Mitochondrial alterations in human gastric carcinoma cell line

Hyoung Kyu Kim,1 Won Sun Park,1 Sung Hyun Kang,1 Mohamad Warda,1,2 Nari Kim,1 Jae-Hong Ko,1 Abd El-bary Prince, and Jin Han1

1Mitochondrial Signaling Laboratory, Mitochondria Research Group, Department of Physiology and Biophysics, College of Medicine, Biohealth Products Research Center, Cardiovascular and Metabolic Disease Research Center, Inje University, Busan, Korea; and 2Department of Biochemistry, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

Submitted 31 January 2007; accepted in final form 24 May 2007

Mitochondrial Morphology Difference Comparisons by Electron Microscope

Cells (1 × 106) were collected, washed twice with PBS, centrifuged for 5 min at 1,800 g, and glutaraldehyde fixed for 2–4 h at 4°C. Mitochondrial morphology differences, numbers, and shapes between outcomes in gastric cell tumors. Therefore, we examined mitochondrial proteome alterations in gastric tumor cells and their correlation with mitochondrial function in the neo-homeostasis environment. This approach provides a better idea regarding the contribution of mitochondrial to neo-homeostasis in gastric cancer, and the impact of neo-homeostasis on mitochondrial proteome alterations may lead to the discovery of new biomarkers specific for mitochondrial involvement. Furthermore, the results suggest possible therapeutic interventions for gastric cancer that affect the mitochondrial membrane potential (ΔΨm) and β-oxidation pathway.

MATERIALS AND METHODS

Cell Culture and Reagents

Cells from the human gastric cancer cell line AGS were cultured in RPMI-1640 (Cambrex, Baltimore, MD) containing 10% FBS and 100 U/ml penicillin-streptomycin in a humidified 5% CO2 atmosphere. As countercells of AGS cells, normal rat gastric mucosal cells from cell line RGM-1 were cultured in DMEM-F-12 (Cambrex) under the same conditions (32).

Solutions and Drugs

Normal Tyrode solution contained (in mM) 143 NaCl, 5.4 KCl, 1.8 CaCl2, 0.5 MgCl2, 5.5 glucose, and 5 HEPES (pH 7.4) adjusted with NaOH. Fluorescent dyes [rhod-2 AM and tetramethylrhodamine ethyl ester (TMRE)] were purchased from Molecular Probes, prepared as 1 mM stock solutions in DMSO, and diluted into test solutions before each experiment. The final concentration of DMSO in the bath solution (<0.1%) has been previously proved not to affect recorded intensities. Acrylamide, methylene bis-acrylamide, glycine, Tris, urea, glycerol, CHAPS, thioare, and bromophenol blue were purchased from Sigma (St. Louis, MO). N,N,N',N'-tetramethylthylene diamine (TEMED), iodoacetamide, ammonium persulfate, DTT, and SDS were obtained from Merck (Darmstadt, Germany). IPG strips, IPG buffer, Destreak rehydration solution, and the two-dimensional (2-D) Quant kit were purchased from Amersham Biosciences (Uppsala, Sweden). The one-dimensional gel electrophoresis kit and membrane transfer kit were from Hoefer (San Francisco, CA). Immobilon-P membranes were purchased from Millipore (Bedford, MA). All other reagents used were of analytic grade.

Mitochondrial Signaling Laboratory, Mitochondria Research Group, Department of Physiology and Biophysics, College of Medicine, Biohealth Products Research Center, Cardiovascular and Metabolic Disease Research Center, Inje University, Busan, Korea.

Address for reprint requests and other correspondence: J. Han, Mitochondrial Signaling Laboratory, Mitochondria Research Group, Dept. of Physiology and Biophysics, College of Medicine, Biohealth Products Research Center, Cardiovascular and Metabolic Disease Research Center, Inje Univ. 633-165 Gaegueng-Dong, Busanjin-Gu, Busan 613-735, Korea (e-mail: phyhanj@ijmc.inje.ac.kr).

http://www.ajpcell.org 0363-6143/07 $8.00 Copyright © 2007 the American Physiological Society
AGS and RGM-1 cells were observed under an electron microscope (JEOL 1200 EX2) (26).

**Measurement of Mitochondrial Ca\(^{2+}\) Concentration and \(\Delta\Psi\)_m**

Loading of cells with fluorescent indicators. Mitochondrial Ca\(^{2+}\) was traced in both AGS and RGM-1 cells using the mitochondrial Ca\(^{2+}\)-sensitive fluorescent dye rhod-2 AM. A cold/warm incubation protocol (59, 67) was used to exclusively load mitochondria with rhod-2 AM. Briefly, cells (1 \(\times\) 10\(^6\) cells/sample) were washed using HEPES buffer [containing (in mmol/l) 130 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 10 HEPES, 11 glucose, and 0.2 CaCl\(_2\)] at pH 7.4 and then stained using 5 \(\mu\)mol/l rhod-2 AM for 120 min at 4°C, followed by a 10-min incubation at 37°C. To complete the deestriatin of intracellular rhod-2 AM, cells were washed using HEPES buffer after being stained and stabilized for an additional 30 min.

To monitor \(\Delta\Psi\)_m, AGS and RGM-1 cells were incubated for 20 min with 200 nM of the cationic dye TMRE perchlorate (1). Cells were then washed twice and centrifuged.

Confocal imaging. A confocal microscopy assay was performed with laser scanning confocal microscope (LSM 510 META) connected to an inverted microscope (Axiovert 200M, Carl Zeiss, Jena, Germany) with a \(\times\)40 water-immersion objective lens, optimal laser lines, and filter. The fluorescence of TMRE was excited at 564 nm, and emitted at 588 nm with a long-pass filter. Images were digitized at 8 bits and analyzed using LSMC 510 META software (Carl Zeiss).

FACS analysis. For FACS tracing of \(\Delta\Psi\)_m, TMRE-loaded cells were analyzed using FACSalign (3,000 cells/sample, Becton Dickinson). After 5 min at a stable state, the mitochondrion uncoupler protein FCCP was added (1 \(\mu\)M final concentration), and \(\Delta\Psi\)_m depolarization was then monitored. The fluorescence intensity of TMRE was monitored at 582 nm (FL-2 channel). FACS data were analyzed using Cell Quest Pro version 5.2.1 (Becton Dickinson) (1).

Fluorimetric measurements. Fluorescence measurements were performed using a spectrofluorometer (Ratio Master, Photon Technology) at an excitation constant of 550 nm and an emission constant of 560 nm. The mitochondrial Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]_{\text{mit}}\)) was determined as previously described (64). \([\text{Ca}^{2+}]_{\text{mit}}\) (in mmol/l) was calculated using the following equation: \([\text{Ca}^{2+}]_{\text{mit}} = K_d \times [(R - R_{\text{min}})/(R_{\text{max}} - R)]\), where \(K_d\) (570 nmol/l) is a dissociation constant, \(R\) is the measured fluorescence intensity (after background subtraction), \(R_{\text{min}}\) is the fluorescence at 0 mm Ca\(^{2+}\) and was derived by exposing cells to 25 \(\mu\)M digitonin in Ca\(^{2+}\)-free HEPES buffer containing 10 \(\mu\)M EGTA, and \(R_{\text{max}}\) is the fluorescence under saturating [Ca\(^{2+}\)] (32 \(\mu\)M) and was derived by exposing cells to HEPES buffer containing 2.5 mmol/l CaCl\(_2\) without EGTA. Acquired data were analyzed using Felix version 1.43b (Photon Technology).

**Mitochondria Oxygen Consumption Measurement**

Respiration rates were measured in both cell types using 0.5-ml Clark oxygen electrodes (Rank Brothers, Cambridge, UK) thermostatted at 25°C and connected to a Kipp and Zonen dual-channel chart recorder, assuming 250 nmol O\(_2\)/ml at air saturation (53). Cell respiration rates were expressed as a function of mitochondria in cells. The supernatant was recentrifuged at 10,000 \(g\) for 10 min to obtain mitochondria-enriched pellets.

2-D gel electrophoresis proteomics of mitochondria proteins. The mitochondria-enriched pellet was dissolved in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris base, 1% DTT, 0.5% IPG buffer, 0.5% Triton X-114, and protease inhibitor cocktail) and kept at room temperature for 1 h. The protein content was assayed using a 2-D Quant kit (GE Healthcare), and 2-D gel electrophoresis (2-DE) was performed as described by Berkelman and Stenstedt (5).

Each sample was run triplicate for a total of six gels. A 13-cm (pH 3–10) IPG strip was rehydrated overnight at 30 V in 250 \(\mu\)l of an isoelectric focusing solution that contained \(\sim 50 \mu\)g of the solubilized proteins. Isoelectric focusing was carried out at 60,000 V/h at 20°C as follows: 500 V for 1 h, 1,000 V for 1 h, and finally 8,000 V increased to 60,000 V/h. IPG strips were placed in 10 ml of an equilibration solution [50 mM Tris-HCl (pH 8.8) containing 6 M urea, 30% glycerol, 2% SDS, and bromophenol blue] that contained 1% (vol/vol) DTT during the first equilibration step and 2.5% (vol/vol) iodoacetamide during the second equilibration step (15 min per equilibration step). The 2-DE separation was performed using the SE 600 Ruby electrophoresis set (Amersham). IPG strips were rehydrated onto a 12.5% SDS-PAGE gel, and running buffer (25 mM Tris, 192 mM glycine, and 3.5 mM SDS at pH 8.3) was added. Low current (15 mA/gel) was applied to the gel for first 15 min and increased up to 60 mA for 5 h. Gels were then stained with silver nitrate. Stained gels were scanned on a flatbed scanner (UMAX power look 1100), and images were analyzed using commercially available software (Image Master 2D Platinum, Amersham Bioscience).

Protein identification. For mass spectrometry fingerprinting, stained portions of the 2-D gel (see Fig. 5) were excised and then digested with trypsin as described by Rosenberg et al. (57). Isolated protein spots were destained with 100 mM sodium thiosulfate and 30 mM potassium ferricyanide. After being washed with 50% acetonitrile (ACN), gel fragments were dried in a vacuum centrifuge. Dried gel fragments were rehydrated in 20 \(\mu\)l of 25 mM NH\(_4\)HCO\(_3\) that contained 0.5 \(\mu\)g of sequencing grade trypsin (Promega). Fragments were then incubated overnight at 37°C. Remaining peptides were extracted twice with 30 \(\mu\)l of a 1:1 mixture of 50 mM NH\(_4\)HCO\(_3\) and ACN. Extracts were evaporated in a vacuum centrifuge for further drying. Aliquots of the peptide-containing samples were applied to a target disk, and aliquots were left to evaporate. Spectra were obtained using a Voyager DE PRO MALDI-MS (Applied Biosystems, Foster City, CA). National Center for Biotechnology Information (NCBI) protein databases were searched with Mascot PMF (http://www.matrixscience.com) using monoisotope peaks. A mass tolerance within 50 ppm was allowed initially, after which a recalibration was performed using the list of proteins that was obtained at 20 ppm. Proteins with Mouse scores over 63 were accepted as significant.

**Statistical Analysis**

Results are expressed as means ± SE. Statistical significance was estimated by Student’s t-test for comparison of two groups. Differences were classified as significant at \(P < 0.05\).

**RESULTS**

**Mitochondria Shape and Numbers in AGS and RGM-1 Cells**

Electron microscopy showed differences in the shape and numbers of mitochondria in cells, where mitochondria appeared red. Figure 1 clearly shows that the region containing mitochondria in RGM-1 cells (number of mitochondria: 23.5 ± 4, \(n = 6\)) was much larger than in AGS cells (number of mitochondria: 16.3 ± 3, \(n = 6\)). Cancer cells contained 69% as many mitochondria as RGM-1 cells, and those mitochondrial size was smaller than in normal cells (RGM-1 cells: 3.5 ±
0.3 μm², n = 6; and AGS cells: 1.3 ± 0.5 μm², n = 6, respectively).

\( \Delta \Psi_m \) of AGS and RGM-1 Cells

We determined \( \Delta \Psi_m \) in AGS and RGM-1 cells under laser scanning confocal microscopy with TMRE staining (Fig. 2A). The intensity of the fluorescent TMRE probe was used as an indirect measure of \( \Delta \Psi_m \) in AGS and RGM-1 cells (1). As shown in Fig. 2B, \( \Delta \Psi_m \) of AGS cells (1,008.88 ± 60.81, n = 6) was higher than that of RGM-1 cells (834.93 ± 68.57, n = 6). To support the above data, we additionally carried out experiments using the FACS system. As shown in Fig. 2C, \( \Delta \Psi_m \) of AGS cells (1,065.34 ± 34.52) was proved to be hyperpolarized compared with that of RGM-1 cells (813.25 ± 38.32), similar to results shown Fig. 2, A and B. Furthermore, the application of FCCP (1 μM) induced more rapid depolarization of \( \Delta \Psi_m \) in AGS cells than in RGM-1 cells (Fig. 2D). These results suggested that there were some changes of mitochondrial function in the cancer cell line.

\( [Ca^{2+}]_{im} \)

\( [Ca^{2+}]_{im} \) in both AGS and RGM-1 cells was traced using rhod-2 AM-loaded AGS and RGM-1 cells. The resulting intensity was an indirect measure of \( [Ca^{2+}]_{im} \) (34). As shown in Fig. 3, A and B, the intensity of AGS cells (1,343.51 ± 81.95, n = 6) was higher than that in RGM-1 cells (1,054.62 ± 43.28, n = 6). Coincidently, fluorimetric measurements revealed that
the calibrated \( [\text{Ca}^{2+}]_m \) of AGS cells (152.26 ± 8.21 mM, \( n = 6 \)) was higher than that in RGM-1 cells (114.13 ± 7.45 mM, \( n = 6 \); Fig. 3C).

**Mitochondria Oxygen Consumption**

As shown in Fig. 4, the oxygen consumption was higher in the normal cell line (2.6 ± 0.2 nmol O\(_2\)\cdot10^{-6}\) cells\cdot min\(^{-1}\)) than in the cancer cell line (1.6 ± 0.15 nmol O\(_2\)\cdot10^{-6}\) cells\cdot min\(^{-1}\)).

**2-DE Proteomics Analysis**

Mitochondrial-enriched fractions isolated from AGS and RGM-1 cells were used for 2DE proteomics analysis to compare the expression of different mitochondrial proteins. Using the automated spot-counting algorithm in Image Master 2D Platinum (Amersham Biosciences), 475 ± 8.5 and 401 ± 22.3 protein spots were detected from AGS and RGM-1 cells, respectively. Expression patterns of mitochondrial proteins are shown in Fig. 5A for RGM-1 cells and Fig. 5B for AGS cells. Mitochondrial proteins from RGM-1 and AGS cell lines were distributed in the region of pI 4-9 and had relative molecular masses between 30 and 150 kDa.

2-DE gel image analysis quantified the percent volume of each spot in both groups and revealed that the expression of 37 spots common to AGS and RGM-1 cells changed significantly by over 170%, including 20 spots that were downregulated and 17 spots that were upregulated in the AGS group. These 37 spots were sampled using a spot picker.
Fig. 3. Mitochondrial Ca$^{2+}$ concentration ([Ca$^{2+}$]$_{m}$) measured by rhod-2 AM fluorescence showing higher [Ca$^{2+}$]$_{m}$ in AGS cells than in RGM-1 cells. A: confocal microscopic images of [Ca$^{2+}$]$_{m}$ in normal and cancer cells. B: summary of the experiments shown in A. C: fluorometric measurements of [Ca$^{2+}$]$_{m}$ in AGS and RGM-1 cells. *P < 0.05 vs. RGM-1 cells.
subjected to matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy protein identification analysis.

Protein Identification

Note that although the mitochondrial fraction was isolated to enrich the mitochondrial yield as much as possible, the proteomics profiling results still covered a range of expressed nonmitochondrial proteins. Thirty-seven selected spots were subjected to MALDI-TOF mass spectroscopy for protein identification. Peptide mass peaks were compared with those in the NCBI database (Fig. 6), and the protein identification data, including GenBank identification numbers, molecular masses, pI values, Mowse scores, numbers of matched peptides, and sequence coverage ratios (%), are shown in Table 1. The 14 identified protein spots were categorized into two groups based on their localization as mitochondrial or cytosolic proteins. The functions and expression levels of the eight proteins that were upregulated (Fig. 7A) and the six proteins that were downregulated (Fig. 7B) are shown in Table 2.

We identified significant changes of cancer-related proteins or cancer biomarkers in AGS cells compared with RGM-1 cells, including tumor rejection antigen (321%) (72), cytokeratin 8 protein (appear) (4, 25), cytokeratin 18 protein (716%) (4), enolase 1 (−57%) (51), testis-specific bromodomain protein (−64%) (60), transferrin (−63%) (22), and annexin I (−88%) (44, 77). Stress-related and chaperoning proteins were highly expressed in the gastric cancer cell line, including heat shock protein (HSP)70 (173%), HSP60 (appear), and mitochondrial translation elongation factor Tu protein (193%).

The altered energy metabolism in the cancer cell line was reflected in the expression of various related proteins, including aldehyde reductase 1 (−70%), mitochondrial enoyl-CoA hydratase 1 (996%), and ubiquinol-cytochrome c reductase (appear).

DISCUSSION

General Discussion

Understanding cancer progression is essential for the successful control of cancer. Since the common feature of malignancy is the ability of malignant cells to proliferate without restraint, apoptosis is considered to be the predominant pathway for the elimination of malignant cells. Despite a previous study (29) of proteomics profiling in gastric tumors, the common link between proteome alterations and the role of mitochondria in apoptosis is still unclear. Mitochondria have dual functions in carcinogenesis, namely, cancer-associated changes in cellular metabolism: the Warburg effect (71, 73), which affects mitochondrial function, and the apoptosis-linked mitochondrial permeability transition pore (MPTP). Destabilizing mitochondria as a key determinant of the point of no return to apoptosis is still under debate (56). Moreover, the mechanisms responsible for the initiation and development of cancer, drug resistance, and disease progression remain to be elucidated. Therefore, understanding the changes in mitochondrial behav-

![Fig. 4. Oxygen consumption rates of AGS and RGM-1 cells. AGS cells had a lower oxygen consumption rate than RGM-1 cells. *P < 0.01.](http://ajpcell.physiology.org/)

![Fig. 5. Two-dimensional gel electrophoresis proteomic analysis of RGM-1 (A) and AGS (B) cells. Thirty-seven significantly changed spots (labeled green in AGS cells) were detected from a total of 475 ± 8.5 spots. Mw, molecular weight.](http://ajpcell.physiology.org/)
ior in gastric cancer is a promising approach for developing a cure.

In this study, we compared the mitochondria of gastric cancer cells and control cells. On the basis of several previous studies (48, 74, 79), we compared a human gastric cancer-derived cell line (AGS) with a control rat gastric epithelial cell line (RGM-1) to build our models. Our data suggest a possible correlation between differential proteomes and antiapoptotic cellular adaptations that lead gastric tumor cells toward apparent immortality.

A State of Cellular Hypoxia

It is well known that oxygen is the first determinant substrate contributing to mitochondrial performance and, hence, cell function and survival. The rate of oxygen consumption is a direct function of mitochondrial performance. Therefore, tumor oxygen status is strongly associated with the growth, malignant progression, and resistance of a cancer tumor to various therapies. A previous study (69) has suggested that hypoxia enhances malignant progression and increase aggressiveness. The reported decreased rate of oxygen consumption likely coincides with the decreased abundance and smaller size of mitochondria in the AGS cell line.

Mitochondrial Response to Apparent Cellular Hypoxia

Despite a decrease in the oxygen consumption rate and relative low abundance of mitochondria, a marked overexpression of ubiquinol-cytochrome c reductase in the gastric cancer cell line was observed. Ubiquinol cytochrome c reductase, the Rieske Fe-S protein, is a key subunit of the cytochrome bc1 complex (complex III) of the mitochondrial respiratory chain (55, 68). This complex generates an electrochemical potential

Table 1. Identification of significantly alternated proteins from AGS compared with RGM-1 cells

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>GenBank Identification No.</th>
<th>Protein Identification</th>
<th>Mr, kDa</th>
<th>pl</th>
<th>Mowse Score</th>
<th>Match Peptide</th>
<th>Sequence Coverage Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>61656607</td>
<td>Tumor rejection antigen (gp96)</td>
<td>91.3</td>
<td>4.75</td>
<td>121</td>
<td>KYPFS/SEYRL</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>114326282</td>
<td>Transferrin</td>
<td>79.8</td>
<td>6.75</td>
<td>72</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>74143673</td>
<td>HSP70</td>
<td>72.4</td>
<td>5.07</td>
<td>128</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>77702086</td>
<td>Chaperonin-HSP60</td>
<td>61.1</td>
<td>5.7</td>
<td>190</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>9</td>
<td>62913980</td>
<td>KRT8 protein</td>
<td>41</td>
<td>4.94</td>
<td>163</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>12</td>
<td>13325287</td>
<td>Enolase 1</td>
<td>47.4</td>
<td>7.01</td>
<td>124</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>303111</td>
<td>KRT18</td>
<td>47.3</td>
<td>5.27</td>
<td>109</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>899285</td>
<td>Elongation factor Tu (mitochondrial)</td>
<td>49.8</td>
<td>7.26</td>
<td>126</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>20</td>
<td>62020939</td>
<td>BRDT protein</td>
<td>53.1</td>
<td>9.34</td>
<td>73</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>23</td>
<td>6978501</td>
<td>Annexin I</td>
<td>39.1</td>
<td>6.97</td>
<td>73</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>25</td>
<td>6978491</td>
<td>Aldehyde reductase 1</td>
<td>36.2</td>
<td>6.26</td>
<td>62</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>28</td>
<td>76642070</td>
<td>Nonmuscle myosin II</td>
<td>22.8</td>
<td>5.52</td>
<td>72</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>35</td>
<td>14286220</td>
<td>Mitochondrial short-chain enoyl-CoA hydratase 1</td>
<td>31.8</td>
<td>8.34</td>
<td>76</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>37</td>
<td>45768728</td>
<td>Ubiquinol-cytochrome c reductase (Rieske iron-sulfur polypeptide 1)</td>
<td>29.9</td>
<td>8.55</td>
<td>86</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

Mr, relative mass; HSP, heat shock protein; KRT, cytokeratin; BRDT protein, testis-specific bromodomain protein.
coupled to ATP synthesis to transfer electrons from ubiquinol to cytochrome c. Logically, the observed upregulation of ubiquinol-cytochrome c reductase, which is in agreement with a report (40) suggesting amplification of the ubiquinol-cytochrome c reductase gene in different kinds of tumors, matched the elevation of $\Delta\Psi_m$, as shown in the results of the present study. Whether this overexpression accompanies a defect in the mitochondrial energetic potential due to a possible complex III defect associated with mitochondrial DNA mutations (8) is still an open question. Nevertheless, the discrepancy between the cellular tendency toward local hypoxia (as demonstrated by the decreased respiration rate and reduced mitochondrial availability) and the overexpression of the complex III component (ubiquinol-cytochrome c reductase) likely reflects a decrease in ROS production as a cellular scavenger against apoptosis. Since a positive correlation between ROS production and apoptosis in other kinds of tumor has been demonstrated (41), this assumption needs further investigation to correlate the role of ROS production with apoptosis in our AGS cell model. Apoptosis is strongly dependent on activation of the MPTP, which is a Ca$^{2+}$-sensitive channel in the mitochondrial inner membrane that plays a crucial role in cell death. A novel MPTP model of an intrinsic mitochondrial pathway of apoptosis has been recently proposed (3), which was regulated by a respira-

Table 2. Different protein expressions and their function in AGS compared with RGM-1 cells

<table>
<thead>
<tr>
<th>Protein Identification</th>
<th>$\Delta$(A/R)</th>
<th>Known Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitochondrial proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spot 7</td>
<td>Chaperonin-HSP60</td>
<td>Appear</td>
</tr>
<tr>
<td>Spot 15</td>
<td>Elongation factor Tu (mitochondrial)</td>
<td>193</td>
</tr>
<tr>
<td>Spot 35</td>
<td>Mitochondrial short-chain enoyl-CoA hydratase 1</td>
<td>996</td>
</tr>
<tr>
<td>Spot 37</td>
<td>Ubiquinol-cytochrome c reductase (Rieske iron-sulfur polypeptide 1)</td>
<td>Appear</td>
</tr>
<tr>
<td><strong>Cytosolic proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spot 2</td>
<td>Tumor rejection antigen (gp96)</td>
<td>321</td>
</tr>
<tr>
<td>Spot 4</td>
<td>Transferrin</td>
<td>37</td>
</tr>
<tr>
<td>Spot 5</td>
<td>HSP70</td>
<td>173</td>
</tr>
<tr>
<td>Spot 9</td>
<td>KRT8 protein</td>
<td>Appear</td>
</tr>
<tr>
<td>Spot 12</td>
<td>Enolase 1</td>
<td>43</td>
</tr>
<tr>
<td>Spot 13</td>
<td>KRT18</td>
<td>716</td>
</tr>
<tr>
<td>Spot 20</td>
<td>BRDT protein</td>
<td>36</td>
</tr>
<tr>
<td>Spot 23</td>
<td>Annexin I</td>
<td>12</td>
</tr>
<tr>
<td>Spot 25</td>
<td>Aldehyde reductase 1</td>
<td>30</td>
</tr>
<tr>
<td>Spot 28</td>
<td>Nonmuscle myosin 14</td>
<td>32</td>
</tr>
</tbody>
</table>

$\Delta$(A/R), spot volume of AGS/RGM-1 cells $\times$ 100 (%).
tory supercomplex formed by NADH:ubiquinone dehydrogenase, cytochrome bc1, and adenine nucleotide translocator (ANT). The model suggested that complex III regulates two distinct pathways to the MPTP: one unregulated and involving mitochondrial ROS, and the other regulated and activated by Ca2+. As recently addressed in gastric tumors, the possible oxidative damages to the redox centers of the respiratory chain that promoted the development of cell hypoxia (11) and the observed Ca2+ overload clearly indicate the gastric tumor cells’ tolerance to Ca2+ overload and possible ROS generation. Moreover, our data refute the role of Ca2+ in triggering mitochondria-mediated apoptosis. It is also possible to speculate on the sensitivity of gastric cancer cells to ROS generation in our model.

The Mitochondrial Interplay With Neo-Homeostasis

The twofold increase in mitochondrial elongation factor Tu provides evidence of translational machinery remodeling in the AGS cell line to meet its primitive prokaryotic form (21). This assumed remodeling requires 1) an alternative energy source and 2) backing by sound chaperone systems that keep pace with the neo-homeostasis.

The increased rate of fatty acid degradation can meet the first requirement. Consistent with a recent study (78) postulating that mitochondrial short-chain enoyl-CoA hydratase 1 (EC 4.2.1.17) overexpression is a function of carcinogenesis, our data clearly confirm its higher expression in gastric cancer cells. The overexpression of this enzyme, which catalyzes the second step of the β-oxidation pathway, suggests a novel biomarker for an aggressive type of gastric cell carcinoma and shows how cancer cells exploit an in-use energy source for their altered performance. We would expect the accelerated rate of β-oxidation, together with the increased expression of a complex III component that does not meet with the consequent increase in oxygen consumption, to be related to the high coupling efficiency of the cancer cell mitochondrial inner membrane. To confirm this assumption, however, further investigation is required.

As biomarkers of carcinogenesis and components of cellular neo-homeostasis, HSPs are elevated during different kinds of stress (12, 16, 46), including gastric infection and tumors (36). HSP60, a chaperonin, is an evolutionarily conserved 60-kDa mitochondrial protein (27, 35) that affords protein chaperoning remodeling activity (31). Although the overexpression of HSP60 has been reported in other tumors (47), the mechanism of its upregulation in our model is still controversial. Previous studies (58, 76) have confirmed that unlike HSP70, which also showed increased expression, HSP60 helps in the induction of apoptosis by acting as a chaperone to pro-caspase-3 and aiding in its maturation into active caspase-3. HSP60 has both pro- and antiapoptotic roles in tumor cells (17, 19); therefore, it is tempting to speculate whether its overexpression could serve as a proapoptotic regulator rather than an antiapoptotic regulator. Moreover, our results provide additional evidence of neohomeostatic regulation via the decreased expression of aldehyde reductase 1, the first limiting enzyme of the polyol pathway. Many known cascades that induce or exacerbate intracellular oxidative stress accelerate the polyol pathway, e.g., glutathione depletion, increased superoxide accumulation, increased JNK activation, and DNA damage (10). This finding strengthens the postulation that a tumor is susceptible to any adverse oxidative stress.

Note that the disagreement between our finding and previous investigations suggests that the upregulation of glycolytic enzyme enolase 1 expression in gastric adenocarcinoma (29) is attributable to the difference in samples examined, since all the tumors in the previous study were classified as intestinal type tumors.

Cytoskeleton Remodeling as a Cellular Adaptation for Neo-Homeostasis

The cytoskeleton remodeling via downregulation of both annexin I and nonmuscle myosin II supports a cellular adaptation for neo-homeostasis. Annexin I belongs to a family of cytosolic Ca2+-binding proteins that plays an important role in actin remodeling and polymerization (24, 28). Its decreased expression, which suggests gastric transformation, is very similar to data for breast cancer (61), prostate cancer (44), lymphoma (70), and esophageal cancer (77). Moreover, deregulated annexin expression was recently observed in a highly invasive gastric cancer cell line (65). As recently noted (18), nonmuscle myosin II likely senses matrix elasticity. Therefore, its decreased expression is clearly indicative of reduced cellular differentiation (45), which renders tumor cells more malleable at different possible surroundings.

Additional Cellular Adaptations for Neo-Homeostasis

Another observed adaptation was the increased expression of transferrin, a specific iron transfer protein that regulates the cellular free iron level (49, 54). Although iron is essential for the metabolism, viability, and proliferation of normal and cancer cells, it acts as a prooxidant that can damage biomolecules (22). Referring to the essential role of iron in cell metabolism and viability, the increased expression of transferrin is not surprising. Furthermore, the disruption of cellular iron homeostasis has been shown to inhibit the growth of different malignant cell types (7, 15, 75).

Expectedly, there was a marked expression of tumor rejection antigen, which is very characteristic of gastric tumors (33). The overexpression of both tumor rejection antigen and cytotkeratin 18 in gastric cell tumors provides insight into possible prognostic and diagnostic approaches for gastric cancer.

GRANTS

This work was supported by the Korean Government through Korea Research Foundation Grants KRF-2005-210-E00003, KRF-2005-211-E00006, KRF-2005-037-E00002, KRF-2005-042-E00010, KRF-2006-312-E00018, KRF-2006-312-C00601, KRF-2006-312-E00003, and KRF-2006-351-E00002 and by Korea Institute of Industrial Technology Evaluation and Planning through the Biohealth Products Research Center of Inje University.

REFERENCES


Downloaded from http://ajpcell.physiology.org/ on June 23, 2017


