Molecular characterization of rat gastric mucosal response to potent acid inhibition

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Molecular characterization of rat gastric mucosal response to potent acid inhibition. Physiol Genomics 22: 24–32, 2005. First published April 12, 2005; 10.1152/physiolgenomics.00245.2004.—Potent acid inhibition cause a number of adaptive responses in gastric mucosal cells, some of which are related to increases in cDNA microarray; gastrin; omeprazole; proliferation; proton pump inhibitors

The introduction of potent drugs [H₂ blockers and proton pump inhibitors (PPIs)] for the control of gastric acid secretion in the 1970s and 1980s completely changed medical therapy of peptic ulcer and reflux esophagitis. During the last decade, H₂ blockers have been largely replaced by PPIs, which, by inhibiting the enzyme responsible for the final step in acid secretion (14), can virtually extinguish acid secretion. In patients with gastroesophageal reflux disease, long-term maintenance therapy with PPIs is often used as an alternative to surgery to reduce the risk of complications of long-standing inflammatory and erosive processes like strictures, ulcers, and bleeding.

The profound hypoacidity induced by PPIs results in significant changes in the intragastric environment. Colonization of the stomach by bacteria that catalyze nitrosation may occur (54), with formation of potentially carcinogenic N-nitroso compounds (39). Also, the barrier function of acid against microorganisms and possibly prions is reduced (34, 42). Secondary to this, the immune and inflammatory responses in the mucosa are affected. With an increased pH, it is also reasonable to believe that the cellular stress response of the gastric mucosa is changed. In addition to its direct effect on the intragastric environment, hypoacidity leads to release of the acid secretagogue hormone gastrin and increases serum gastrin (62). This hormone acts primarily on the gastrin (CCK₂) receptors on the enterochromaffin-like (ECL) cell of the gastric corpus mucosa (48). In addition to having a specific and pronounced effect on function and proliferation of ECL cells, gastrin has a general trophic effect on the corpus mucosa (12, 21). This trophic effect may be due to a direct action on the endodermal-derived stem cell or may be mediated indirectly by substances released from, e.g., the ECL cell. In rats, long-term hypergastrinemia induced by very high doses of PPIs not only increases mucosal thickness and ECL cell density but also results in the development of gastric tumors (ECLomas) (12, 21). ECL cell hyperplasia has also been reported in patients treated with PPIs (11). Carcinoid tumors have been seen in patients with hypergastrinemia induced by gastrin-producing tumors or by atrophic gastritis (17). It is, however, debated whether carcinoid tumors may occur in humans in response to therapeutic acid inhibition.

We have only a very limited knowledge of molecular mechanisms involved in the complex physiological and pathophysiological responses to acid inhibition. The response of ECL cells to hypergastrinemia is known to involve increased abundance of histidine decarboxylase (Hdc), chromogranin A (CgA), and regenerating gene protein (Reg1) mRNA (9, 10, 37). However, ECL cells only represent a small fraction of the different exocrine and neuroendocrine cells present in gastric glands. Among the exocrine cells we find surface mucous cells, isthmus and neck cells, acid-secreting parietal cells, and peptinogen-secreting chief cells. The neuroendocrine cells, which constitute ~2% of the cells in the rat gastric mucosa, comprise ECL cells, which produce and release histamine, D cells (somatostatin), G cells (gastrin), and other neuroendocrine cells.

The changes in intragastric environment induced by acid inhibition cause a number of adaptive responses in gastric mucosal cells, some of which are related to increases in...
mucosal thickness and tumorigenesis. However, these adaptive responses and their underlying molecular mechanisms are still incompletely understood. In this study, we have investigated genome-wide transcript profile changes in gastric corpus mucosa after a 10-wk dosing of rats with a potent acid inhibitor, the PPI omeprazole. Using cDNA microarrays representing 11,848 rat genes, we show that omeprazole modulates the expression of many genes not previously known to respond to acid inhibition, including genes involved in proliferation, apoptosis, and stress response.

MATERIALS AND METHODS

Animals. The study was approved by the Animal Welfare Committee of St. Olav’s University Hospital, Trondheim, Norway. Male Sprague-Dawley rats weighing 200–250 g were housed in wire-mesh cages at 24°C and 40–60% humidity with a 12:12-h light-dark cycle and fed ad libitum with a commercial rat diet and tap water. Nine rats received 400 μmol·kg⁻¹·day⁻¹ of the PPI omeprazole (Astra, Gothenburg, Sweden) suspended in Methocel (Dow Corning, Midland, MI). The substance was administered by gavage daily for 10 wk. Seven rats received Methocel only. After 10 wk the rats were anesthetized with 0.2 ml/100 g body wt of a combination of (per ml) 2.5 mg of fluanison, 0.05 mg of fentanyl, and 1.25 mg of midazolam. Blood samples were collected from the abdominal aorta, and the stomachs were removed for sampling of full-thickness corpus from the upper part of the greater curvature. The animals were killed by exsanguination while still anesthetized.

Serum gastrin measurement. Radioimmunoassay for gastrin was done as previously described (28), using antibodies directed against the bioactive part of gastrin.

RNA isolation. Frozen tissue was homogenized in a guanidium-isothiocyanate buffer using an Ultra-Turrax rotating-knife homogenizer. Total RNA was extracted by ultrasound centrifugation on a 5.7 M cesium chloride cushion, precipitated, and kept on ethanol at −80°C until further processing. Total RNA was further purified using the TRizol method (phenol-guanidinium-thiocyanate) (GIBCO BRL Life Technologies, New York, NY) and examined for degradation by TRIzol method (phenol-guanidinium-thiocyanate) (GIBCO BRL Life Technologies, New York, NY) and examined for degradation by electrophoresis, and concentration was adjusted to 0.05–0.50 mg/ml.

Microarray procedures. Microarrays were obtained from the Norwegian Microarray Consortium (http://www.mikromatriske.no/). Briefly, 14,018 plasmids with sequence-verified rat probes were obtained from Research Genetics (Huntsville, AL), amplified by PCR with M13 plasmid primers, purified by Montage PCR (96-well filtration plate; Millipore, Bedford, MA), and redissolved in 50% DMSO. Obtained from Research Genetics (Huntsville, AL), amplified by PCR with M13 plasmid primers, purified by Montage PCR (96-well filtration plate; Millipore, Bedford, MA), and redissolved in 50% DMSO. Briefly, 14,018 plasmids with sequence-verified rat probes were obtained from Research Genetics (Huntsville, AL), amplified by PCR with M13 plasmid primers, purified by Montage PCR (96-well filtration plate; Millipore, Bedford, MA), and redissolved in 50% DMSO. DNA probes were made by amplifying cDNA inserts by PCR from plasmid clones obtained from Research Genetics (Huntsville, AL). Northern blot analysis. DNA probes were made by amplifying cDNA inserts by PCR from plasmid clones obtained from Research Genetics (Huntsville, AL). Northern blot analysis was performed as described previously (64). Group means were compared by use of the Student’s t-test. P < 0.01 was considered statistically significant.

Quantitative real-time RT-PCR. The same total RNA used for the microarray analysis was reverse transcribed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) as described in the manufacturer’s protocol. TaqMan real-time PCR amplification was performed by a Smart Cycler instrument (Cepheid, Sunnyvale, CA), using a QuantiTect Probe PCR kit (Qiagen, Valencia, CA) as described by the manufacturer. The following primers and TaqMan probes were used: Resp18-primer, 5'-CAC GAT CAT TGC TCC TCC TGA; Resp18-as, 5'-GTA A-3; Resp18-probe, 5'-CAC CAA ACA AGA GTC GAT CCA CCA TTC-3; Ntrk2-s, 5'-AAC CTG CGG CAC ATC AAT-3; Ntrk2-as, 5'-GTC AAG GTG GCC GAA ATG-3; Ntrk2-probe, 5'-GCA GAT GCC GAG TTT GTC CAG GAG A-3; β-actin-s, 5'-CTG GCT CCT AGC ACC ATG A-3; β-actin-as, 5'-AGC CAC CAA TCC ACA CAG A-3; β-actin-probe, 5'-GAA CAT CAT TGC TCC TCC TGA GGG-3. Samples from each rat were run in duplicate and averaged.

Statistical analysis. To assess the significance of up- or downregulation of the genes, tests for differential expression were performed using moderated t-tests, as implemented in the Limma R package of Smyth (53). This is based on empirical Bayes analysis, where the power of the tests is improved by replacing gene-specific variance estimates with estimates found by borrowing strength from data on the remaining genes.

Database submission of microarray data. The microarray data were prepared according to minimum information about a microarray experiment (MIAME) recommendations (6) and deposited in the GEO database and can be accessed at http://www.ncbi.nlm.nih.gov/geo/.

The GEO accession no. for the platform is GPL1427. The nine samples can be retrieved with GEO accession nos. GSM31158–GSM31166 and constitute the series with GEO accession no. GSE1808.

GASTRIC MUCOSAL GENE EXPRESSION DURING ACID INHIBITION

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Antibodies against rat receptor b (Ntrk2) were visualized using an EnVision-HRP kit (K5007; Dako, Glostrup, Denmark), human Syk (1:100, Acris Antibodies, Hiddenhausen, Germany), human Syk (1:100, Acris Antibodies, Ntrk2 (1:200; United States Biological, Swampscott, MA), Hdc (1:10,000; Eurodiagnostica, Malmö, Sweden), and CgA (1:500; Dia Sorin, Stillwater, MN) were diluted in Tris-buffered saline, pH 7.4, with 0.025% Tween-20 (DakoCytomation, Glostrup, Denmark) and 1% bovine serum albumin (Sigma, St. Louis, MO), and the sections were incubated with antibodies for 60 min. The immunoreactions were visualized using an EnVision-HRP kit (K5007; Dako, Glostrup, Denmark) and an AEC peroxidase kit (SK4200; Vector, Burlingame, CA).

RESULTS

Animal characteristics. Gastric corpus tissue specimens from seven control rats and nine omeprazole-treated rats were used. Serum gastrin measurements showed that control rats had serum gastrin levels within the normal range, 45 (16–62) pM [mean (range)]. The omeprazole-treated rats all had elevated serum gastrin levels within the normal range, 45 (16–62) pM [mean (range)].

Genes differentially regulated by omeprazole. Genes with changed expression levels in response to omeprazole were identified by microarray analysis by hybridization of RNA from the gastric corpus of omeprazole-dosed rats against a pool of RNA from the gastric corpus of control rats. We chose to pool RNA from control rats, since our goal was to identify genes differentially regulated in omeprazole-dosed rats at levels over the background biological variability in the controls. The use of a common reference or control is also the most commonly used design in microarray experiments, and it has the advantage of allowing efficient comparison of samples. For each gene, a P value was calculated using the moderated t-test of Smyth (53). Using a cutoff of the P value at 0.01, we found 134 differentially expressed genes. Recently, methods for estimating the proportion of all genes that truly are differentially expressed have become available. Using the convex decreasing density estimator of Fergkingstad et al. (15), we found this proportion to be 35%. The false discovery rate (FDR) of the list of 134 differentially expressed genes was estimated to be 4.8%. An FDR of 4.8% means that, among genes called significant, 4.8% of these are on average truly not differentially expressed. The FDR has been calculated using the method of Storey (55), with the estimated proportion of truly differentially expressed genes found above inserted. Of the 134 genes that were differentially expressed between control rats and omeprazole-dosed rats, 63 were induced, and 71 were repressed. Twenty-three (37%) of the upregulated genes and forty-nine (69%) of the downregulated genes were genes without known function.

Thus the highest number of genes encoding products without any known functions was found in the list of downregulated genes. This phenomenon has also been observed by others (23). For the complete list of differentially expressed genes, see Supplemental Table S1 (available at the Physiological Genomics web site).

To facilitate interpretation of the results, the genes with significantly changed expression in rats dosed with omeprazole were annotated with the cellular processes in which they are likely to be involved, based on information from the literature and from the SwissProt and LocusLink databases. Table 1 shows all genes that were found to be annotated with the processes proliferation/apoptosis, stress response, and immune and inflammatory responses. We chose to focus on these processes because they were considered to be the most interesting ones in the response to acid inhibition. Of the 62 differentially expressed genes with known function, 27 (44%) could be classified into these broad functional groups. Mediators of proliferation and apoptosis comprised the largest functional gene cluster, whereas the numbers of genes involved in stress response and immune/inflammatory responses were somewhat lower. Several genes were involved in more than one of these biological processes (Table 1). The remaining 35 genes encoding products with known function were spread over many categories including metabolism, development, regulation of gene expression, signal transduction, transport, and digestion, where no single category amounted to >10% of all genes with known function.

Verification of results by Northern blot analysis and real-time RT-PCR. We validated the microarray results using Northern blot analysis for the genes Reg1, Resp18, Hdc, Ela1, Fat, Cxcr4, Atg, and 18S. Six of the seven probes tested by Northern hybridization gave detectable signals (Fig. 1A). One probe, angiotensinogen (Atg), did not give detectable signals by Northern hybridization. Five of the genes (Reg1, Resp18, Hdc, Ela1, and Cxcr4) detected by Northern hybridization showed qualitatively similar expression changes in Northern blot and microarray analysis, while one (Fat), which appeared to be downregulated by microarray analysis, was found to be upregulated by Northern hybridization (Fig. 1). Expression changes detected by microarray were for some genes underestimated by a factor of nearly 2 compared with Northern blot. Overall, ~70% of the genes (5 of 7) tested by Northern blot analysis were confirmed. This is within the range of false positives commonly found in microarray experiments (8, 51).

Two gene probes (UI-R-A0-am-d-07-0-UI and UI-R-A0-as-a-03-0-UI; Table 1) representing known genes are associated with two different Unigene clusters, Resp18 or Den and Ntrk2 or Id3, respectively. These genes were further validated by quantitative real-time RT-PCR with the use of TaQMan probes. Gel analysis of PCR products, using primers and probes against Resp18 and Ntrk2, and cycle threshold measurements show that both genes were induced by omeprazole in eight of nine dosed rats (data not shown). In addition, the size of the mRNA obtained by Northern blot analysis with the probe representing UI-R-A0-am-d-07-0-UI demonstrates that the hybridized mRNA is Resp18. Together, these results strongly indicate that these probes represent Resp18 and Ntrk2. All probes that are associated with two different Unigene clusters are listed in Table 1. The Supplemental Material for this article (Supplemental Table S1) is available online at http://physiolgenomics.physiology.org/cgi/content/full/00245.2004/DC1.
Differentially expressed genes in omeprazole-dosed rats involved in proliferation and apoptosis, stress response, and immune and inflammatory responses. A full account of all genes (including genes without known function) differentially regulated in omeprazole-dosed rats is given in Supplemental Table S1. Unigene build 134.

Known (named) genes that are likely to be involved in proliferation and apoptosis, stress response, and immune and inflammatory responses. A full account of all genes (including genes without known function) differentially regulated in omeprazole-dosed rats is given in Supplemental Table S1. Unigene build 134 was used.

are indicated in Supplemental Table S1. Most of these represent unknown genes. Gene probes representing one known gene and one unknown gene were counted as known genes.

Quality control of results using exogenous RNA spikes. Further validation of our microarray protocol was done using the SpotReport Array Validation System (Stratagene). Analysis of exogenous (*A. thaliana*) poly(A)⁺ RNAs spiked into labeling reactions at predetermined ratios showed that the observed ratios highly correlated with the expected ratios (data similar to that in Ref. 64). The expected ratios for the RNA controls were 6, 5, 4, 3, 2, 1, 0.5, 0.33, 0.25, and 0.125. The pairwise correlations among the 10 *A. thaliana* spikes (repre-

Table 1. Differentially expressed genes in omeprazole-dosed rats involved in proliferation and apoptosis, stress response, and immune and inflammatory responses.

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Unigene Cluster</th>
<th>Name</th>
<th>Symbol</th>
<th>Proliferation/ Apoptosis</th>
<th>Stress Response</th>
<th>Inflammatory/ Immune Response</th>
<th>Mean Log Ratio</th>
<th>SD</th>
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<tr>
<td>UI-R-E1-fc-08-0-01-Rn.87407</td>
<td>spleen tyrosine kinase</td>
<td>Syk</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>2.3 ± 0.4</td>
<td>0.11</td>
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<td>UI-R-C1-ec-12-0-01-Rn.58139</td>
<td>angiotensinogen</td>
<td>Agt</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>1.1 ± 0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-A1-ew-04-0-01-Rn.81250</td>
<td>dehydrated myc downstream regulated 4</td>
<td>Ndr4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>0.5 ± 0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-A0-as-03-0-01-Rn.11246</td>
<td>neurotrophic tyrosine kinase, receptor, type 2</td>
<td>Ntrk2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>0.4 ± 0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-E1-fr-06-0-01-Rn.54431</td>
<td>chemokine receptor (LCR1)</td>
<td>Cxc4r4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>0.7 ± 0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-A1-ca-01-0-01-Rn.13685</td>
<td>transmembrane 4 superfamily member 2</td>
<td>Tm4sf2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>0.4 ± 0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-A1-ee-01-0-03-Rn.11352</td>
<td>regenerating islet-derived 1</td>
<td>Reg1</td>
<td>x</td>
<td>1.7 ± 0.18</td>
<td>0.15</td>
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<tr>
<td>UI-R-C1-lg-07-0-01-Rn.31982</td>
<td>uromodulin</td>
<td>Umod</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>1.1 ± 0.11</td>
<td>0.11</td>
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<tr>
<td>UI-R-C0-gw-09-0-01-Rn.11250</td>
<td>histone 2b</td>
<td>H2b</td>
<td>x</td>
<td>0.4 ± 0.08</td>
<td>0.15</td>
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<td>UI-R-E0-cr-b-04-0-01-Rn.93004</td>
<td>paired related homeobox 2</td>
<td>Prxr2</td>
<td>x</td>
<td>0.3 ± 0.05</td>
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<td>UI-R-A1-ev-06-0-01-Rn.5892</td>
<td>major histocompatibility complex, class II, DM beta</td>
<td>Hla-dmb</td>
<td>x</td>
<td>0.5 ± 0.11</td>
<td>0.15</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Downregulated

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Unigene Cluster</th>
<th>Name</th>
<th>Symbol</th>
<th>Proliferation/ Apoptosis</th>
<th>Stress Response</th>
<th>Inflammatory/ Immune Response</th>
<th>Mean Log Ratio</th>
<th>SD</th>
</tr>
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<tr>
<td>UI-R-A1-dr-f-11-0-01-Rn.22382</td>
<td>integrin, alpha 6</td>
<td>Itgα6</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.5 ± 0.10</td>
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<tr>
<td>UI-R-E1-fr-03-0-01-Rn.9096</td>
<td>early growth response 1</td>
<td>Egr1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.4 ± 0.08</td>
<td>0.11</td>
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<td>UI-R-Y0-ir-09-0-01-Rn.17145</td>
<td>connective tissue growth factor</td>
<td>Cg5f</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.6 ± 0.13</td>
<td>0.11</td>
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<tr>
<td>UI-R-C1-nm-e-02-0-01-Rn.31788</td>
<td>calponin 1</td>
<td>Cnn1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.4 ± 0.07</td>
<td>0.11</td>
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<tr>
<td>UI-R-A1-em-f-01-0-01-Rn.109452</td>
<td>glutathione S-transferase, mitochondrial</td>
<td>GST13-13</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.6 ± 0.11</td>
<td>0.11</td>
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<tr>
<td>UI-R-E1-fa-b-06-0-01-Rn.54439</td>
<td>chemokine (C-X-C motif) ligand 12</td>
<td>Cxc12</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.6 ± 0.14</td>
<td>0.11</td>
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<tr>
<td>UI-R-E0-cc-f-08-0-01-Rn.780</td>
<td>alpha-2-macroglobulin</td>
<td>A2m</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.4 ± 0.07</td>
<td>0.11</td>
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<td>UI-R-A0-aa-b-04-0-01-Rn.2743</td>
<td>follistatin</td>
<td>Fst</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>−0.4 ± 0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>UI-R-E0-cf-c-01-0-01-Rn.3193</td>
<td>WAP four-disulfide core domain 1</td>
<td>Wdc1</td>
<td>x</td>
<td>−0.6 ± 0.03</td>
<td>0.12</td>
<td></td>
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<tr>
<td>UI-R-CG-ii-10-0-01-Rn.95269</td>
<td>bromodomain-containing 2</td>
<td>Brd2</td>
<td>x</td>
<td>−0.6 ± 0.03</td>
<td>0.12</td>
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<tr>
<td>UI-R-E1-gc-a-10-0-01-Rn.30004</td>
<td>CUG triplet repeat, RNA-binding protein 2</td>
<td>Cugbp2</td>
<td>x</td>
<td>−0.5 ± 0.12</td>
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<tr>
<td>UI-R-A0-aa-a-07-0-01-Rn.92342</td>
<td>histone deacetylase 7</td>
<td>Hdac7</td>
<td>x</td>
<td>−0.5 ± 0.09</td>
<td>0.12</td>
<td></td>
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</tr>
</tbody>
</table>

A

Control Omeprazole

B

<table>
<thead>
<tr>
<th>Probe</th>
<th>Microarray ratio</th>
<th>Northern blot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg1</td>
<td>3.2 ± 0.4</td>
<td>5.8 ± 2.6</td>
</tr>
<tr>
<td>Resp18</td>
<td>1.4 ± 0.1</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Hdc</td>
<td>2.7 ± 0.2</td>
<td>3.1 ± 0.7</td>
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<tr>
<td>Ela1</td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7 ± 0.03</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Cxc4r4</td>
<td>1.7 ± 0.2</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>18S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Validation of microarray results using Northern blot analysis. A: gastric mucosa total RNA from a pool of control rats (n = 7) or omeprazole-dosed rats (n = 9) was examined by Northern blot analysis using cDNA probes for the following genes: *Reg1* (regenerating islet-derived 1), *Resp18* (regulated endocrine-specific protein 18), *Hdc* (histidine decarboxylase), *Ela1* (elastase 1), *Fat* (*Rattus norvegicus* similar to fatty acid translocase/CD36 (LOC362310), *mRNA*), *Cxc4r4* (chemokine receptor (LCR1)), and *18S* (18S RNA). B: comparison of expression levels for selected genes by microarray and Northern blot hybridizations in omeprazole-dosed rats and a pool of control rats. Ratios were calculated for Northern blot by quantifying radioactivity in corresponding bands shown in A, as detailed in MATERIALS AND METHODS. Ratios for microarray analysis represent mean values from 9 omeprazole-dosed rats compared with the control pool of 7 rats. Data are means ± SD.
The median pairwise correlation was 0.996, with an interquartile range of 0.0045. The external controls also showed that a twofold change in mRNA levels could be reliably detected and correlated well with expected fold changes. However, the observed fold changes for the external controls were underestimated at both ends of the expected range (data not shown). This is analogous to the observed differences between Northern blot and microarray measurements and is similar to our observations in a previous study (64).

Sequencing. Errors in the IMAGE collections have been reported, and additional errors may have occurred during production. For this reason, 43 (32%) of the clones representing differentially expressed genes were sequence verified using standard methods. Good sequencing results were obtained for 29 of the clones, and 22 (76%) of these showed the expected sequence. For 7 of the 29 clones the sequence was wrong. Four of the incorrect clones were shown to represent a different gene than expected. In these cases, the new gene information is reported in Table 1 and Supplemental Table S1. Three sequenced clones did not show similarity to any genes, and these clones were removed from the tables.

**Immunohistochemistry.** The Cxcr4 immunoreactivity was localized in the basal one-half of the glands, where chief cells and neuroendocrine cells are found. However, parietal cells were not labeled (Fig. 2A). Syk seemed to be expressed in parietal cells that had a more intense immunoreactivity in the upper one-third of the glands, whereas no immunoreactivity could be seen in other cell types (Fig. 2B). CgA-immunoreactive cells were found in the basal one-half of the glands; some were seen with cytoplasmic extensions (Fig. 2C). The ECL cell marker Hdc was distributed similarly to CgA, but fewer cells were labeled (Fig. 2D). Ntrk2 was expressed in a few distinct cells with an elongated shape, and located in the middle and lower part of the glands, which could be a subpopulation of neuroendocrine cells in the oxyntic mucosa (Fig. 2, E and F).

**DISCUSSION**

In the present study, molecular responses in gastric mucosa of rats receiving the PPI omeprazole were studied by measuring genome-wide transcript-level changes using cDNA microarrays with probes representing 11,848 genes. Serum gastrin levels increased significantly in the group dosed with this drug, showing that the pharmacological intervention was suc-
cessful. The 134 genes identified as differentially expressed include the ECL-specific genes Hdc and Reg1. Both Hdc and Reg1 are known to be induced by gastrin (9, 10, 37), and in the present study both were found to be upregulated by microarray analysis. Another gastrin-responsive gene, Cga, also showed significantly elevated transcript levels in the omeprazole-dosed rats. Because Cga gene probes were not present on the microarrays, this measurement was performed by Northern blot analysis (data not shown). It is also interesting to note that microarray analysis indicated upregulation of P-450 (cytochrome) oxidoreductase (Por), since cytochrome P-450 enzymes mediating the major metabolic transformations of several PPIs, including omeprazole, are known to be induced by omeprazole, not only in the liver but also in the rat corpus mucosa (4, 30). These results show that potent acid suppression was obtained and confirm earlier observations on gene expression changes of gastric mucosa in response to acid inhibition. Moreover, the results strongly indicate that the microarray method can be used to detect gene expression changes in a minor cell population of the complex gastric tissue such as ECL cells.

The 134 genes found to be differentially regulated in omeprazole-dosed rats are most likely directly or indirectly regulated by acid inhibition and/or hypergastrinemia. The genes include both known gastrin-regulated genes and a number of genes previously not known to be regulated by acid inhibition that are involved in several different cellular processes. One of the interesting upregulated genes is regulated endocrine-specific protein-18 (Resp18), which is expressed in endocrine cells and has been suggested to participate in a neuroendocrine intracellular signaling pathway (49). The present study is the first to report that Resp18 is expressed in the rat gastric mucosa and elevated in response to treatment with acid inhibitors.

Because gastrin is known to stimulate proliferation of both the ECL cells and the gastric mucosa in general, genes involved in this process were regarded as the most interesting. The immune/inflammatory cellular processes are also of great interest, since, for instance, Helicobacter pylori infection of the human stomach is very common and is related to various diseases such as gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphomas, and gastric cancer. The immune/inflammatory processes are most likely affected by the dramatic changes in the intragastric environment induced by potent acid inhibition. Moreover, with an increased pH, there is also reason to believe that the cellular stress response of the gastric mucosa is changed. In the following sections, these selected processes and the genes involved will be discussed.

Acid inhibition is associated with alteration in expression of genes involved in proliferation and apoptosis. Mediators of proliferation and apoptosis comprised the largest functional gene cluster (Table 1). Gastric epithelial turnover is a dynamic process characterized by continuous cell proliferation balanced by cell loss. In the normal gastric mucosa, ~1−3% of gastric epithelial cells in the antrum or corpus/fundus are apoptotic (46). Inflammatory conditions of the stomach, including chronic gastritis, are commonly associated with increased epithelial proliferation. However, chronic gastritis is usually not associated with mucosal thickening, indicating that the increased epithelial proliferation is balanced by cell loss (61). On the other hand, hypergastrinemia induced by potent and long-term inhibition of gastric acid secretion increases proliferation rate and mucosal thickness, indicating that the rate of apoptosis is not correspondingly increased. It is therefore interesting to note that we observe a number of changes in gene expression indicating activation of proliferation and inhibition of apoptosis. These genes are discussed below.

As expected, we confirmed the upregulation of Reg1. In patients with chronic hypergastrinemia, Reg1 production is stimulated, with an increased proliferation of gastric mucosal cells. Reg1 protein may be a growth factor regulating the proliferation and differentiation of normal and neoplastic gastric epithelial cells (27). The upregulated gene encoding neurotrophic tyrosine kinase receptor TrkB (Ntrk2) is localized to a subpopulation of human gastrointestinal endocrine cells and has been found to induce proliferation and/or suppress apoptosis (13, 16). Upregulation of the homeobox transcription factor gene Prx2 during proliferation of gastric mucosa may indicate its function in the development of new blood vessels and connective tissue secondary to proliferation of the mucosa, since the homeobox transcription factors Prx are shown to control vascular smooth muscle cell proliferation and expression of the extracellular matrix protein tenascin C (24).

A number of genes with decreased expression levels after omeprazole dosing inhibit proliferation or activate apoptosis. The downregulated gene Wdfy1 (WAP four-disulfide core domain 1) is known to have growth inhibitory activity (32). The transcription factor early growth response gene 1 (Egr1), also downregulated in omeprazole-dosed rats, is described as a positive regulator of tumor suppressor p53-dependent growth regulatory mechanisms in replicative senescence and cell growth (29). For another gene downregulated in our study, histone deacetylase 7 (Hdac7), it has been reported that its downregulation inhibits repression of Stat3 activity and results in induced growth and suppressed apoptosis (63). Previous results suggest that connective tissue growth factor (Ctgf), downregulated in omeprazole-dosed rats, acts as a negative regulator of cell growth in oral squamous cell carcinoma (38). The present results may indicate that this growth factor inhibits proliferation also in gastric mucosa.

Taken together, the expression patterns of positive and negative regulators of proliferation and apoptosis indicate a complex involvement of the identified genes in increased proliferation and decreased apoptosis, inducing the mucosal hyperplasia. However, the complexity of the gastric mucosa makes precise interpretation of these results difficult. Numerous studies report that gastrin stimulates proliferation of both the ECL cells and the gastric mucosa in general, the effect on ECL cells being more pronounced than the general trophic effect (2, 12, 21). The growth-related genes found here represent good candidates for further confirmation and follow-up studies that may shed light on the precise cellular molecular responses to potent acid inhibition.

Altered expression of genes involved in stress responses. Of 62 differentially expressed genes with known function, 13 encode proteins involved in stress responses. Egr1 and Ctgf, known to be induced in response to stress (26, 43, 44, 58), are downregulated in response to acid inhibition. The high number of differentially expressed stress responsive genes is most likely due to the decreased level of gastric acid in the stomach. Gastric acid is the main aggressive factor in the intragastric environment and a prerequisite for disorders like peptic ulcer disease, stress ulcer, and ulcerative gastritis. On the other hand,
the success of pharmacological treatment to prevent or heal ulcerative lesions may not depend only on the blockade of acid secretion but also on the enhancement of mucosal protective factors. It has been suggested that part of the protective effects of PPIs can be ascribed to a reduction of gastric oxidative injury, which results also in an increased bioavailability of mucosal sulfhydryl compounds (41). Omeprazole has previously been shown to prevent oxidative stress (25, 31, 47). It is therefore interesting to note that omeprazole-dosed rats show upregulation of genes known to protect the cell against oxidative stress, such as endothelial PAS domain protein 1 (Epas1) (50), carbonyl reductase 1 (Cbr1) (3), and spleen tyrosine kinase (Syk) (57). These proteins may be involved in the molecular mechanisms underlying acid-independent gastroprotective effects of PPIs.

Genes encoding proteins regulating inflammatory and immune responses. Altered gene expression was observed for, surprisingly, many genes known to be involved in immune and inflammatory responses, and many of these were upregulated. Reduced gastric acidity creates an environment susceptible to bacterial colonization, and the high number of genes encoding proteins involved in cellular responses to pathogens may be due to activation of submucosal inflammatory cells. Examples of such genes are Ntrk2, involved in modulation of immune cell function (40); chemokine receptor Cxcr4, which plays an important role in immune cell trafficking (52); uromodulin (Umod), proposed to have a dual immunomodulating effect (51); and chemokine receptor Cxcr4, involved in intracellular signaling in the inflammatory response (59); and Agrp, known to be activated during inflammation and proposed to be an acute-phase protein (5). Reducing the amount of acid with potent PPIs may also reduce inflammatory responses in the gastric mucosa, since gastric acid is the main aggressive factor in the intragastric environment. Downregulation of the multifunctional transcription factor Egr1 and Cxcl12 (Cxcl12), both of which may be involved in immune and/or inflammatory responses (7, 36), may be interpreted to indicate that these proteins may be involved in protecting the gastric mucosa against acid-induced damage.

The differentially expressed genes are found in different cell types. The gastric mucosa is very complex, with intermingling cells of different types with various roles in the acid secretory process. Parietal cells comprise ~40% of the rat corpus mucosa, whereas chief cells make up ~18%. The remainder of the gastric mucosal cells comprise ~22% surface mucous cells, 9% mucosal neck cells, and 7% lamina propria cells (35). Endocrine cells constitute ~2% of the cells in the rat gastric corpus region and mainly belong to one of the following three types: ECL cells (66%), A-like cells (24%), and somatostatin (D) cells (7–8%) (18, 19). A few of the differentially expressed genes, chosen to cover a spectrum of biological functions, were studied further by localizing the gene products to cell type(s). Previous studies show that Cxcr4 can be found in gastric epithelium (1) and that this protein is important for cellular migration and preservation of mucosal integrity in the intestine (52). The present work shows that Cxcr4 is most likely localized to chief cells and possibly neuroendocrine cells, definitely not to parietal cells. On the other hand, we find Syk mostly in parietal cells, where its role is unclear but could be related to vascular proliferation (22), intercellular adhesion, or inflammatory process signaling (60). The general neuroendocrine cell marker CgA and the gastrin-responsive and ECL cell-specific enzyme Hdc are found in scattered cells in the region where neuroendocrine cells are localized. Ntrk2 has previously been found in a subpopulation of endocrine cells in the gastrointestinal system (13). The present results indicate that Ntrk2 is indeed localized to a neuroendocrine cell subtype that responds to acid inhibition but is not the ECL cell.

The present experiment gives a picture of the gene expression changes in the total cell population of the gastric mucosa and must be followed by further analysis, using other methods like single-cell studies, immunohistochemistry, or in situ hybridization that may indicate in which cell types the different genes of interest are expressed. It is, however, highly interesting that alterations in transcript levels in this complex tissue are informative due to a robust response in a few cells. Thus the increased gene expression of the ECL cell-specific genes Hdc and Reg1 was clearly observable, even though the ECL cells only comprise ~1% of the total cell mass in the rat gastric mucosa. This finding in our physiological model is in accordance with the detection of gene expression alterations in serial dilutions of mixed cellular RNA as studied by Hamadeh et al. (20).

Inhibitors of gastric acid secretion are among the most commonly used drugs in clinical practice. We have presented the first large-scale assay of transcriptional changes in gastric mucosa during potent acid inhibition, using rats as a physiological model system. Our results indicate a global change in the induction of proliferation and apoptosis and inflammatory, immune, and stress responses in the presence of PPIs. The genes presented in this study are likely to be directly or indirectly associated with acid inhibition, may help to elucidate the physiological and molecular responses to this pharmacological intervention, and may be useful in the assessment of drug safety.

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REFERENCES

GASTRIC MUCOSAL GENE EXPRESSION DURING ACID INHIBITION


