Hirata, K., **Suzuki, H.**, Hosoe, N., Imaeda, H., Ueno, M., Murata, H., Ogata, H., Mukai, M., Hibi, T.	Microvascular rich pyogenic granuloma of the distal small intestine	第37回日本微小循環学会総会	2012
高本俊介、渡辺知佳子、佐藤伸悟、八月朔日秀明、上田俊秀、東山正明、富田謙吾、中村光康、穂苅量太、川口　淳、永尾重昭、**三浦総一郎**	当院における高齢者炎症性腸疾患の臨床的特徴	第４５回日本成人病(生活習慣病) 学会学術集会	2011
Sato, S., Hokari, R., Hozumi, H., Ueda, T., Higashiyama, M., Okada, Y., Kurihara, C., Komoto, S., Watanabe, C., Nakamura, M., Tomita, K., Kawaguchi, A., Nagao, S., **Miura, S.**	The combination of dietary lipids and a sweetner creates a synergy on the intestinal glucagon-like peptide (GLP-2) secretion	Digestive Disease Week	2011
Hozumi, H., Hokari, R., Sato, S., Ueda, T., Higashiyama, M., Okada, Y., Kurihara, C., Watanabe, C., Komoto, S., Nakamura, M., Tomita, K., Kawaguchi, A., Nagao, S., **Miura, S**.	Increased expression of autotaxin/lysophospholipase D on intestinal vessels involves in aggravation of intestinal damage through lymphocytes migration	Digestive Disease Week	2011
Ueda, T., Hokari, R., Higashiyama, M., Kurihara, C., Okada, Y., Hozumi, H., Sato, S., Watanabe, C., Komoto, S., Tomita, K., Nakamura, M., Kawaguchi, A., Nagao, S., **Miura, S.**	Dietary fat aggravates NSAID-induced small intestinal damage via modulation of leukocyte migration in mice	Digestive Disease Week	2011
Higashiyama, M., Hokari, R., Hozumi, H., Kurihara, C., Ueda, T., Watanabe, C., Tomita, K., Nakamura, M., Komoto, S., Okada, Y., Kawaguchi, A., Nagao, S., Suematsu, M., Goda, N., **Miura, S**	HIF-1 in T cells ameliorates intestinal inflammation by controlling regulatory T cell homeostasis.	Digestive Disease Week	2011
Okada, Y., Tsuzuki, Y., Higashiyama, M., Ueda, T., Hozumi, H., Sato, S., Hokari, R., Kurihara, C., Komoto, S., Nakamura, M., Watanabe, C., Tomita, K., Kawaguchi, A., Nagao, S., **Miura, S**	A novel vegetable-derived pribiotics (VDP) modulates proinflammatory cytokines and substance P expression on colonic tissue and attenuated DSS-induced colitis	Digestive Disease Week	2011

Kurihara, C., Hokari, R., Higashiyama, M., Ueda, T., Hozumi, H., Sato, S., Okada, Y., Watanabe, C., Komoto, S., Kawaguchi, A., Nagao, S., **Miura, S.**	Cytokine mRNA expression after exposure to fatty acids is differently modulated in macrophages from small intestine and colon.	Digestive Disease Week	2011
八月朔日秀明、穂苅量太、**三浦総一郎**	炎症性腸疾患における Autotaxin の関与、シンポジウム：消化器疾患と微小循環・酸化ストレス	第９７回日本消化器病学会総会	2011
栗原千枝、穂苅量太、東山正明、上田俊秀、八月朔日秀明、佐藤伸悟、岡田義清、渡辺知佳子、川口　淳、永尾重昭、**三浦総一郎**	腸管マクロファージにおけるＬＰＳ誘導サイトカイン mRNA 発現に関する liver X receptor の関与、ミニシンポジウム:大腸ＩＢＤ病態１	第９７回日本消化器病学会総会	2011
上田俊秀、穂苅量太、東山正明、栗原千枝、岡田義清、八月朔日秀明、佐藤伸悟、渡辺知佳子、高本俊介、富田謙吾、中村光康、川口　淳、永尾重昭、**三浦総一郎**	脂肪摂取の小腸 NSAID 潰瘍における白血球マイグレーションへの影響について、ミニシンポジウム:小腸粘膜障害	第９７回日本消化器病学会総会	2011
岡田義清、都築義和、上田俊秀、東山正明、八月朔日秀明、佐藤伸悟、穂苅量太、栗原千枝、渡辺知佳子、中村光康、富田謙吾、川口　淳、永尾重昭、**三浦総一郎**	伝統的発酵食品中に存在するプロバイオティクスの大腸炎抑制作用、ミニシンポジウム:大腸ＩＢＤ病態４	第９７回日本消化器病学会総会	2011
和田晃典、清水基規、堀内和樹、吉松亜希子、山口隼人、丸田紘史、安武優一、佐藤伸悟、渡辺知佳子、高本俊介、穂苅量太、川口　淳、永尾重昭、**三浦総一郎**	食道カンジダ症を併発し抗真菌薬で改善を認めた良性食道狭窄の１例	第９２回日本消化器内視鏡学会関東地方会	2011
穂苅量太、八月朔日秀明、渡辺知佳子、栗原千枝、上田俊秀、高本俊介、富田謙吾、中村光康、岡田義清、川口　淳、永尾重昭、**三浦総一郎**	炎症性腸疾患腸粘膜における白血球マイグレーション関連分子発現と内視鏡像、シンポジウム：IBD における内視鏡検査の有用性	第２９回日本大腸検査学会総会	2011
Okada, Y., Tsuzuki, Y., Hokari, R., Kurihara, C., Komoto, S., Watanabe, C., Nakamura, M., Tomita, K., Kawaguchi, A., Nagao, S., **Miura, S**	A novel vegetable-derived Lactobacillus strain attenuates DSS induced colitis.	Asain Pacific Digestive Disease Week	2011
三浦総一郎	教育講演：機能性消化管障害への今日的アプローチ	第４２回日本消化吸収学会、第１９回日本消化器病週間内合同企画 JDDW 2011	2011
八月朔日秀明、穂苅量太、**三浦総一郎**	潰瘍性大腸炎および Crohn 病の abberant lymphocyte migration に対する autotaxin の役割	第５３回日本消化器病学会大会、第１９回日本消化器病週間内合同企画 JDDW 2011	2011

岡田義清、都築義和、上田俊秀、八月朔日秀明、佐藤伸悟、穂苅量太、栗原千枝、中村光康、富田謙吾、川口　淳、永尾重昭、**三浦総一郎**	大腸上皮系細胞 Caco２へのトランス脂肪酸暴露の影響について	第４２回日本消化吸収学会総会、第１９回日本消化器病週間内合同企画 JDDW 2011	2011
穂苅量太、渡辺知佳子、高本俊介、上田俊秀、八月朔日秀明、佐藤伸悟、栗原千枝、岡田義清、川口　淳、永尾重昭、**三浦総一郎**	精神的ストレスの大腸粘膜障害に与える影響	第１３回日本神経消化器病学会	2011
佐藤伸悟、穂苅量太、八月朔日秀明、上田俊秀、岡田義清、栗原千枝、渡辺知佳子、中村光康、富田謙吾、高本俊介、川口　淳、永尾重昭、**三浦総一郎**	Glucagon-like peptide-2 分泌に与える甘味成分の影響について	第４９回小腸研究会	2011
上田俊秀、穂苅量太、**三浦総一郎**	リコンビナント・リコモデュリンはマウス DSS 腸炎を改善する	第 39 回日本潰瘍学会	2011
Kamiya T, Shikano M, Hirata Y, Mizushima T, Shimura T, Mizoshita T, Tanida S, Kataoka H, Ueda T, Ugawa S, Ishida Y, Shimada S, **Joh T**	Acid-sensitive TRPV4 channel is expressed in mouse esophageal epithelium cells	DDW	2011
Kataoka H, Tanaka M, Mizushima T, Shimura T, Mizoshita T, Tanida S, Kamiya T, Mizuno K, **Joh T**	A comparison of cardiovascular tolerance and autonomic nervous responses among three groups: transnasal small-caliber endoscopy, transoral small-caliber endoscopy, and transoral normal endoscopy.	DDW	2011
Ebi M, Kataoka H, Higashiyama S, **Joh T**	TGF beta induces EGFR transactivation and HB-EGF C-terminal fragment nuclear translocation through ADAM17 activation in gastric cancer cells.	DDW	2011
Kamiya T, Shikano M, Hirata Y, Mizushima T, Murakami K, Shimura T, Mizoshita T, Mori Y, Tanida S, Kataoka H, **Joh T**	A multicenter open-label randomized trial comparing Rabeprazole versus Itopride in Japanese functional dyspepsia: The Nagoya Study	DDW	2011
Kamiya T, Shikano M, Hirata Y, Mizushima T, Murakami K, Shimura T, Mizoshita T, Mori Y, Tanida S, Kataoka H, **Joh T**	Functional TRPV4 channels are expressed in mouse esophageal epithelial cells.	UEGW 2011	2011
谷田諭史、溝下　勤、水島隆史、尾関啓司、塚本宏延、志村貴也、神谷 武、片岡洋望、**城 卓志**	抗酸菌性腸炎の２例	第 7 回日本消化管学会総会	2011

尾関　啓司、谷田　諭史、溝下　勤、塚本　宏延、**城　卓志**	IBD 腸管炎症に関わる炎症性サイトカインによる EGF signal を介した大腸癌細胞増殖機序　-EGF-C 末端 signal を標的とした新規薬剤探索-.	第 7 回消化管学会総会	2011
森　義徳，片岡洋望，神谷　武，谷田諭史，志村貴也，溝下　勤，村上賢治，水島隆史，平田慶和，海老正秀，馬渕元志，岡本泰幸，塚本宏延，尾関啓司，田中　守，Randal N Johnston，**城　卓志**	腫瘍溶解性ウイルス reovirus による新規胃癌集学的治療の検討	第 7 回日本消化管学会総会学術集会	2011
田中　守，片岡洋望，尾関啓司，塚本宏延，馬渕元志，海老正秀，平田慶和，水島隆史，志村貴也，溝下　勤，村上賢治，森　義徳，谷田諭史，神谷　武，**城　卓志**	消化管癌に対する糖鎖連結クロリンを用いた新規光線力学的治療法(Photodynamic Therapy; PDT)の検討	第 7 回日本消化管学会総会学術集会	2011
鹿野美千子，神谷　武，田中守，海老正秀，平田慶和，水島隆史，志村貴也，村上賢治，溝下　勤，森　義徳，谷田諭史，片岡洋望，**城　卓志**	六君子湯が胃運動機能におよぼす効果	第 108 回日本内科学会総会.	2011
神谷　武、鹿野美千子、田中守、塚本宏延、海老正秀、平田慶和、水島隆史、村上賢治、志村貴也、溝下　勤、森　義徳、谷田諭史、片岡洋望、**城卓志**	機能性ディスペプシアにおける胃活動電位、自律神経機能の検討	第 13 回日本神経消化器病学会総会	2011
片岡洋望，久保田英嗣，田中守，森　義徳，志村貴也，青山峰芳，高橋　智，浅井清文，**城　卓志**	ES cell-specific Ras, ERas accelerates liver metastases of gastric cancer through epithelial-mesenchymal transition	第 70 回日本癌学会学術総会	2011
田中　守，片岡洋望，岡本泰幸，海老正秀，平田慶和，志村貴也，溝下　勤，森　義徳，神谷　武，川本圭祐，柴原隆志，矢野重信，**城　卓志**	新規白金族元素パラジウム錯体，糖鎖連結パラジウム抗癌剤のシスプラチン耐性胃癌細胞株に対する抗腫瘍効果	第 70 回日本癌学会学術総会	2011
吉田道弘，志村貴也，福田信治，東山繁樹，溝下　勤，片岡洋望，**城　卓志**	胃癌化学療法における核移行 amphiregulin の意義	第 70 回日本癌学会学術総会	2011
志村貴也，吉田道弘，福田信治，溝下　勤，片岡洋望，東山繁樹，**城　卓志**	HB-EGF-CTF の核内移行は胃癌浸潤を促進する	第 70 回日本癌学会学術総会	2011
尾関　啓司、谷田　諭史、溝下　勤、塚本　宏延、城　卓志. 尾関　啓司、谷田　諭史、溝下　勤、塚本　宏延、**城卓志**	IBD 腸管炎症に関わる炎症性サイトカインによる EGF-C 末端 signal を介した大腸癌細胞増殖機序—HB-EGF-CTF シグナル抑制薬網羅的探索から—	JDDW　2011	2011
平田慶和、片岡洋望、**城　卓志**	循環器疾患患者における低用量アスピリンによる消化管出血性病変の検討	JDDW　2011	2011

海老　正秀、片岡　洋望、志村　貴也、溝下　勤、谷田　諭史、平田　慶和、神谷　武、水島　隆史、東山　繁樹、**城　卓志**	TGFβによる ROS および ADAM17 を介した EGF 受容体 transactivation のメカニズムの解析.	JDDW　2011	2011
溝下　勤 、佐々木誠人、谷田諭史、志村貴也、森　義徳、片岡洋望、神谷　武、**城　卓志**	ランソプラゾール持続投与が胃腫瘍発生に与える影響 – スナネズミモデルでの検討	第 22 回日本消化器癌発生学会総会	2011
尾関　啓司、志村　貴也、田中　守、塚本　宏延、海老　正秀、平田　慶和、村上　賢治、溝下　勤、谷田　諭史、森　義徳、片岡　洋望、神谷　武、**城　卓志**	検診にて発見された限局性十二指腸アミロイドーシスの一例	第 54 回 日本消化器内視鏡学会東海地方会	2011
海老　正秀、志村　貴也、**城　卓志**	食道表在癌の深達度診断における画像強調観察拡大内視鏡診断分類の検討	第 54 回日本消化器内視鏡学会東海地方会	2011
木下芳一、三輪洋人、**春日井邦夫**	逆流性食道炎初期治療におけるエソメプラゾールの有効性と安全性の検討	第 97 回日本消化器病学会総会	2011
木下芳一、三輪洋人、**春日井邦夫**	逆流性食道炎治癒患者の寛解維持におけるエソメプラゾールの有効性と安全性の検討	第 97 回日本消化器病学会総会	2011
井澤晋也、徳留健太郎、杉山智哉、足立百合加、近藤好博、伊藤義紹、増井竜太、土方康孝、河村直彦、飯田章人、水野真理、小笠原尚高、舟木康、佐々木誠人、**春日井邦夫**	PPI 抵抗性 NERD 症例に対する PPI 倍量投与の用性の検討	第 97 回日本消化器病学会総会	2011
徳留健太郎、舟木康、足立百合加、野田久嗣、近藤好博、伊藤義紹、増井竜太、井澤晋也、土方康孝、河村直彦、飯田章人、水野真理、小笠原尚高、佐々木誠人、**春日井邦夫**	高年齢者 PPI 不応性 NERD に対する食道運動機能検査を用いた病態解析	第 97 回日本消化器病学会総会	2011
小林佑次、田中創始、林伸彦、石井紀光、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志、有川卓、野浪敏明	尾側膵管拡張にて発見され、EUS-FNA にて診断された T1 膵癌の 1 例　ポスター	第 97 回日本消化器病学会総会	2011
小笠原尚高、土方康孝、**春日井邦夫**	胃腫瘍に対する Hybrid NOTES としての腹腔鏡補助下内視鏡的胃全層切除術の検討	日本消化器病学会東海支部第 114 回例会	2011
岡庭紀子、増井竜太、川村百合加、伊藤義紹、近藤好博、井澤晋也、土方康孝、河村直彦、徳留健太郎、飯田章人、水野真理、小笠原尚高、舟木康、佐々木誠人、**春日井邦夫**	Crohn 病と鑑別を要した腸結核の 1 例	日本消化器病学会東海支部第 114 回例会	2011

小林佑次、田中創始、野田久嗣、石井紀光、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志、永田博、野浪敏明	腹痛を契機に発見されたリンパ節転移を伴う膵ガストリノーマの1例	日本消化器病学会東海支部第114回例会	2011
高田真由子、水野真理，近藤好博，土方康孝，河村直彦，徳留健太郎，小笠原尚高，佐々木誠人，米田政志，**春日井邦夫**	ニューモシスチス肺炎を併発した潰瘍性大腸炎(UC)の1例	第214回日本内科学会東海地方会	2011
舟木康、徳留健太郎、近藤好博、伊藤義紹、井澤晋也、増井竜太、土方康孝、河村直彦、飯田章人、水野真理、小笠原尚高、佐々木誠人、米田政志、**春日井邦夫**	健康成人を対象としたプロトンポンプインヒビターの胃内 pH に及ぼす影響	第53回日本消化器病学会大会（JDDW2011）	2011
舟木康、小笠原尚高、**春日井邦夫**	食道運動機能検査を用いた機能性胸やけの病態分類とプライマリーケア診療のあり方	第53回日本消化器病学会大会（JDDW2011）	2011
石井紀光、佐藤顕、小林佑次、田中創始、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	膵尾部癌脾静脈浸潤に伴い出現した孤立性胃静脈瘤破裂に対して内視鏡的硬化療法（EIS）を施行し救命し得た一例	第53回日本消化器病学会大会（JDDW2011）	2011
野田久嗣、川村百合加，河村直彦，岡庭紀子，田村泰弘，小笠原尚高，佐々木誠人，中尾春壽，米田政志，**春日井邦夫**	急性膵炎を契機に発見された根治手術が可能であった早期十二指腸乳頭部癌の一例	第53回日本消化器病学会大会（JDDW2011）	2011
鳥井貴司、川村百合加，河村直彦，岡庭紀子，田村泰弘，小笠原尚高，佐々木誠人，中尾春壽，米田政志，**春日井邦夫**	GIST との鑑別に苦慮したガーゼオーマの1例	第215回日本内科学会東海地方会	2011
井上匡央、小林佑次，吉峰崇，田邉敦資，野田久嗣，石井紀光，佐々木誠人，中尾春壽，**春日井邦夫**，米田政志	超音波内視鏡下ドレナージが奏功した感染性膵仮性嚢胞の1例	第215回日本内科学会東海地方会	2011
Naotaka Ogasawara, Makoto Sasaki, Mari Mizuno, Kentaro Tokudome, Ryuta Masui, Yasutaka Hijikata, Yoshihiro Kondo, Yoshitsugi Ito, Akihito Iida,**Kunio Kasugai**	Rebamipide suppressed inflammatory bowel disease via regulation of TBK1-IRF3/7 signaling pathway	19th United European Gastroenterology Week (UEGW 2011)	2011
Yasutaka Hijikata, Naotaka Ogasawara, Makoto Sasaki, Mari Mizuno, Ryuta Masui, Yoshihiro Kondo, Shinya Izawa, Yoshitsugi Ito, **Kunio Kasugai**	Endoscopic submucosal dissection with sheath-assisted counter traction using a novel sheath for early gastric cancers.	19th United European Gastroenterology Week (UEGW 2011)	2011

佐々木誠人、舟木康、小笠原尚高、飯田章人、**春日井邦夫**	NSAID による小腸粘膜傷害に対するレバミピドの効果	第３９回日本潰瘍学会	2011
伊藤義紹、小笠原尚高、**春日井邦夫**	早期胃癌に対する薬品加工シースを用いたシースアシスト法による ESD の有用性	日本消化器病学会東海支部 115 回例会	2011
小林佑次、石井紀光、井上匡央、山本高也、野田久嗣、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志、有川卓、野浪敏明	当院における胆・膵病変の診断に対する EUS-FNA の有用性の検討	日本消化器病学会東海支部 115 回例会	2011
山本高也、大橋知彦、川村百合加、坂野文美、金森寛幸、佐藤顕、中出幸臣、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	AL 型アミロイドーシスに合併した自然肝出血の一例	日本消化器病学会東海支部 115 回例会	2011
井上匡央、小林佑次、小松原利典、新村哲也、川村百合加、岡庭紀子、吉峰崇、田邉敦資、野田久嗣、石井紀光、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	感染性膵仮性嚢胞に対してドレナージ術を施行した 2 例	日本消化器病学会東海支部 115 回例会	2011
吉峰崇、小林佑次、石井紀光、井上匡央、野田久嗣、田邉敦資、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	膵性腹水・膵性胸水の一例	日本消化器病学会東海支部 115 回例会	2011
杉山智哉、増井竜太、岡庭紀子、田村泰弘、近藤好博、伊藤義紹、井澤晋也、土方康孝、徳留健太郎、河村直彦、飯田章人、水野真理、小笠原尚高、舟木康、佐々木誠人、中尾春壽、米田政志、**春日井邦夫**、安藤景一	アニサキス症が原因と考えられた小腸イレウスの一例	日本消化器病学会東海支部 115 回例会	2011
岡庭紀子、小林佑次、石井紀光、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	胆嚢摘出術にて救命し得た出血性胆嚢炎の 2 例	日本消化器病学会東海支部 115 回例会	2011
小松原利典、土方康孝、井上匡央、野田久嗣、小林佑次、石井紀光、佐々木誠人、中尾春壽、**春日井邦夫**、米田政志	EUS-FNA で診断した内視鏡治療後 7 年で再発した S 状結腸癌の 1 例	第 54 回日本消化器内視鏡学会東海地方会	2011
野口誠司、田村泰弘、飯田章人、水野真理、伊藤義紹、土方康孝、小笠原尚高、佐々木誠人、米田政志、**春日井邦夫**	消化管出血を来たした有茎性 Brunner 腺過形成の 1 例	第 216 回日本内科学会東海地方会	2011
伊藤義紹、佐々木誠人、舟木康、小笠原尚高、水野真理、飯田章人、河村直彦、、徳留健太郎、土方康孝、井澤晋也、増井竜太、近藤好博、吉峰崇、岡庭紀子、野口誠司、川村百合加、井上匡央、**春日井邦夫**	NSAIDs 誘発小腸傷害におけるカプセル内視鏡と糖負荷試験による粘膜透過性との比較	第 8 回日本消化管学会	2011

井澤晋也、舟木康、徳留健太郎、近藤好博、伊藤義紹、増井竜太、土方康孝、河村直彦、飯田章人、水野真理、小笠原尚高、佐々木誠人、**春日井邦夫**	食道外病変（胸痛）を伴った PPI 抵抗性 NERD に対し PPI 倍量分割投与が著効した 1 例	第 8 回日本消化管学会	2012
岡庭紀子、水野真理、高田真由子、川村百合加、田村泰弘、近藤好博、伊藤義紹、増井竜太、井澤晋也、土方康孝、徳留健太郎、河村直彦、飯田章人、小笠原尚高、舟木康、佐々木誠人、**春日井邦夫**	当院における高齢者発症潰瘍性大腸炎患者の臨床的特徴	第 8 回日本消化管学会	2012
川村百合加、河村直彦、飯田章人、水野真理、小笠原尚高、佐々木誠人、中尾春壽、米田政志、**春日井邦夫**	GIST と鑑別に苦慮したガーゼオーマの一例	第 8 回日本消化管学会	2012
堀江隆介、**内藤裕二**、髙木智久、福田　亘、稲田　裕、飯田貴弥、辻　俊史、久貝宗弘、寄木浩行、春里暁人、水島かつら、岡田ひとみ、大矢友子、吉田直久、堅田和弘、鎌田和浩、内山和彦、半田　修、石川　剛、小西英幸、八木信明、古倉　聡、吉川敏一	Young World Symposium「遺伝子、シグナルの異常と胃疾患との病態相関」六君子湯による実験胃潰瘍の治癒促進に関する検討	第 43 回胃病態機能研究会	2011
堀江隆介、髙木智久、**内藤裕二**、辻　俊史、久貝宗弘、寄木浩行、井上　健、福本晃平、山田真也、岡田ひとみ、大矢友子、水島かつら、石川　剛、内山和彦、半田　修、小西英幸、若林直樹、八木信明、古倉　聡、吉川敏一	六君子湯による胃潰瘍治癒促進作用ならびに関連酸化修飾タンパク質の解析. ミニシンポジウム;胃粘膜障害	第 97 回日本消化器病学会総会	2011
内藤裕二、高木智久、吉川敏一	シンポジウム「消化器疾患と微小循環・酸化ストレス」翻訳語修飾タンパク質の解析で見えてきた酸化ストレス研究の新展開	第 97 回日本消化器病学会総会	2011
大矢友子、髙木智久、**内藤裕二**	チルグリオキザールによる Hsp27 タンパク質の翻訳後修飾の解析とその細胞生物学的意義. ワークショップ;肥満、代謝異常と消化管疾患:病態解明への新たなアプローチ	第 7 回日本消化管学会総会学術集会	2011
髙木智久、**内藤裕二**、吉川敏一	ベーシックサイエンス企画　シンポジウム 1「抗酸化環境応答と加齢」消化管炎症における抗酸化環境応答	第 11 回日本抗加齢医学会総会	2011
井上　健、髙木智久、**内藤裕二**	TNBS 誘発ラット大腸炎における腸管線維化の評価と大建中湯の治療効果. ワークショップ;下部消化管疾患に対する漢方治療の最前線	第 7 回日本消化管学会総会学術集会	2011

堀江隆介、髙木智久、**内藤裕二**、辻　俊史、久貝宗弘、寄木浩行、井上　健、福本晃平、山田真也、岡田ひとみ、大矢友子、水島かつら、石川　剛、内山和彦、半田　修、小西英幸、若林直樹、八木信明、古倉　聡、吉川敏一	六君子湯による胃潰瘍治癒促進作用ならびに関連酸化修飾タンパク質の解析．ミニシンポジウム;胃粘膜障害	第97回日本消化器病学会総会	2011
Horie R, **Naito Y**, Takagi T, Mizushima K, Tsuji T, Kugai M, Yoriki H, Yamada S, Fukumoto K, Inoue K, Harusato A, Uchiyama K, Handa O, Kokura S, Ichikawa H, Yoshikawa T.	Rikkunshito, a Japanese traditional herbal medicine, promotes murine gastric ulcer healing through the inhibition of the oxidative modification to proteins	Digestive Disease Week 2011	2011
西澤俊宏、鈴木雅之、高橋正彦、鈴木秀和、松崎潤太郎、田中伸、日比紀文	*Helicobacter pylori* 除菌自費診療への取り組み	第17回日本ヘリコバクター学会学術集会	2011
高橋正彦，武田篤也，木下聡，谷口智香，岩畔慶太，真一まこも，中里圭宏，南雲大暢，小松英嗣，**西澤俊宏**，藤山洋一，金子博，海老沼浩利，斎藤英胤，日比紀文	体幹部定位放射線治療（stereotactic body radiotherapy）を用いた肝癌治療	第97回日本消化器病学会総会	2011
西澤俊宏	*Helicobacter pylori* 除菌の基礎と臨床	千代田区学術講演会	2011
小田義英，**西澤俊宏**，南雲大暢，小松英嗣，藤山洋一，金子博，高橋正彦，田中伸，寺田総一郎，岡崎勲，織田正也	門脈腫瘍血栓を生じた HCC で TACE 後に PEIT，経皮熱湯注入療法を施行して効果が見られた3例	第97回日本消化器病学会総会	2011
真一まこも，木下聡，谷口智香，岩畔慶太，中里圭宏，南雲大暢，小松英嗣，**西澤俊宏**，藤山洋一，金子博，箭頭正徳，鈴木雅之，関敦子，前島新史，高橋正彦	多発転移を伴い AFP および PIVKA-2 高値を呈した胃肝様腺癌の1剖検例	第97回日本消化器病学会総会	2011
中里圭宏，小松英嗣，木下聡，谷口智香，岩畔慶太，真一まこも，南雲大暢，**西澤俊宏**，藤山洋一，金子博，箭頭正徳，鈴木雅之，高橋正彦	ステロイド・免疫抑制剤併用での HBV 再活性化による B 型重症肝炎の一例	第97回日本消化器病学会総会	2011

Ⅳ. 研究成果の刊行物・別刷

ANTIOXIDANTS & REDOX SIGNALING
Volume 14, Number 1, 2011

DOI: 10.1089/ars.2010.3146

ORIGINAL RESEARCH COMMUNICATION

Two Amino Acids Mutation of Ferric Uptake Regulator Determines *Helicobacter pylori* Resistance to Metronidazole

Hitoshi Tsugawa,[1] Hidekazu Suzuki,[1] Kazue Satoh,[2] Kenro Hirata,[1] Juntaro Matsuzaki,[1] Yoshimasa Saito,[1] Makoto Suematsu,[3] and Toshifumi Hibi[1]

Abstract

Metronidazole (Mtz) is a prodrug that is converted to its active form when its nitro group is reduced and superoxide radicals are generated. The superoxide radicals are directly toxic to the bacterium. On the other hand, the transcriptional regulator, ferric uptake regulator (Fur), of *Helicobacter pylori* is a direct suppressor of the iron-cofactored superoxide dismutase SodB, which is essential for protection against superoxide attack. Here, we demonstrate that in some Mtz-resistant strains, SodB activity is induced in a dose-dependent manner on exposure to Mtz. Further, under Mtz exposure, the generation of superoxide radicals in Mtz-resistant strains was significantly reduced as compared with that in the Mtz-susceptible strains. These Mtz-resistant strains were found to carry amino acids mutation of Fur (C78Y, P114S; mutant-type Fur). The binding affinity of the mutant-type Fur to an operator sequence on the *sodB* promoter (Fur-Box) was significantly reduced. Our approach demonstrated that SodB expression is derepressed by mutant-type Fur, which is associated with the development of Mtz resistance. *Antioxid. Redox Signal.* 14, 15–23.

Introduction

HELICOBACTER PYLORI IS A GRAM-NEGATIVE BACTERIUM that colonizes the gastric mucosa in more than half of the entire population of the world; it is a major cause of chronic active gastritis and peptic ulcer disease and also an early risk factor for gastric cancer (16, 43). Eradication of this bacterium from the stomach results in recovery from gastritis and peptic ulcer disease in over 90% of patients. Metronidazole (Mtz) was initially used against a variety of anaerobic microorganisms, but the drug was later found to also exhibit activity against certain microaerophilic organisms such as *H. pylori*. Currently, one of the most effective treatment regimens for *H. pylori* consists of a combination of a proton pump inhibitor and any two of the following three antimicrobial agents: amoxycillin, Mtz, and clarithromycin (15).

Recently, a gradually increasing prevalence of Mtz resistance has begun to be reported from Asia and Europe (11, 26, 47). Kim *et al.* suggested that Mtz is also widely prescribed for other infections such as parasitic or genital infections and that such widespread use and abuse of this inexpensive drug may contribute to the increasing prevalence of Mtz resistance (26). This increase in the prevalence of Mtz resistance is likely to become an issue of concern in the clinical management of *H. pylori* infection. Mtz enters the cells by diffusion, and its antimicrobial toxicity is dependent on the reduction of its nitro group to nitro anion radicals and the generation of superoxide radicals (37, 38). According to Goodwin *et al.*, since nicotinamide adenine dinucleotide phosphate (reduced form) nitroreductase (RdxA) of *H. pylori* reduces the nitro group of Mtz to anion radicals that produce DNA strand breaks and oxidative stress, which ultimately cause rapid cell death (14), mutational inactivation of the *rdxA* gene would be expected to be associated with the development of resistance to Mtz. However, a number of Mtz-resistant strains have been reported in which the RdxA protein appears to be unchanged (23, 45, 49). In addition, Masaoka *et al.* has also isolated Mtz-resistant strains with an intact RdxA protein (31). These reports strongly suggest the existence of a resistance mechanism in the organisms other than RdxA inactivation. In the Mtz-resistant strains, superoxide radicals are generated through the reduction of Mtz; therefore, we focused on the radical scavenging activity in these Mtz-resistant strains.

H. pylori expresses only a single superoxide dismutase (SOD), the iron-cofactored superoxide dismutase (SodB) protein, which exhibits 53.5% identity to the *Escherichia coli* FeSod (41). SodB, as the primary defense against superoxide radicals, prevents interaction between iron and superoxide as

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.
[2]Department of Anatomy, School of Medicine, Showa University, Tokyo, Japan.
[3]Department of Biochemistry and Integrative Medical Biology, Keio University School of Medicine, Tokyo, Japan.

well as catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. In addition, expression of SodB is also essential for gastric colonization by *H. pylori* and for its growth under microaerobic conditions (40).

Recently, Ernst *et al.* reported that *sodB* expression in *H. pylori* is directly regulated by the ferric uptake regulator (Fur) protein. Fur functions as a global transcriptional regulator and is involved in acid tolerance, detoxification of reactive oxygen species (ROS), and energy metabolism in *H. pylori* (5, 7, 12, 29). It is reported that Fur binds to iron (Fe^{2+}) and that the genes for iron uptake are repressed by the iron-binding form of Fur (10, 48). On the other hand, *sodB* expression is known to be repressed by the iron-free form of Fur (apo-Fur) (13). Apo-Fur binds to a specific consensus sequence called the Fur-Box located on the *sodB* promoter and blocks the binding of RNA polymerase (2, 13, 46).

In the present study, we attempted to confirm the hypothesis that Mtz-resistant strains which show no evident change of the RdxA protein exhibit an enhanced ability to defend themselves against superoxide radicals by SodB. The present study was designed to examine the expression of SodB and the structure and functions of Fur, which acts as a *sodB* transcriptional repressor, in Mtz-resistant strains.

Materials and Methods

Bacterial strains and culture conditions

H. pylori strains ATCC700392, KS0163, and KS0189 were used as the Mtz-susceptible strains; and strains KS0033, KS0048, and KS0145 were used as the Mtz-resistant strains. None of these Mtz-resistant strains showed any evident changes of the RdxA protein as determined by amino acid alignment analysis of the RdxA protein (31). According to the report of Masaoka *et al.*, KS0033 and KS0048 showed a moderate-level resistance ($16 \leq$ minimum inhibitory concentration [MIC] $< 32\,\mu g/mL$), and KS0145 showed a high-level resistance ($32\,\mu g/mL \leq$MIC) to Mtz (31). In this study, KS strains isolated from patients were maintained at −80°C in Brucella Broth (Becton-Dickinson) containing 25% (vol/vol) glycerol. The bacteria were cultured on Columbia HP agar (Becton-Dickinson) for 2 days at 37°C, under microaerobic conditions maintained with AnaeroPack MicroAero (Mitsubishigas).

Total RNA isolation and quantitative RT-polymerase chain reaction

Since Fur activity is dependent on the concentration of iron in the medium, the bacteria, normalized to an OD_{600} of 0.5, were incubated with 0, 0.01 and 0.05 μg/mL Mtz for 3 h in an iron-free medium (saline). The total RNA of the bacteria incubated with Mtz (Sigma) was isolated using the SV Total RNA Isolation system (Promega). The reverse transcription (RT) reaction was performed using the PrimeScript RT reagent Kit (Takara), in accordance with the manufacturer's guidelines. For real-time polymerase chain reaction (PCR), the PCR amplification was performed using the SYBR Premix Ex Taq Perfect Real Time kit (Takara) in a Thermal Cycler Dice Real Time System (Takara). The primer sequences used were as follows: *sodB* mRNA: forward 5′-CGACTGCCCTAAGC GATG and reverse 5′-CCAATTCCAACCAGAGCC; the 16S rRNA gene mRNA primers have been previously described in detail (35). The *H. pylori* 16S rRNA gene was used as the internal control for the quantitative RT-PCR.

Measurement of SOD activity

Since the Fur activity is dependent on the concentration of iron in the medium, the bacteria, normalized to an OD_{600} of 0.5, were incubated with 0, 0.05, and 0.5 μg/mL Mtz for 5 h in an iron-free medium (saline). After sonication (1.5 min at 25% power) of the bacteria incubated with Mtz, the resultant bacterial lysates were centrifuged, and the SOD activities were measured using an SOD Assay Kit-WST (Dojindo), in accordance with the manufacturer's guidelines.

Electron spin resonance assay

A spin trapping agent, 5 μM 4-Hydroxy-TEMPO (Sigma) or 40 mM CYPMPO (Radical Research), was added to the bacteria, normalized to an OD_{600} of 0.5, and incubated with 0, 0.05 or 0.5 μg/mL Mtz for 5 h. After sonication of the bacteria, the resultant bacterial lysates were transferred to a quartz flat cell (disposables) (Radical Research), and the radical intensity was determined by electron spin resonance (ESR) spectroscopy (JESRE1X, X-band; 100 kHz modulation frequency; Jeol) at 20°C.

Measurement of RdxA activity

After sonication (1.5 min at 25% power) of the bacteria cultivated for 2 days in the Brucella Broth plate, the resultant bacterial lysates were centrifuged, and the protein concentrations were measured using the BCA method (Pierce). RdxA activity was spectrophotometrically measured with reduction of Mtz observed at 320 nm. The reaction mixture contained Tris/acetate (100 mM Tris-HCl, 50 mM acetate), pH 7.0, 0.05 mM Mtz, and 0.3 mM nicotine adenine dinucleotide (reduced form), as described by Goodwin *et al.* (14).

Construction of SodB overexpression strain and rdxA deletion mutant strain

The shuttle vector pHel3 (19) was used as a scaffold to construct a SodB-overexpressing strain of *H. pylori*. The *sodB* gene was PCR-amplified with specific primers (forward 5′-CTCGAGATTAACTTTTAAAAAATTTAAAAAGAATTTG and reverse 5′-GGTACCTTAAGCTTTTTTATGCACC) and cloned into the pHel3 shuttle vector as a *Kpn*I-*Xho*I fragment. A nucleic acids sequencing of a *Kpn*I-*Xho*I fragment was performed on the pHelSodB construct, and then the construct was electroporated into *H. pylori*, which was grown on kanamycin to obtain a SodB-overexpressing strain. On the other hand, *H. pylori* transfected with only the pHel3 shuttle vector was grown on kanamycin to obtain the control strain.

The target-region gene cassette (5′*rdxA-chloramphenicol acetyltransferase* (*cat*)-3′*rdxA*) for construction of *rdxA* deletion mutant strain was cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA), and then the sequences were determined (target-vector). The target-vector was electroporated into *H. pylori* ATCC700392, which was grown on 20 μg chloramphenicol (Sigma) to obtain an *rdxA* deletion mutant strain of *H. pylori* ATCC700392.

Measurement of the MIC to Mtz

The bacteria (at an OD_{600} of 0.1) were inoculated on an agar plate containing Mtz in serial twofold dilutions (0.5–128 μg/mL). All the plates were incubated at 37°C under microaerobic conditions, and the MIC values were determined (32).

DNA sequencing and protein modeling of H. pylori *Fur*

The complete *fur* gene and the promoter region of *sodB* were PCR-amplified with specific primers (*fur* gene: forward 5′-ATGAAAAGATTAGAAACTTTG and reverse 5′-ACATT CACTCTCTTGGCATTCT; *sodB* promoter gene: forward 5′-CCCTTAAAATCCACAAAATTTGC and reverse 5′-GTA ATGTAAACATGTTTTCTCCTTGTG) using Ex Taq DNA polymerase (Takara). The PCR products were cloned into the pCR4-TOPO vector (Invitrogen), and then the sequences of the *fur* and *sodB* promoter genes were determined using the BigDye terminator V1.1 Cycle Sequencing Kit (Applied Biosystems); the deduced amino acids were then aligned using GENETYX Version 5.1. The protein structures were modeled and displayed using Swiss-Model (www.expasy.org/swissmod) and DeepView-Swiss-PdbViewer (www.expasy.org/spdbv/), respectively.

Expression and purification of H. pylori *Fur*

The *fur* gene was PCR-amplified with specific primers (ExFur gene: forward 5′-CATATGAAAAGATTAGAAACTT TGG and reverse 5′- AGATCTGGACATTCACTCTCTTG) and cloned into the pET-30b (+) (Novagen) as an *NdeI-BglII* fragment. The pETFur construct was transformed into *E. coli* BL21 (DE3), and the expression was achieved by induction, by the addition of 0.5 mM IPTG, of a 200 mL culture incubated for 6–8 h at 30°C and grown to an OD_{600} of 0.6. The Fur protein expressed in this strain as a C-terminal Six-His tagged protein was purified using the MagneHis Protein Purification System (Promega).

Apo-Fur binding analysis by surface plasmon resonance assay

A Biacore 2000 instrument (Biacore AB) was used to perform the Surface Plasmon Resonance assay in accordance with the manufacturer's guidelines. To construct the biotinylated *sodB* promoter gene of each strain, each *sodB* promoter gene was PCR-amplified with specific biotinylated primers (forward 5′-CCCTTAAAATCCACAAAATTTGC and reverse 5′-Bio-GTAATGTAAACATGTTTTCTCCTTGTG). To conduct the analyses under a low-iron condition, the following buffer was used for the analyses: HBS-EP running buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM ethylenediaminetetraacetic acid, 0.005% surfactant P20) and biotinylated PCR products of the *sodB* promoter were immobilized on to Sensor Chip SA (GE Healthcare). At least five concentrations of each purified Fur protein were applied to the *sodB* promoter-immobilized Sensor Chip SA in HBS-EP buffer at a flow rate of 10 μl/min. The response value of the reference cell (flow cell 3, blank) was subtracted from the response value of each flow cell 4 (*sodB* promoter) to correct for nonspecific binding. The data were analyzed, and the dissociation constant (*Kd*) values were calculated using a BIAevaluation software (Baicore).

Results

Expression of SodB under Mtz exposure

In the Mtz-susceptible strain ATCC700392, *sodB* mRNA expression was scarcely derepressed under Mtz exposure (Fig. 1a). On the other hand, in the Mtz-resistant strains, which showed no evident change of the RdxA protein (KS0033, KS0048, and KS0145), the *sodB* mRNA expression was derepressed in a dose-dependent manner under exposure to Mtz (Fig. 1a). Further, no increase of the SodB activity was observed in the Mtz-susceptible strain, whereas significant increase of the SodB activity was found in the Mtz-resistant strains in the presence of 0.5 μg/mL Mtz (Fig. 1b).

Generation of superoxide radicals in H. pylori *under Mtz exposure*

To assess whether ROS generation was suppressed by the overexpression of SodB in the Mtz-resistant strains, we measured the amount of ROS produced in each type of *H. pylori*

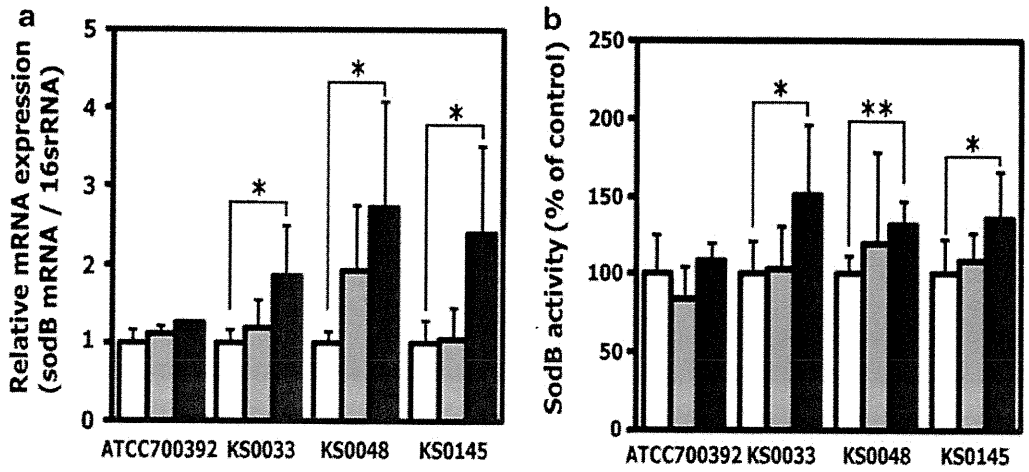

FIG. 1. Expression of SodB under Mtz exposure. (**a**) Expression of *sodB* mRNA in an Mtz-susceptible strain (ATCC700392) and Mtz-resistant strains (KS0033, KS0048, and KS0145) exposed to 0 (white), 0.01 (gray), and 0.05 (black) μg/mL Mtz was measured by quantitative reverse transcription–polymerase chain reaction. (**b**) Expression of SodB activity in an Mtz-susceptible strain (ATCC700392) and Mtz-resistant strains (KS0033, KS0048, and KS0145) exposed to 0 (white), 0.05 (gray), and 0.5 (black) μg/mL Mtz was measured by the method described in the Materials and Methods section. Results are means ± SD of three independent assays. Asterisks indicate statistical significance from each strain with no Mtz exposure, $*p < 0.05$, $**p < 0.01$. Mtz, metronidazole; SodB, iron-cofactored superoxide dismutase.

strain under Mtz exposure by ESR assay. Although significant dose-dependent increase in the generation of ROS was observed after exposure to Mtz in the Mtz-susceptible strains, the ROS generation was significantly reduced in the Mtz-resistant strains (Fig. 2a). Further, Figure 2b shows the presence of the superoxide radical-specific signal of ESR detected with the CYPMPO reagent in the Mtz-susceptible strain, whereas no such specific signals can be seen in the Mtz-resistant strains.

Effect of SodB overexpression on H. pylori *susceptibility to Mtz*

To assess the contribution of the SodB overexpression to Mtz resistance, a SodB-overexpressing strain was constructed using a pHel3 shuttle vector (19). The SodB activity of the SodB-overexpressing strain (ATCC700392 pHel3::*sodB*) was twofold higher as compared with that of the control strain (ATCC700392 pHel3 control) (data not shown). Although the MIC of Mtz for the ATCC700392 strain and pHel3 control strain was the same as that for the Mtz-susceptible strains (MIC $<8\,\mu g/mL$), the MIC values for KS0033, KS0048, KS0145, and ATCC700392 pHel3::*sodB* were 64, 32, 128, and $32\,\mu g/mL$, respectively (Table 1). Thus, these strains showed a high level resistance to Mtz (MIC $\geq 32\,\mu g/mL$). In addition, to assess the Mtz reduction activity associated with Mtz resistance of KS0033, KS0048, and KS0145, the RdxA activity was spectrophotometrically measured with reduction of Mtz at 320 nm. The RdxA activity for KS0033, KS0048, KS0145, ATCC700392 pHel3::*sodB*, and ATCC700392 pHel3 control were not decreased compared with ATCC700392 (Table 1). On the other hand, the RdxA activity of ATCC700392 Δ*rdxA*, which showed a moderate-level resistance to Mtz ($8 \leq$MIC $<32\,\mu g/mL$), was significantly decreased compared with ATCC700392 (Table 1). This result indicated that RdxA inactivation did not contribute to development of the Mtz resistance in the KS0033, KS0048, KS0145, and ATCC700392 pHel3::*sodB*. Therefore, these findings strongly suggest that SodB overexpression contributes to Mtz resistance in the KS0033, KS0048, KS0145, and ATCC700392 pHel3::*sodB*.

Alignment of the nucleic acid sequence of the SodB promoter and the amino acid sequence of Fur

To assess the mechanism of SodB overexpression in the Mtz-resistant strains, we focused on the regulation of *sodB* expression by Fur. We aligned the nucleic acid sequence of the *sodB* promoter (Fur-Box) and the predicted amino acid sequence of Fur for the Mtz-susceptible strains (ATCC700392, KS0163, and KS0189) and Mtz-resistant strains (KS0033, KS0048, and KS0145). The A-5C mutation of the Fur-Box was detected in all of the clinical isolates from Keio University hospital (Fig. 3a). Although KS0145 showed a G-3A mutation adjacent to the Fur-Box, no distinct mutation of the Fur-Box was observed in the Mtz-resistant strains (Fig. 3a). On the other hand, two distinct mutations of the amino acid sequence of Fur were noted in the Mtz-resistant strains (Fig. 3b). KS0145 had a mutant-type Fur protein, with Cys 78 replaced by Tyr (C78Y) and Asn 118 replaced by His (N118H). KS0033 and KS0048 also showed a mutant-type of Fur, with Pro 114 replaced by Ser (P114S) and N118H (Fig. 3b). The HHDHXXCXXC motif, which is believed to be involved in the binding of the iron cofactor, was highly conserved (Fig. 3b) (4).

Kd value of apo-wild-type Fur and apo-mutant-type Fur

To assess the effect of the amino acid mutations of Fur (mutant-type Fur) on the affinity of apo-Fur for the Fur-Box, we examined the affinity of each of the apo-Fur proteins for the *sodB* promoter (Fur-Box) by Surface Plasmon Resonance assay (Biacore 2000). Beforehand, it was confirmed that the *Kd* value of apo–wild type (WT)-Fur to Fur-Box was similar to the value that Ernst *et al.* reported (13), and then the *Kd* value of

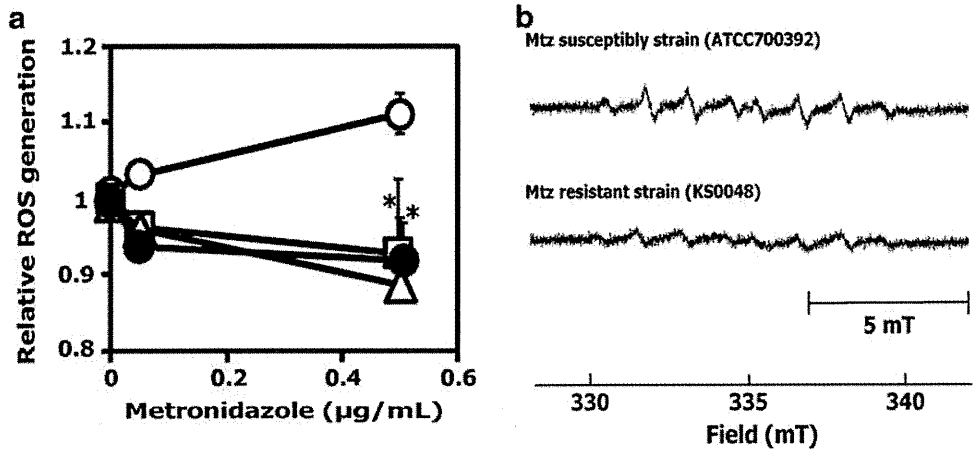

FIG. 2. Generation of superoxide radicals under Mtz exposure. (**a**) The induction of ROS was measured by electron spin resonance using $5\,\mu M$ 4-Hydroxy-TEMPO in an Mtz-susceptible strain (ATCC700392) (white circle) and Mtz-resistant strains (KS0033 [white square], KS0048 [black circle], and KS0145 [white triangle]) exposed to 0, 0.05, and $0.5\,\mu g/mL$ Mtz. The ROS generation was calculated as reference in the ROS generation of each strain without Mtz exposure. Results are means ± SD of three independent assays. Asterisks of KS0048 and KS0145 indicate statistical significance for the comparison with Mtz-susceptible strain (ATCC700392) as determined by Student's *t*-test ($^*p < 0.05$). (**b**) Representative signal patterns of generation of superoxide radicals in the Mtz-susceptible stain and Mtz-resistant strains exposed to $0.5\,\mu g/mL$ Mtz as measured by electron spin resonance using 40 mM CYPMPO. ROS, reactive oxygen species.

TABLE 1. THE EFFECT OF SUPEROXIDE DISMUTASE-OVEREXPRESSION AND RdxA ACTIVITY ON MINIMUM INHIBITORY CONCENTRATION (μG/mL) OF METRONIDAZOLE

Strains	*RdxA activity (nmol/min/mg protein)*	p-*Value*	*Minimum inhibitory concentration (μg/mL)*	*Metronidazole susceptibility*
ATCC700392	2.57 ± 0.26		2	Susceptible level
ATCC700392 Δ*rdxA*	1.29 ± 0.19	<0.01	16	Moderate level resistance
KS0033	2.45 ± 0.41	0.59	64	High level resistance
KS0048	2.35 ± 0.08	0.11	32	High level resistance
KS0145	2.40 ± 0.23	0.23	128	High level resistance
ATCC700392 pHel3::sodB	2.39 ± 0.05	0.16	32	High level resistance
ATCC700392 pHel3 control	2.77 ± 0.05	0.23	4	Susceptible level

SodB, iron-cofactored superoxide dismutase; RdxA, nicotinamide adenine dinucleotide phosphate (reduced form) nitroreductase.

apo-mutant-type Fur to Fur-Box as control with that of apo-WT-Fur was measured. The results of the assay revealed a significant increase of the *Kd* value for the apo-mutant-type Fur in the Mtz-resistant strains as compared with that of apo-WT-Fur in the Mtz-susceptible strains (Fig. 4). These results indicate a significantly decreased affinity of apo-mutant-type Fur for the Fur-Box and that the SodB expression in the Mtz-resistant strains is not repressed to the same extent as that in the Mtz-susceptible strains (Fig. 5).

Further, to assess the effect of nucleic acid mutations of the *sodB* promoter on the affinity of apo-Fur for the Fur-Box, we examined the affinity of apo-ATCC700392 Fur for the KS0145

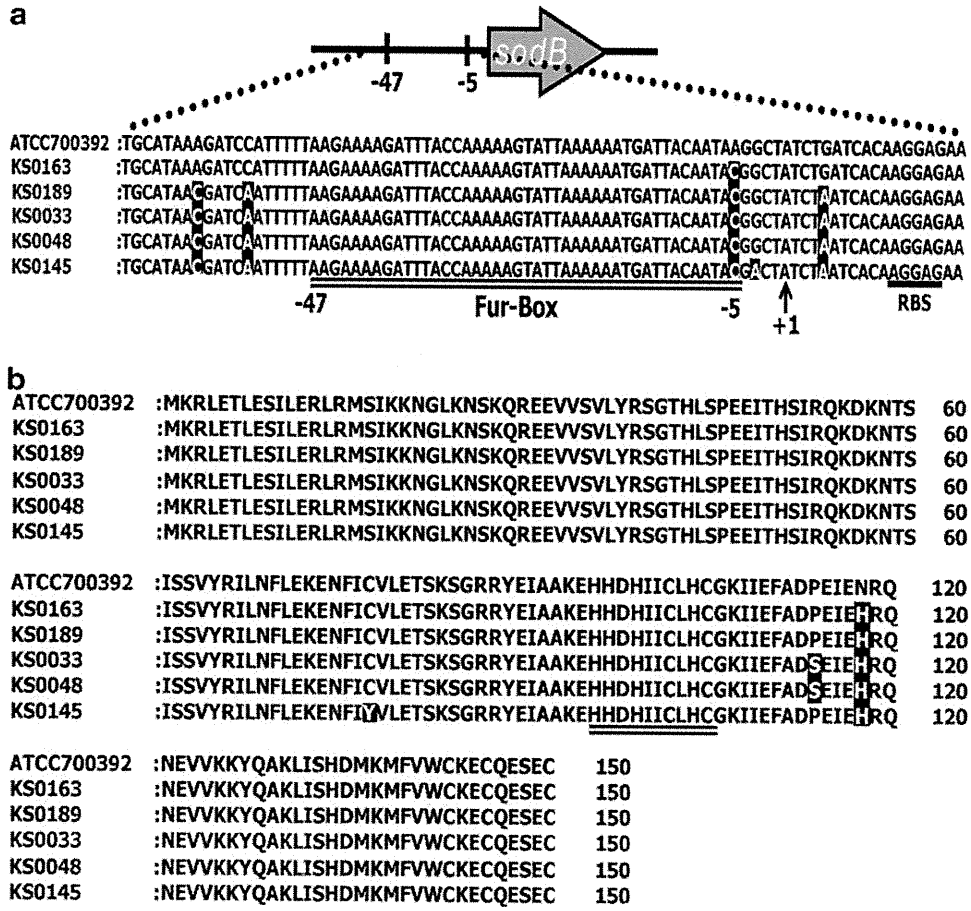

FIG. 3. Alignments of the *Helicobacter pylori sodB* promoter and Fur protein. (**a**) Alignment of the *sodB* promoter from the Mtz-susceptible strains (ATCC700392, KS0163, and KS0189) and Mtz-resistant strains (KS0033, KS0048, and KS0145). Each mutation point is marked in white. The predicted Fur-Box ranges from −5 to −47 and is indicated by the double line. +1 indicates the *sodB* transcriptional start site, and RBS indicates the ribosmal binding site. (**b**) Alignment of the predicted Fur amino acid sequences of Mtz-susceptible strains (ATCC700392, KS0163, and KS0189) and Mtz-resistant strains (KS0033, KS0048, and KS0145). Each mutation point is marked in white. The highly conserved motif (HHDHXCXXC) believed to be involved in the binding of the iron cofactor is indicated by the double lines. Fur, ferric uptake regulator.

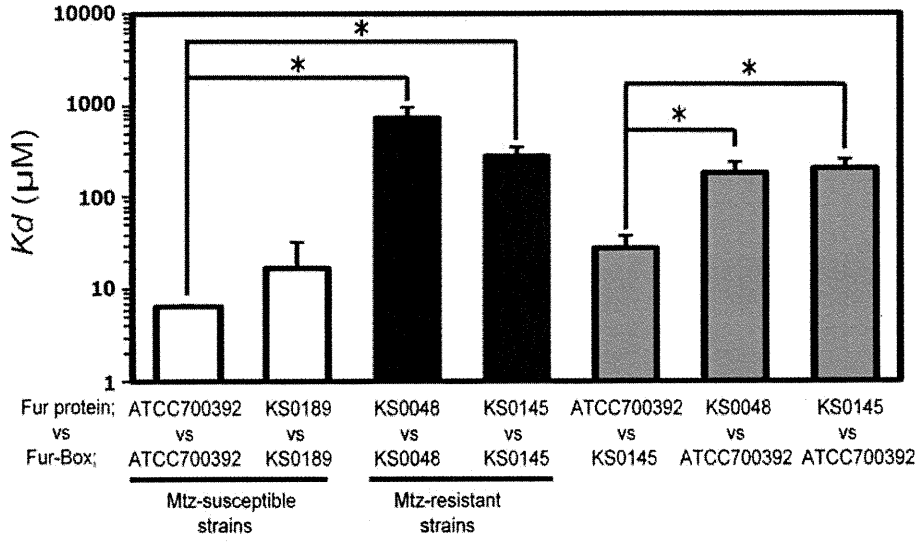

FIG. 4. Mutation of Fur affects its affinity for the Fur-Box. The *Kd* value for binding of each apo-Fur protein to each Fur-Box was calculated as reference in the Flow Cell in which *sodB* promoter was not immobilized on to Sensor Chip SA using a BIAevaluation software, and the combination of apo-Fur protein and Fur-Box is denoted as Fur protein *versus* Fur-Box. White bar indicates the affinity of apo–wild type (WT)-Fur for the Fur-Box of the Mtz-susceptible strains, black bar indicates the affinity of apo-mutant-type Fur for the Fur-Box in the Mtz-resistant strains, and the gray bar indicates the effect of the nucleic acid mutations of the Fur-Box on the affinity of apo-Fur for the Fur-Box. Results are means ± SD of three independent assays. Asterisks indicate statistical significance from using an apo-WT-Fur, $*p < 0.05$. *Kd*, dissociation constant.

Fur-Box and the affinity of apo-mutant-type Fur for the ATCC700392 Fur-Box. The *Kd* value of apo-ATCC700392 Fur for binding to the KS0145 Fur-Box was fourfold higher as compared with that for the binding to the ATCC700392 Fur-Box, although the difference was not significant (Fig. 4). On the other hand, the *Kd* values of apo-mutant-type Fur for binding to the ATCC700392 Fur-Box were scarcely reduced as compared with that for its binding to the KS0145 or KS0048 Fur-Box (Fig. 4). The results of the assay revealed a significant increase of the *Kd* values of apo-mutant-type Fur for binding to the ATCC700392 Fur-Box as compared with that of apo-ATCC700392 Fur for binding to the KS0145 Fur-Box (Fig. 4).

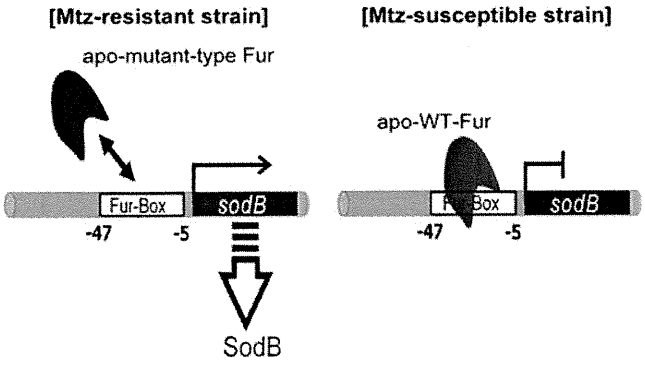

FIG. 5. Schematic representation of the proposed mode of action of apo-mutant type-Fur in the Mtz-resistant strains and apo-WT-Fur in the Mtz-susceptible strains. The apo-Fur binds to an operator sequence called Fur-Box in the *sodB* promoter, and then binding of apo-Fur suppresses *sodB* expression. The affinity of the apo-mutant-type Fur to the Fur-Box is significantly decreased, and then *sodB* expression of Mtz-resistant strains is more derepressed than Mtz-susceptible strains.

Prediction of the three-dimensional structure of H. pylori Fur

To predict the positions of the mutations in the three-dimensional structure of Fur, the structure was determined using a Swiss Model and DeepView-Swiss-PdbViewer. The N-terminal domain possessing four helices followed by a loop was formed by the residues located between two antiparallel β-strands. The C-terminal domain, which was separated by a coil from the N-terminus possessing two antiparallel β-strands, was followed by another β-strand located between the two helices (Fig. 6). C78Y is predicted to belong to a β strand in the N-terminal domain, whereas P114S and N118H are predicted to belong to a C-terminal domain (Fig. 6).

Discussion

The present study revealed amino acid mutations of Fur in some Mtz-resistant strains with the RdxA activity remaining with reduced affinity of the mutant Fur for the Fur-Box, and enhancement of the superoxide radical scavenging activity in these strains, as *sodB* was not repressed to the same extent by the apo-mutant-type Fur in these strains as by the wild-type apo-fur in the Mtz-susceptible strains (Figs. 1–4, Table 1).

Recently, Carpenter *et al.* reported that the A-5C mutation of the Fur-Box decreases the affinity of apo-Fur for the Fur-Box in *H. pylori* (6). In the present study, the A-5C mutation of the Fur-Box was detected in all of the tested clinical isolates (Fig. 3a). In the Surface Plasmon Resonance assay, the *Kd* value for the binding of apo-ATCC700392 Fur to the KS0145 Fur-Box was fourfold higher as compared with that for its binding to the ATCC700392 Fur-Box (Fig. 4), suggesting that the A-5C mutation in the Fur-Box is important for the binding with apo-ATCC700392 Fur, which is consistent with the report of Carpenter *et al.* (6). On the other hand, in the Mtz-resistant strains, the A-5C mutation hardly influenced the interaction between

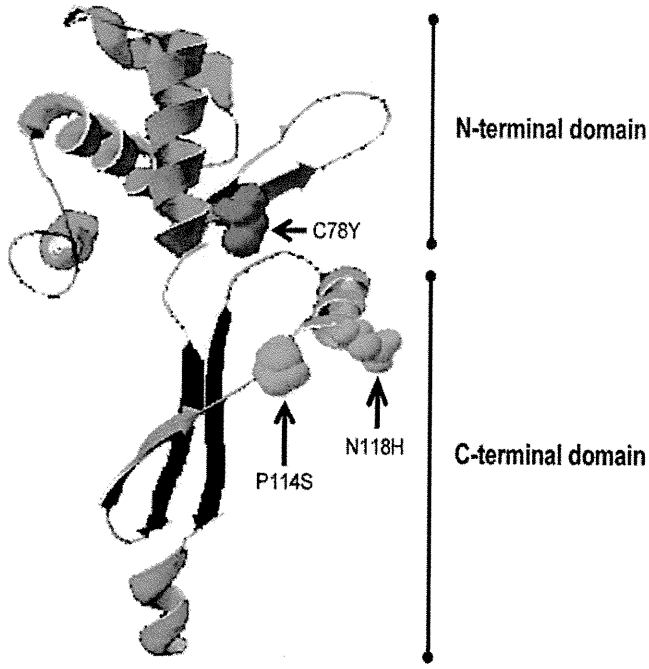

FIG. 6. Prediction of the three-dimensional structure of the Fur protein. Each mutation point is marked with an arrow. C78Y is predicted to exist in the N-terminal domain, whereas P114S and N118H are predicted to be located in the C-terminal domain. The three-dimensional structure was determined using a Swiss-Model and DeepView-Swiss-PdbViewer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

the Fur-Box and the apo-mutant-type Fur (Fig. 4). These results indicate that the Fur mutations C78Y, P114S, and N118H could play a greater role on the affinity of apo-Fur for the Fur-Box than the A-5C mutation in the Fur-Box.

The Fur protein has been best characterized in *E. coli*, in which it has been shown to possess three functional domains (the DNA-binding domain, iron-binding domain, and the oligomerization domain), and the protein binds to the Fur-Box after dimerization (17, 36, 42, 46). The Fur monomer of *E. coli* has been reported to consist of a helix-turn-helix motif and two β strands separated by a turn that forms the wings on the N-terminal domain, which is considered to be involved in the DNA binding (21, 42, 46). On the other hand, the C-terminal domain of *E. coli* Fur, separated by a coil from the N-terminal, consists of two antiparallel β-strands, which are considered to be involved in the oligomerization of the protein (21, 42). From the results of the homology modeling of *H. pylori* Fur, it was inferred that *H. pylori* Fur also has three functional domains (the DNA-binding domain near the N-terminal, iron cofactor-binding domain (HHDHXXCXXC), and the oligomerization domain near the C-terminal) (4). Therefore, it was inferred that the C78Y mutation of the KS0145 strain was located in the DNA-binding domain and that the P114S and N118H mutations of KS0033 and KS0048 strains were located in the oligomerization domain using a homology modeling (Fig. 6). Therefore, these mutations are predicted to affect the affinity of the Fur protein for the Fur-Box. However, the amino acid sequence of *H. pylori* Fur exhibited moderate identity (23%–37%) to the Fur protein from other bacteria present in the database, such as *Campylobacter jejuni*, *E. coli*, *Haemophilus influenzae*, *Vibrio cholerae*, *Bordetella pertussis*, *Klebsiella pneumoniae*, *Neisseria meningitides*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, suggestive of a moderate homology (4). This finding indicates that the amino acids which are important for DNA binding or dimerization may differ between *H. pylori* Fur and other bacterial Fur proteins.

ROS damage of pathogenic bacteria constitutes a key part of the immune response of the host. Many studies have shown that *H. pylori* infection elicits a strong oxidative stress response from the host (1, 3, 9, 44). To survive the effects of production of ROS by the host, *H. pylori* depends on a significant repertoire of detoxification enzymes, such as SodB, catalase (KatA), and neutrophil-activating protein (NapA) (18, 34, 41). Upstream of *katA*, a low-affinity putative Fur-Box has been identified (30, 33). In addition, Cooksley *et al.* reported that Fur is involved in *napA* regulation and that a potential Fur-Box by which this control could be mediated has been identified (8). Accordingly, the expression of *katA* and/or *napA* might be derepressed by mutant-type Fur, leading to enhancement of the ability of *H. pylori* to colonize the human stomach.

In the present study, we demonstrated that the overexpression of SodB mediated by mutant-type Fur may underlie the RdxA-independent resistance of *H. pylori* to Mtz. Recently, it has come to be recognized that in addition to RdxA, some other proteins such as pyruvate oxidoreductase, nicotinamide adenine dinucleotide phosphate (reduced form) flavin oxidoreductase (FrxA), and ferredoxin-like protein (FdxB) may also be associated with the activation of Mtz (22, 25). Many researchers have demonstrated an association between inactivation of these proteins and resistance to Mtz (20, 24, 27, 28). On the other hand, Jenks *et al.* reported that RdxA-independent mechanisms may play only a relatively minor role in Mtz resistance or may be involved only in the transition to high-level resistance (22). Although it is difficult to determine whether overexpression of SodB associated with mutant-type Fur entirely accounts for RdxA-independent Mtz resistance, it is, nevertheless, an important mechanism that participates in not only Mtz resistance but also resistance of the host immune responses to ROS.

Recently, overexpression of Fe-SOD was reported to be associated with the Mtz resistance in Mtz-resistant strains of the protozoan parasite *Entamoeba histolytica*, which is the causative agent of human amoebiasis (39, 50). Based on these reports, it is considered that overexpression of SOD may affect the Mtz resistance mechanism in many bacterial species.

In conclusion, the present study demonstrates a novel mechanism of Mtz resistance of *H. pylori*, namely, aberrant increase of SodB expression resulting from mutations of Fur.

Acknowledgments

The authors are grateful to Dr. Rainer Haas of Ludwig-Maximilians-University, Munich, for providing us the pHel shuttle vectors.

This work was supported by a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science (21790133, to H.T.), a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (22300169, to H.S.), a grant from the Smoking Research Foundation (to H.S.), Keio Gijuku Academic Development

Funds (to H.S.), and Keio University Research grants for Life Sciences and Medicine (99-095-0009, to H.T.).

This work was awarded the Prize for Best Investigator at the 18th Digestive and Free Radical Workshop, the Young Investigator Award at the 62nd Society for Free Radical Research Japan, and also the Uehara *H. pylori* Award at the 15th Japanese Society for Helicobacter Research. A part of this work was presented at a Research Forum in Digestive Disease Week 2009 in Chicago, IL.

Author Disclosure Statement

No competing financial interests exist.

References

1. Allen LA. Phagocytosis and persistence of *Helicobacter pylori*. *Cell Microbiol* 9: 817–828, 2007.
2. Baichoo N and Helmann JD. Recognition of DNA by Fur: a reinterpretation of the Fur box consensus sequence. *J Bacteriol* 184: 5826–5832, 2002.
3. Baik SC, Youn HS, Chung MH, Lee WK, Cho MJ, Ko GH, Park CK, Kasai H, and Rhee KH. Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. *Cancer Res* 56: 1279–1282, 1996.
4. Bereswill S, Lichte F, Vey T, Fassbinder F, and Kist M. Cloning and characterization of the fur gene from *Helicobacter pylori*. *FEMS Microbiol Lett* 159: 193–200, 1998.
5. Bijlsma JJ, Waidner B, Vliet AH, Hughes NJ, Hag S, Bereswill S, Kelly DJ, Vandenbroucke-Grauls CM, Kist M, and Kusters JG. The *Helicobacter pylori* homologue of the ferric uptake regulator is involved in acid resistance. *Infect Immun* 70: 606–611, 2002.
6. Carpenter BM, Gancz H, Gonzalez-Nieves RP, West AL, Whitmire JM, Michel SL, and Merrell DS. A single nucleotide change affects fur-dependent regulation of *sodB* in *H. pylori*. *PLoS One* 4: e5369, 2009.
7. Choi YW, Park SA, Lee HW, and Lee NG. Alteration of growth-phase-dependent protein regulation by a fur mutation in *Helicobacter pylori*. *FEMS Microbiol Lett* 294: 102–110, 2009.
8. Cooksley C, Jenks PJ, Green A, Cockayne A, Logan RP, and Hardie KR. NapA protects *Helicobacter pylori* from oxidative stress damage, and its production is influenced by the ferric uptake regulator. *J Med Microbiol* 52: 461–469, 2003.
9. Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, Blake DR, and Rampton DS. *Helicobacter pylori* stimulates antral mucosal reactive oxygen metabolite production *in vivo*. *Gut* 35: 179–185, 1994.
10. Delany I, Pacheco AB, Spohn G, Rappuoli R, and Scarlato V. Iron-dependent transcription of the frpB gene of *Helicobacter pylori* is controlled by the Fur repressor protein. *J Bacteriol* 183: 4932–4937, 2001.
11. Dunn BE, Cohen H, and Blaser MJ. *Helicobacter pylori*. *Clin Microbiol Rev* 10: 720–741, 1997.
12. Ernst FD, Bereswill S, Waidner B, Stoof J, Mader U, Kusters JG, Kuipers EJ, Kist M, van Vliet AH, and Homuth G. Transcriptional profiling of *Helicobacter pylori* Fur- and iron-regulated gene expression. *Microbiology* 151: 533–546, 2005.
13. Ernst FD, Homuth G, Stoof J, Mader U, Waidner B, Kuipers EJ, Kist M, Kusters JG, Bereswill S, and van Vliet AH. Iron-responsive regulation of the *Helicobacter pylori* iron-cofactored superoxide dismutase SodB is mediated by Fur. *J Bacteriol* 187: 3687–3692, 2005.
14. Goodwin A, Kersulyte D, Sisson G, Veldhuyzen van Zanten SJ, Berg DE, and Hoffman PS. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol Microbiol* 28: 383–393, 1998.
15. Group EHPS. Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report. *Gut* 41: 8–13, 1997.
16. Group TES. An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet* 341: 1359–1362, 1993.
17. Hamed MY and Al-Jabour S. Iron(II) triggered conformational changes in *Escherichia coli* fur upon DNA binding: a study using molecular modeling. *J Mol Graph Model* 25: 234–246, 2006.
18. Hazell SL, Evans DJ Jr., and Graham DY. *Helicobacter pylori* catalase. *J Gen Microbiol* 137: 57–61, 1991.
19. Heuermann D and Haas R. A stable shuttle vector system for efficient genetic complementation of *Helicobacter pylori* strains by transformation and conjugation. *Mol Gen Genet* 257: 519–528, 1998.
20. Hoffman PS, Goodwin A, Johnsen J, Magee K, and Veldhuyzen van Zanten SJ. Metabolic activities of metronidazole-sensitive and -resistant strains of *Helicobacter pylori*: repression of pyruvate oxidoreductase and expression of isocitrate lyase activity correlate with resistance. *J Bacteriol* 178: 4822–4829, 1996.
21. Jabour S and Hamed MY. Binding of the Zn^{2+} ion to ferric uptake regulation protein from *E. coli* and the competition with Fe^{2+} binding: a molecular modeling study of the effect on DNA binding and conformational changes of Fur. *J Comput Aided Mol Des* 23: 199–208, 2009.
22. Jenks PJ and Edwards DI. Metronidazole resistance in *Helicobacter pylori*. *Int J Antimicrob Agents* 19: 1–7, 2002.
23. Jenks PJ, Ferrero RL, and Labigne A. The role of the *rdxA* gene in the evolution of metronidazole resistance in *Helicobacter pylori*. *J Antimicrob Chemother* 43: 753–758, 1999.
24. Jeong JY, Mukhopadhyay AK, Dailidiene D, Wang Y, Velapatino B, Gilman RH, Parkinson AJ, Nair GB, Wong BC, Lam SK, Mistry R, Segal I, Yuan Y, Gao H, Alarcon T, Brea ML, Ito Y, Kersulyte D, Lee HK, Gong Y, Goodwin A, Hoffman PS, and Berg DE. Sequential inactivation of *rdxA* (HP0954) and *frxA* (HP0642) nitroreductase genes causes moderate and high-level metronidazole resistance in *Helicobacter pylori*. *J Bacteriol* 182: 5082–5090, 2000.
25. Jorgensen MA, Trend MA, Hazell SL, and Mendz GL. Potential involvement of several nitroreductases in metronidazole resistance in *Helicobacter pylori*. *Arch Biochem Biophys* 392: 180–191, 2001.
26. Kim JJ, Reddy R, Lee M, Kim JG, El-Zaatari FA, Osato MS, Graham DY, and Kwon DH. Analysis of metronidazole, clarithromycin and tetracycline resistance of *Helicobacter pylori* isolates from Korea. *J Antimicrob Chemother* 47: 459–461, 2001.
27. Kwon DH, El-Zaatari FA, Kato M, Osato MS, Reddy R, Yamaoka Y, and Graham DY. Analysis of *rdxA* and involvement of additional genes encoding NAD(P)H flavin oxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in metronidazole resistance of *Helicobacter pylori*. *Antimicrob Agents Chemother* 44: 2133–2142, 2000.
28. Kwon DH, Kato M, El-Zaatari FA, Osato MS, and Graham DY. Frame-shift mutations in NAD(P)H flavin oxidoreductase encoding gene (*frxA*) from metronidazole resistant *Helicobacter pylori* ATCC43504 and its involvement in metronidazole resistance. *FEMS Microbiol Lett* 188: 197–202, 2000.

29. Lee HW, Choe YH, Kim DK, Jung SY, and Lee NG. Proteomic analysis of a ferric uptake regulator mutant of *Helicobacter pylori*: regulation of *Helicobacter pylori* gene expression by ferric uptake regulator and iron. *Proteomics* 4: 2014–2027, 2004.
30. Manos J, Kolesnikow T, and Hazell SL. An investigation of the molecular basis of the spontaneous occurrence of a catalase-negative phenotype in *Helicobacter pylori*. *Helicobacter* 3: 28–38, 1998.
31. Masaoka S, Suzuki H, Kurabayashi K, Nomoto Y, Nishizawa T, Mori M, and Hibi T. Could frameshift mutations in the *frxA* and *rdxA* genes of *Helicobacter pylori* be a marker for metronidazole resistance? *Aliment Pharmacol Ther* 24: 81–87, 2006.
32. Nagayama A, Yamaguchi K, Watanabe K, Tanaka M, Kobayashi I, and Nagasawa Z. Final report from the Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, on the agar dilution method (2007). *J Infect Chemother* 14: 383–392, 2008.
33. Odenbreit S, Wieland B, and Haas R. Cloning and genetic characterization of *Helicobacter pylori* catalase and construction of a catalase-deficient mutant strain. *J Bacteriol* 178: 6960–6967, 1996.
34. Olczak AA, Wang G, and Maier RJ. Up-expression of NapA and other oxidative stress proteins is a compensatory response to loss of major *Helicobacter pylori* stress resistance factors. *Free Radic Res* 39: 1173–1182, 2005.
35. Osaki T, Hanawa T, Manzoku T, Fukuda M, Kawakami H, Suzuki H, Yamaguchi H, Yan X, Taguchi H, Kurata S, and Kamiya S. Mutation of *luxS* affects motility and infectivity of *Helicobacter pylori* in gastric mucosa of a Mongolian gerbil model. *J Med Microbiol* 55: 1477–1485, 2006.
36. Pecqueur L, D'Autreaux B, Dupuy J, Nicolet Y, Jacquamet L, Brutscher B, Michaud-Soret I, and Bersch B. Structural changes of *Escherichia coli* ferric uptake regulator during metal-dependent dimerization and activation explored by NMR and X-ray crystallography. *J Biol Chem* 281: 21286–21295, 2006.
37. Perez-Reyes E, Kalyanaraman B, and Mason RP. The reductive metabolism of metronidazole and ronidazole by aerobic liver microsomes. *Mol Pharmacol* 17: 239–244, 1980.
38. Rao DN and Mason RP. Generation of nitro radical anions of some 5-nitrofurans, 2- and 5-nitroimidazoles by norepinephrine, dopamine, and serotonin. A possible mechanism for neurotoxicity caused by nitroheterocyclic drugs. *J Biol Chem* 262: 11731–11736, 1987.
39. Samarawickrema NA, Brown DM, Upcroft JA, Thammapalerd N, and Upcroft P. Involvement of superoxide dismutase and pyruvate:ferredoxin oxidoreductase in mechanisms of metronidazole resistance in *Entamoeba histolytica*. *J Antimicrob Chemother* 40: 833–840, 1997.
40. Seyler RW Jr., Olson JW, and Maier RJ. Superoxide dismutase-deficient mutants of *Helicobacter pylori* are hypersensitive to oxidative stress and defective in host colonization. *Infect Immun* 69: 4034–4040, 2001.
41. Spiegelhalder C, Gerstenecker B, Kersten A, Schiltz E, and Kist M. Purification of *Helicobacter pylori* superoxide dismutase and cloning and sequencing of the gene. *Infect Immun* 61: 5315–5325, 1993.
42. Stojiljkovic I and Hantke K. Functional domains of the *Escherichia coli* ferric uptake regulator protein (Fur). *Mol Gen Genet* 247: 199–205, 1995.
43. Suzuki H, Hibi T, and Marshall BJ. *Helicobacter pylori*: present status and future prospects in Japan. *J Gastroenterol* 42: 1–15, 2007.
44. Suzuki H, Mori M, Seto K, Kai A, Kawaguchi C, Suzuki M, Suematsu M, Yoneta T, Miura S, and Ishii H. *Helicobacter pylori*-associated gastric pro- and antioxidant formation in Mongolian gerbils. *Free Radic Biol Med* 26: 679–684, 1999.
45. Tankovic J, Lamarque D, Delchier JC, Soussy CJ, Labigne A, and Jenks PJ. Frequent association between alteration of the *rdxA* gene and metronidazole resistance in French and North African isolates of *Helicobacter pylori*. *Antimicrob Agents Chemother* 44: 608–613, 2000.
46. Tiss A, Barre O, Michaud-Soret I, and Forest E. Characterization of the DNA-binding site in the ferric uptake regulator protein from *Escherichia coli* by UV crosslinking and mass spectrometry. *FEBS Lett* 579: 5454–5460, 2005.
47. van der Wouden EJ, van Zwet AA, Vosmaer GD, Oom JA, de Jong A, and Kleibeuker JH. Rapid increase in the prevalence of metronidazole-resistant *Helicobacter pylori* in the Netherlands. *Emerg Infect Dis* 3: 385–389, 1997.
48. van Vliet AH, Stoof J, Vlasblom R, Wainwright SA, Hughes NJ, Kelly DJ, Bereswill S, Bijlsma JJ, Hoogenboezem T, Vandenbroucke-Grauls CM, Kist M, Kuipers EJ, and Kusters JG. The role of the ferric uptake regulator (Fur) in regulation of *Helicobacter pylori* iron uptake. *Helicobacter* 7: 237–244, 2002.
49. Wang G, Wilson TJ, Jiang Q, and Taylor DE. Spontaneous mutations that confer antibiotic resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* 45: 727–733, 2001.
50. Wassmann C, Hellberg A, Tannich E, and Bruchhaus I. Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. *J Biol Chem* 274: 26051–26056, 1999.

Address correspondence to:
Dr. Hidekazu Suzuki
Division of Gastroenterology and Hepatology
Department of Internal Medicine
Keio University School of Medicine
35 Shinanomachi, Shinjuku-ku
Tokyo 160-8582
Japan

E-mail: hsuzuki@sc.itc.keio.ac.jp

Date of first submission to ARS Central, February 8, 2010; date of final revised submission, May 27, 2010; date of acceptance, June 2, 2010.

Abbreviations Used

ESR = electron spin resonance
Fur = ferric uptake regulator
Kd = dissociation constant
Mtz = metronidazole
PCR = polymerase chain reaction
RdxA = nicotinamide adenine dinucleotide phosphate (reduced form) nitroreductase
ROS = reactive oxygen species
SodB = iron-cofactored superoxide dismutase

GASTROENTEROLOGY 2011;140:189–198

BASIC—ALIMENTARY TRACT

Dysfunctional Gastric Emptying With Down-regulation of Muscle-Specific MicroRNAs in *Helicobacter pylori*-Infected Mice

YOSHIMASA SAITO, HIDEKAZU SUZUKI, HITOSHI TSUGAWA, SACHIKO SUZUKI, JUNTARO MATSUZAKI, KENRO HIRATA, and TOSHIFUMI HIBI

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan

BACKGROUND & AIMS: Little is known about the pathogenic mechanisms of functional dyspepsia. We investigated the role of microRNAs (miRNAs) in gastric motility disorders associated with *Helicobacter pylori* infection. **METHODS:** Male C57BL/6 mice were infected with *H pylori*. After long-term infection, gastric emptying was examined and compared with that of uninfected mice (controls). The miRNA expression profile was analyzed by miRNA microarray and quantitative reverse-transcriptase polymerase chain reaction. The results obtained from the animal study were confirmed by in vitro experiments. **RESULTS:** Gastric emptying was significantly accelerated in mice after chronic infection with *H pylori*. Histologic examination showed that the muscular layers of the stomachs of *H pylori*-infected mice were significantly thickened. The miRNA expression profile revealed that the muscle-specific miRNAs *miR-1* and *miR-133* were significantly down-regulated in the stomachs after long-term infection with *H pylori*. However, expression of histone deacetylase 4 and serum response factor, which are reported target genes of *miR-1* and *miR-133*, increased. Down-regulation of *miR-1* and *miR-133* and increased cell proliferation were observed in C2C12 mouse myoblast cells after coculture with *H pylori*. **CONCLUSIONS: Chronic infection with *H pylori* down-regulates expression of muscle-specific miRNAs and up-regulates expression of histone deacetylase 4 and serum response factor. These might cause hyperplasia in the muscular layer of the stomach and dysfunction in gastric emptying. These findings provide insight into the molecular pathogenesis of gastric motility disorders, including functional dyspepsia.**

Keywords: Functional Gastrointestinal Disorder; Noncoding RNA; Muscle Cell; Liquid Gastric Emptying.

Helicobacter pylori has been shown to be involved not only in the pathogenesis of chronic gastritis, peptic ulcer, and gastric cancer[1–5] but also in gastric motility disorders such as functional dyspepsia (FD).[6–8] Although delayed gastric emptying has been reported in patients with FD,[9–11] several studies have failed to confirm such a relationship.[12,13] Recent studies have shown that accelerated gastric emptying in patients with FD is associated with postprandial fullness, bloating, nausea, and stomach pain[14] and that gastric emptying is significantly accelerated in *H pylori*-positive children with nonulcer dyspepsia in comparison with noninfected children.[15] Lee et al[16] have reported that spontaneous duodenal acid exposure is increased in a subset of FD patients who are characterized with more severe dyspeptic symptoms. They suggested that increased duodenal acid may enhance the pathophysiology of FD, producing symptoms such as delayed gastric emptying. Despite many clinical studies of affected patients, the pathogenesis of FD is still poorly understood.

MicroRNAs (miRNAs) are small noncoding RNAs that function as endogenous silencers of target genes, thus playing critical roles in cell proliferation, apoptosis, and differentiation during mammalian development.[17] Links between miRNAs and human cancers are becoming increasingly apparent, and aberrant expression of miRNAs is known to be involved in the initiation and progression of gastrointestinal (GI) cancers.[18–24] Moreover, recent studies have revealed that *miR-29a* and *miR-510* are involved in the pathophysiology of irritable bowel syndrome (IBS), indicating that miRNAs play important roles not only in GI cancers but also in functional GI disorders such as IBS.[25,26] To investigate the molecular mechanism underlying the pathogenesis of functional gastric disorders associated with *H pylori* infection, we analyzed the miRNA expression profile in the stomachs of mice after long-term infection with *H pylori*.

Abbreviations used in this paper: α-SMA, α-smooth muscle actin; BrdU, bromodeoxyuridine; CFU, colony-forming unit; DAPI, 4′,6-diamidino-2-phenylindole; ELISA, enzyme-linked immunosorbent assay; FD, functional dyspepsia; GI, gastrointestinal; HDAC4, histone deacetylase 4; *H pylori*, *Helicobacter pylori*; IBS, irritable bowel syndrome; IL, interleukin; miRNA, microRNA; RT-PCR, reverse-transcription polymerase chain reaction; SRF, serum response factor; TNF, tumor necrosis factor.

0016-5085/$36.00
doi:10.1053/j.gastro.2010.08.044