randomized, controlled trials [63, 64] are needed to confirm any advantages of this procedure.

Colonoscopy allows observation of the entire colon and is considered the gold standard for detection of colorectal neoplasia. However, polyps can still be missed when using colonoscopy to screen for CRC. The miss rate of adenomas, as reported in tandem colonoscopy studies, is 20–26% for any adenoma and 2.1% for large adenomas (>10 mm) [65]. These detection rates are reported to depend on the quality of the procedure, including the technique of the colonoscopist and several other factors such as optimal bowel preparation, sufficient withdrawal time, and complete examination of the colon [66–70]. The participation rates of patients in colonoscopy screening are lower compared with FOBT and sigmoidoscopy because of its invasive and burdensome nature. However, most patients only need to undergo a colonoscopy once every 10 years after a negative colonoscopy because the risk of developing CRC after a negative colonoscopy remains low for more than 10 years [71, 72]. In this survey, physicians in Korea, the Philippines, and Thailand recommended a colonoscopy every 10 years for CRC screening, and this procedure was the second most popular CRC screening method overall. Several ongoing studies are evaluating the role of colonoscopy screening on CRC-related incidence and mortality. The Nordic-European Initiative on CRC (NordIGG) trial includes 66,000 individuals randomized to either colonoscopy screening or no screening. A 15-year follow-up is planned, and the results are expected in 2026. In a Spanish trial, CRC-related mortality is being compared between patients who undergo biannual FOBT and colonoscopy; results are expected in 2021.

The use of DNA markers in stool (sDNA) for CRC screening is a relatively new method. Because no single gene mutation is present in all adenomas or cancer cells, a multipanel of DNA markers is needed. The point mutations on APC, KRAS, and P53 genes plus long DNA (PreGen-Plus [73]) are being tested in two large average-risk cohorts [74]. Methylated vimentin, mutant KRAS, and mutant APC (SDT-2) are being tested in a smaller study [75]. Several studies on the efficacy of CRC screening by PreGen-Plus have been done, with one study showing a sensitivity of 52% and a specificity of 94% [74], and another study showing a sensitivity of 20% and a specificity of 96% [75]. The low sensitivity of this method was based on the kinds of panel DNA markers that identify most but not all CRCs. There are no data based on a randomized controlled study regarding the efficacy of sDNA for CRC screening, and it is not known which patients would be better off undergoing CRC screening by sDNA than by FOBT. In this survey, physicians from China recommended fecal DNA test as a second-line method for CRC screening. However, overall, fecal DNA tests were a minor screening method in this survey. Further studies are needed to confirm if fecal DNA tests are effective for screening for CRC compared with other methods.

With the development of new instruments and tests, the diagnostic and therapeutic approach to GC and CRC is continuously changing. In this survey, we conducted an attitude survey of Asian physicians, including gastroenterologists, regarding screening for GC and CRC. Some countries should likely change their screening approaches based on recent results of reliable controlled surveys that show decreases in the mortality GC and CRC with specific screening methods. However, the social situation in each country, including insurance systems, is different. Thus establishment of strong evidence for cancer surveillance using up-to-date methods that have been proven effective in well-controlled studies is imperative.

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Appendix

Questionnaire used in the present survey

Gastric cancer (GC)

1. How old were the patients when you started GC screening? (Circle one)
2. What is the most common age group you screen for GC in your hospital?
3. Which do you think is the best screening method for GC?
   a. Barium X-ray examination
   b. Endoscopy
   c. Serum pepsinogen test
   d. *H. pylori* antibody
   e. Tumor marker
   f. Others
4. Which is the most popular screening method in your hospital?
5. Which combination among a–e is the best for GC screening (for those who answered from a to e)?
6. For those who answered f, what kind of examination did you perform?
7. What kind of follow-up examination did you perform in *H. pylori*-positive patients who did not receive *H. pylori* eradication?
8. What kind of follow-up examination and how often did you perform it in patients who received *H. pylori* eradication?

Colorectal cancer (CRC)

1. How old were the patients when you started CRC screening?
2. What is the most common age group you screen for CRC in your hospital?
3. Which do you think is the best screening method for CRC?
   a. Fecal occult blood test every year
   b. Fecal DNA test every year
   c. Sigmoidoscopy every 5 years
   d. Total colonoscopy every 10 years
   e. Barium enema every 5 years
   f. CT colonography every 5 years
   g. Others
4. Which is the most popular screening method in your hospital?
5. For those who answered g, what kind of examination did you perform?
6. Did you change the screening method of CRC after 10 years or before (for those who have had CRC over 10 years)?
7. For those who answered yes, what kind of examination did you perform?

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