Deficiency of the complex I of the mitochondrial respiratory chain but improved adenylate control over succinate-dependent respiration are human gastric cancer-specific phenomena

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Received: 15 March 2012 / Accepted: 7 July 2012 © Springer Science+Business Media, LLC. 2012

Abstract The purpose of study was to comparatively characterize the oxidative phosphorylation (OXPHOS) and function of respiratory chain in mitochondria in human gastric corpus mucosa undergoing transition from normal to cancer states and in human gastric cancer cell lines, MKN28 and MKN45. The tissue samples taken by endobiopsy and the cells were permeabilized by saponin treatment to assess mitochondrial function in situ by high-resolution oxygraphy. Compared to the control group of endobiopsy samples, the maximal capacity of OXPHOS in the cancer group was almost twice lower. The respiratory chain complex I-dependent respiration, normalized to complex II-dependent respiration, was reduced that suggests deficiency of complex I, but the respiratory control by ADP in the presence of succinate was increased. Similar changes were observed also in mucosa adjacent to cancer tissue. The respiratory capacity of MKN45 cells was higher than that of MKN28 cells, but both types of cells exhibited a deficiency of complex I of the respiratory chain which appears to be an intrinsic property of the cancer cells. In conclusion, human gastric cancer is associated with decreased respiratory capacity, deficiency of the respiratory complex I of mitochondria, and improved coupling of succinate oxidation to phosphorylation in tumor tissue and adjacent atrophic mucosa. Detection of these changes in endobiopsy samples may be of diagnostic value.

Keywords Respiratory chain · Oxidative phosphorylation · Gastric mucosa · Gastric cancer · Gastric cancer cell line

Introduction

The biological model of gastric carcinogenesis can be envisaged as an inflammation → atrophy → metaplasia → dysplasia → carcinoma sequence [1], which is based on several distinct but intermingled processes. One of them is a chronic active inflammation, usually caused by Helicobacter pylori infection, which by establishing the background conditions paves the way for cancer-specific geno- and phenotypic alterations. Prevalence of apoptosis over cell proliferation results in mucosal atrophy, a second class of changes. In parallel to these processes, a progressive decay of differentiation capacity of mucosal cells resulting in replacement of normal glands by...
Accumulating evidence suggests that specific alterations in cellular energy metabolism may underpin the processes listed above. This was first considered by Otto Warburg who observed that mouse ascites cancer cells exhibited much higher (55 %) contribution of glycolytic ATP to total ATP production than normal tissue cells (0.94 %) in aerobic conditions [3]. He concluded that development of cancer is causally related to suppression of oxidative phosphorylation (OXPHOS) and activation of aerobic glycolysis. However, this view has been questioned by later studies. It has been found that the balance between the rates of OXPHOS and glycolysis varies largely depending on cancer cell type and environmental conditions (i.e., normoxia vs. hypoxia in cancer tissue). Many types of tumor cells possess the mechanisms for ensuring high rates of OXPHOS capable of producing even up to 80–95 % of normal cellular ATP [4–6], which appears necessary for maintenance of tumorigenicity [7–12]. Oncogene c-Myc, which up-regulates the glycolytic system and anaerobic fluxes, serves as an important factor for augmenting mitochondrial capacity, thus orchestrating the interaction of bioenergetic and biosynthetic pathways to ensure cell proliferation [13]. At the same time, the role of OXPHOS in maintaining the viability of human cancer cells is largely unclear and, therefore, deserves further investigation.

Our previous study showed that atrophic gastritis was associated with decreased respiratory capacity and relative deficiency of complex I of the respiratory chain of mitochondria [14]. Considering that the mucosal atrophy precedes development of carcinoma, we hypothesized that these functional alterations of mitochondria may also be characteristic of cancer cell phenotype. Therefore, we have characterized the parameters of OXPHOS and the function of the respiratory chain in normal and cancerous mucosa, including gastric endobiopsies taken from patients suffering from adenocarcinoma. In order to relate clinical observations to the cancer cell phenotype, the alterations observed were compared with indices of mitochondrial function in human gastric cancer cell lines.

Materials and methods

Patients and biopsy sampling

Six patients (3 men and 3 women, mean age 68 ± 3 years) with gastric non-cardial adenocarcinoma located in the corpus region of the stomach and sent to department of oncological surgery for diagnostic examination were included in the study as a cancer group. In these patients, mucosal endoscopic biopsies were taken from the visually detectable tumor tissue and from surrounding visually normal tissue. In subsequent assessment, these biopsies were grouped and referred to as “cancer” and “adjacent”, respectively. One part of each endobiopsy specimen was used for assessment of the presence of H. pylori and histological profile of the mucosa as described previously [15]. The presence and severity of chronic gastritis, activity of gastritis, atrophy, and intestinal metaplasia were graded according to the Sidney system from 0 (no changes) through 1 (mild) and 2 (moderate) to 3 (severe changes) [16]. Infiltration of lymphocytes was taken to indicate the chronic status of inflammation and abundant the presence of polymorphonuclear leukocytes to mark an active chronic process. The amount of H. pylori in the mucosa was estimated semi-quantitatively by microscopic counting as described earlier [17]. Another part of the corpus mucosa specimens was placed immediately in ice-cold solution A containing (in mM) CaK2EGTA 2.77, K2EGTA 7.23, MgCl2 6.56, DTT 0.5, K-MES 50, imidazole 20, taurine 20, Na2ATP 5.3, phosphocreatine 15, at pH 7.1 and used for studies of mitochondrial function.

To form a control group, 16 consecutive patients undergoing upper gastrointestinal endoscopy for epigastric complaints were initially recruited. None of them had received non-steroidal anti-inflammatory drugs, H+-pump inhibitors, or antibiotics to cure their illness. Mucosal biopsies were taken from the anterior and posterior walls of the medio-lateral part of the corpus. The biopsies from the corpus were processed similarly to the specimens taken from cancer patients. Based on histological findings from 16 patients only 10 (5 men and 5 women; mean age, 64 ± 3 years) who exhibited no signs of mucosal atrophy and active chronic inflammation in the corpus were selected to represent the control group.

Gastric endoscopic biopsies were carried out in accordance with the European Communities Council Directive 86/609/EEC and with the Declaration of Helsinki [18]. Written informed consent was obtained from all patients, and the Tartu University Ethics Committee approved the study.

Preparation of permeabilized mucosal tissue

The permeabilized cells of mucosal tissue were prepared by the technique described previously [15]. The mucosal tissue biopsy samples were cut into smaller pieces in the ice-cold solution A and the pieces were gently stretched with thin needles, to facilitate the diffusion of the medium into the intercellular space. Next, the tissue was incubated at +4 °C, at mild stirring for 30 min in solution A containing 50 μg/ml saponin for permeabilization of the cell plasma membrane. The permeabilized mucosal tissue samples were then washed for 10 min in solution B.
containing (mM) CaK$_2$EGTA 2.77, K$_2$EGTA 7.23, DTT 0.5, MgCl$_2$ 1.38, K-Mes 100, taurine 20, imidazole 20, K$_2$HPO$_4$ 3, pyruvate 5, and 5 mg/ml fatty acid-free bovine serum albumin (BSA), pH 7.1 at 25°C; this procedure of washing was repeated two more times to remove all metabolites from the cells.

Cell cultures

The human gastric cancer cell lines MKN28 and MKN45 derived from moderately differentiated tubular adenocarcinoma and undifferentiated adenocarcinoma of medullary type, respectively, were used in the study [19]. The cells were grown in flasks in the presence of RPMI 1640 supplemented with 10 % fetal calf serum, 2 mM glutamine and 1 % antibiotic solution (Invitrogen) at 37°C and 5 % CO$_2$. The cells reaching 70–80 % confluence were removed from plastic surface by shaking the flask or using quick trypsin treatment and thereafter used for experiments.

Transmission electron microscopy

The intact and permeabilized with saponin MKN28 and MKN45 cells were fixed with 0.25 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and post-fixed in 2 % OsO$_4$ in the same buffer. After dehydration with ethanol and acetone, the specimens were embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with Tecnai 10 electron microscope (Philips/FEI/) at 80 kV.

Preparation of permeabilized cells

The cells were centrifuged at 200 g for 5 min at room temperature, the supernatant (growth medium) was decanted and sedimented cells resuspended in 5 ml Mitomed solution containing (mM) sucrose 110, EGTA 0.5, K-lactobionate 60, MgCl$_2$ 3, taurine 20, dithiothreitol 0.5, HEPES 20, pH 7.1, and centrifuged again as above. After removing the supernatant, cells were incubated in shaking conditions for 15 min at room temperature in 6 ml Mitomed solution containing 65 μg/ml saponin. After that cells were centrifuged and resuspended in Mitomed, this washing step was repeated twice more and the final pellet was resuspended in Mitomed and used immediately for experiments.

Measurement of mitochondrial respiratory function

The rates of oxygen uptake were recorded using a high-resolution OROBOROS oxygraph-2k (OROBOROS INSTRUMENTS Corp, Austria). The sequential multiple substrate-inhibitor titration protocol (Fig. 3a) was applied for the assessment of the function of the respiratory chain. The permeabilized mucosal tissue was assessed in Solution B containing 0.1 mM free Ca$^{2+}$ and supplemented with 10 mM glutamate and 2 mM malate, and 5 mg/ml BSA [14]. The permeabilized MKN28 and MKN45 cells were incubated in an oxygraph chamber in the Mitomed solution at 25°C containing 2 mg/ml BSA, 0.2 μM free Ca$^{2+}$ [20], in the presence of 10 mM glutamate, and 2 mM malate as the respiratory substrates.

Statistical analysis

Data are expressed as mean ± SE. Where appropriate, one-way ANOVA with Bonferroni post-test and unpaired or paired Student’s t test were used to analyze the differences between the groups. $P < 0.05$ was considered to be statistically significant.

Results

Histological findings

In the control group, no mucosal atrophy, dysplasia, or intestinal metaplasia were detected. Five of ten patients (5/10) had no chronic inflammation of the corpus mucosa; among the rest of the patients 4/5 had chronic inflammation score 1 and 1/5 exhibited inflammation score 2. Infection with H. pylori was found in 6/10 patients (2/6 score 1, 1/6 score 2 and 3/6 score 3). In the adjacent group, all 6 patients with gastric cancer had a chronic inflammation of the corpus mucosa (4/6 score 1 and 2/6 score 2). Five of six patients had mucosal atrophy (score 2) and intestinal metaplasia was detected in 3 of 6 patients (1/3 score 1 and 2/3 score 2). Dysplasia was not detected in the biopsies in the adjacent mucosa group. 1/6 patients was H. pylori positive (score 1). In the cancer group, 1/6 patients had moderately differentiated adenocarcinoma, 3/6 had poorly differentiated adenocarcinoma, and 2/6 had signet ring cell carcinoma of the stomach.

Function of respiratory chain complexes in corpus mucosa of patients with gastric cancer

Table 1 summarizes the mitochondrial function in permeabilized mucosal specimens from healthy corpus, gastric cancer, and adjacent to cancer tissue assessed according to the protocol shown in Fig. 3a. In cancer patients (adjacent and cancer groups), the mean values of all respiratory rate indices [basal rate, $V_0$; maximal complex I-dependent ADP stimulated respiration rate ($V_{Glu}$); maximal complex II-dependent ADP stimulated respiration rate ($V_{Succ}$); proton leak; and respiratory equivalent of activity of cytochrome
Table 1 Characterization of respiratory parameters in the gastric corpus mucosa of patients without gastric cancer (Control) and patients with gastric adenocarcinoma, in specimens taken from the tumor tissue (Cancer) and adjacent to tumor mucosa (Adjacent)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 10)</th>
<th>Adjacent (n = 6)</th>
<th>Cancer (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>0.29 ± 0.03</td>
<td>0.14 ± 0.03**</td>
<td>0.13 ± 0.01***</td>
</tr>
<tr>
<td>$V_{Glut}$</td>
<td>0.89 ± 0.03</td>
<td>0.39 ± 0.06***</td>
<td>0.34 ± 0.04***</td>
</tr>
<tr>
<td>$RCI_{Glut}$</td>
<td>3.21 ± 0.30#</td>
<td>3.21 ± 0.19</td>
<td>2.67 ± 0.24</td>
</tr>
<tr>
<td>$V_{Succ}$</td>
<td>0.71 ± 0.08</td>
<td>0.49 ± 0.05</td>
<td>0.40 ± 0.05*</td>
</tr>
<tr>
<td>$V_{ATR}$</td>
<td>0.48 ± 0.05</td>
<td>0.19 ± 0.04***</td>
<td>0.18 ± 0.01***</td>
</tr>
<tr>
<td>$RCI_{Succ}$</td>
<td>1.48 ± 0.06</td>
<td>2.80 ± 0.31***</td>
<td>2.17 ± 0.24*</td>
</tr>
<tr>
<td>Proton leak</td>
<td>0.23 ± 0.04</td>
<td>0.13 ± 0.02</td>
<td>0.10 ± 0.02*</td>
</tr>
<tr>
<td>$V_{COX}$</td>
<td>1.31 ± 0.13</td>
<td>0.74 ± 0.08**</td>
<td>0.61 ± 0.08***</td>
</tr>
</tbody>
</table>

The mean ± SE of parameter values are given for experiments performed according to the protocol shown in Fig. 3a. The rates of respiration ($V$) are expressed in nmol O₂/min/mg protein. $V_0$—basal respiration without ADP or ATP; $V_{Glut}$—ADP-stimulated respiration in the presence of glutamate and malate (indicating the function of the respiratory chain complex I); $RCI_{Glut}$—respiration control index calculated as a ratio of maximal ADP-dependent respiration rate in the presence of glutamate and malate to basal respiration without ADP; $V_{Succ}$—ADP-stimulated respiration in the presence of rotenone and succinate (characterize function of complex II); $RCI_{Succ}$—ratio of ADP-stimulated respiration in the presence of succinate when respiratory chain complex I is inhibited by rotenone to respiration rate after inhibition of succinate-stimulated respiration by atracyloside; Proton leak—measured indirectly as a state 4 respiration equal to difference between the respiration rates with atractyloside and antimycin A; $V_{COX}$—the respiratory equivalent of cytochrome oxidase (complex IV) activity calculated as $[V_{COX} = V_{TMPD} - V_{TMPD+NaN3}]$ where $V_{TMPD}$ and $V_{TMPD+NaN3}$ are TMPD-stimulated respiration rates before and after addition of NaN₃.

$*$ $P < 0.001$, $**$ $P < 0.01$, $*$ $P < 0.05$, compared to mucosa of the control group. $#$ $P < 0.001$ compared to the $RCI_{Succ}$ of the control group.

oxidase ($V_{COX}$) were lower than in the control group. Since similar changes were found in atrrophic corpus mucosa of patients with pernicious anemia [14], it is highly possible that the decreased respiratory capacity in the adjacent group stems from the loss of mitochondria-rich parietal cells due to mucosal atrophy preceding the development of the cancerous mucosa. Table 1 demonstrates that in the healthy gastric corpus mucosa, the respiratory adenylate control index, $RCI_{Glut}$, almost doubled the relevant index during succinate oxidation, $RCI_{Succ}$. This difference stems from relatively higher succinate-dependent state 4 respiration ($V_{ATR}$) established in the presence of atracyloside, a blocker of ADP transport into the matrix, in comparison with the glutamate/malate-dependent $V_0$. High value of $V_{ATR}$ can be explained by larger $\Delta\Psi$ during succinate oxidation which promotes proton leak [21, 22]. Development of cancer was accompanied with suppression in complex I-dependent respiration ($V_{Glut}$) to relatively larger extent (by 2.7 times) than in complex II-dependent respiration ($V_{Succ}$) (1.8 times) as compared to control parameters. In addition, the succinate-dependent proton leak was diminished. These two changes combined to result in increased $RCI_{Succ}$ compared to that parameter in control group. Thus, mitochondria in cancer group used succinate for supporting the ATP synthesis more effectively than their counterparts in the control group. Surprisingly, no difference was observed between the respiratory indices of cancerous mucosa, and the non-neoplastic mucosal tissue surrounding it (adjacent group) (Table 1) as the latter group was also characterized by low respiratory capacity compared to control. A common finding for cancer and adjacent groups was reduced $V_{Glut}/V_{Succ}$ ratio as compared to control corpus mucosa (Fig. 1). This change is considered to be a reliable index of respiratory chain deficiency at the level of the complex I, frequently observed in gene-modified animals and in case of human muscle disease [23–25].

Morphological and functional characterization of the MKN45 and MKN28 cells

In order to prove that alterations of mitochondrial functions in diseased gastric mucosa represented changes intrinsic to cancer cells, the function of mitochondrial respiratory chain was assessed in human gastric cancer cell lines, MKN45 and MKN28. These cell lines exhibit a distinct level of differentiation, whereas the MKN28 cell line is derived from moderately differentiated tubular
adenocarcinoma representing an intestinal type of cancer, the MKN45 cell line originates from a poorly differentiated adenocarcinoma of medullary type.

Both cell lines showed adhesive growth on plastic surface but the adhesion of MKN28 cells was much more intensive than MKN45 cells. This qualitative difference stems from microscopic inspection (not shown) revealing that in our experimental settings, the MKN28 cells grew forming the sheets like islands of cells closely attached to each other. In contrast, growth of MKN45 cells resembled that of fibroblasts, as adjacent cells contacted with cell projections mainly. Figure 2 shows that the ultrastructure between these two cell lines differs by the presence of plasma membrane microvilli, number of mitochondria and amount of intracellular filaments. In general, much more surface microvilli and intracellular filament bundles were observed in MKN28 cells as compared with MKN45 cells. This indicates that the MKN28 cells are more differentiated than MKN45 cells toward establishment of epithelial cell’s phenotype. Both types of cells had few endosomes and multivesicular bodies or lysosomes. The number of mitochondria in these cells appeared to be similar or somewhat less in MKN28 cells compared with MKN45 cells. Mitochondria of untreated cells had pale matrix with basic architecture of typical mitochondria. The Fig. 2c, d, g, h shows that after saponin treatment, the cells preserved their normal structure. Mitochondria exhibited the electron dense matrix with vacuoles and irregularly swollen cristae. The outer membrane of mitochondria was mostly intact and the mitochondria retained their close contacts with intracellular filaments in MKN28.

Figure 3a demonstrates the original oxygraphic recording for assessment of the respiratory chain in mitochondria in permeabilized gastric cell line MKN45. It can be seen that addition of 2 mM MgADP markedly activated mitochondrial respiration over the basal level. This finding shows that sarcolemma was sufficiently permeabilized to enable diffusion of ADP into the cell and exert a control over the OXPHOS. As expected, rotenone, an inhibitor of the complex I of the respiratory chain, strongly suppressed the glutamate/malate-dependent respiration. Addition of succinate resulted in reactivation of oxygen consumption due to succinate oxidation through complex II. The subsequent inclusion of atractyloside markedly reduced the oxygen consumption due to inhibition of adenine nucleotide translocase (ANT), this effect indicating intactness (impermeability for ADP) of the inner mitochondrial membrane in the permeabilized cells. Antimycin A, an inhibitor of complex III of the respiratory chain, caused additional suppression of respiration. The residual respiration registered corresponds to the non-mitochondrial oxidative processes, whereas the difference between the respiration rates with atractyloside and antimycin A equals to proton leak. In subsequent stage of the experiment, addition of TMPD with ascorbate strongly activated the COX-associated oxygen consumption rate. This was confirmed by inhibition of respiration by azide, and the azide-sensitive respiration was taken as a respiratory equivalent of the COX activity. Figure 3a also shows that addition of exogenous cytochrome c to the medium exerted no influence on TMPD + ascorbate-dependent respiration. Hence, the maximum rate of oxygen consumption was not limited by availability of cytochrome c which means that mitochondrial outer membrane remained intact and mitochondrial cytochrome c pool was preserved within the intermembrane space. The conclusion that mitochondria largely preserved their functional properties inside the
permeabilized cells despite the presence of underlying disease is also valid for saponin treated endobiopsy samples (Table 1) and corresponds to earlier data [14]. Significantly, the respiratory capacity of the MKN45 cells largely exceeded that in MKN28 cells, with similar differences for $V_o$, $V_{Glut}$, $V_{Succ}$, and $V_{COX}$ (Fig. 3b). This finding points to probability that the MKN45 cells contained much more mitochondria than in the MKN28 cells. Higher $RCl_{Glut}$ in MKN28 cells compared with MKN45 cells implies that lower capacity of OXPHOS in MKN28 cells was partially compensated by a better control of OXPHOS with ADP in conditions of complex I-dependent
respiration (Fig. 3b). Interestingly, the cancer cell lines exhibited even a stronger suppression in $V_{\text{Glu}}/V_{\text{Succ}}$ ratio than detected in the cancer and adjacent groups. Thus, impaired function of complex I of the respiratory chain is an inherent property of the gastric cancer cell line, at least in conditions of oxidation of glutamate and malate. Observation that this change became evident in cells adjacent to cancer mucosa suggests that impairment of complex I may be an early sign of phenotypic shift from a normal to cancerous tissue.

**Discussion**

A novel finding in this study is that development of gastric cancer was not only in cancerous mucosa but also in nonmalignant, adjacent to cancerous mucosa, as compared with normal one. The levels of state 2 and state 3 respiration in cancer and adjacent mucosa (Table 1) were similar to those registered in atrophied gastric mucosa of patients having pernicious anemia [14]. Compared to normal mucosa, the respiratory rates corresponding to the activities of different respiratory chain complexes were decreased in cancer tissue and in mucosa adjacent to it. In addition, atrophic gastritis was found in the adjacent mucosa of almost all patients having gastric cancer. It is conceivable therefore that suppression of respiratory activities in cancer and in adjacent mucosa (Table 1) resulted from the loss of zymogenic and parietal cells rich of mitochondria [26] due to the atrophy of mucosa.

Our data are the first to show that the MKN45 gastric cancer cells exhibited much higher capacity of OXPHOS than the MKN28 cells (Fig. 3). At least two molecular mechanisms can account for that difference. One of them is based on a variance in p53-mediated pathways [27–29]. It is known that in normal cells activation of p53 leads to the stimulation of OXPHOS and respiration, through increased expression of synthesis of cytochrome c oxidase 2 (SCO2) [27]. In cancer cells, this function of p53 should be attenuated or even lost due to mutations in that tumor suppressor gene; hence, the overall capacity of OXPHOS might be suppressed. In fact, the MKN45 and MKN28 cells express a wild-type and mutated p53, respectively [30]. It has been shown that although mutant p53 can translocate to mitochondria, its effect on mitochondrial functions differ from that in wild-type p53 [31]. Thus, higher activity of OXPHOS in MKN45 compared with MKN28 cells could be attributed to the presence of normal p53, capable of supporting high activity of mitochondria in the former cells. The second mechanism may be based on different activation of p38 mitogen-activated protein kinases (MAPKs). Atsumi et al. [32] have shown that MKN45 cells exceed the MKN28 cells by levels of phospho-p38 MAPK. Activated p38 stimulates the transcriptional coactivator of PPARγ coactivator 1z (PGC-1z) through hindering binding of p160RF, a repressor of the transcription of PGC-1z target genes, to PGC-1z [33]. As a result, the biogenesis of mitochondria, positively controlled by PGC-1z, increases which could explain enhanced OXPHOS activity in MKN45 compared with MKN28 cells. Interestingly, p38 MAPK was more active in human endobiopsy samples taken from poorly differentiated gastric cancers than in samples from differentiated cancers [32]. In our study, MKN45 cells which are poorly differentiated (Fig. 2) and multipotent—capable of inducing many cell types—exhibited higher OXPHOS activity than the more differentiated MKN28 cells mostly comprising the epithelial cells of intestinal type (Fig. 2; [19]). It appears thus that the level of differentiation in gastric cancer cells is inversely related to the OXPHOS capacity. On the other hand, higher OXPHOS activity in MKN45 may indicate increased malignancy. This assumption is supported by evidence that mitochondria exert a tumor suppression function [34] because OXPHOS and the H++-ATP synthase are required for apoptotic cell death. Therefore, reduction of the overall capacity of OXPHOS strongly hampers the potential of apoptotic pathways of cancer cell elimination [35]. On the other hand, induction of mitochondrial biogenesis suppresses carcinogenesis through inhibition of proliferation and invasiveness [36]. Considering higher OXPHOS capacity of MKN45 cells as compared to MKN28 cells, the MKN45 cells may also be viewed as potentially less malignant cells.

A major new observation was that both the cancerous and adjacent mucosa exhibited a relative deficiency of the complex I of the mitochondrial respiratory chain. Given the similar respiratory control by ADP in the presence of glutamate/malate in all groups (Table 1), this defect was not caused by impaired coupling of respiration to ATP synthesis. Complex I deficiency has been earlier observed in human ovarian cancer tissue [21], renal oncocytoma [37], thyroid oncocytoma [38], corpus mucosa of patients suffering from atrophic gastritis [14], and in conditions of chemically induced carcinogenesis in rat liver [39]. Based on the literature, a variety of mechanisms can underpin suppressed complex I activity of the mitochondria in cancer tissue. One of the mechanisms may be based on processes induced by hypoxia. Indeed, analysis of gastric endobiopsies from normal mucosa, mucosa implicated in H. pylori-associated gastritis, intestinal metaplasia dysplasia, and intestinal and diffuse adenocarcinoma has revealed increased expression of HIF-1z and HIF-2z in a given sequence of samples [40, 41]. Thus, in a course of transition from precancerous states to cancer, the gastric epithelial cells may progressively be exposed to deficit of oxygen,
which in turn may aggravate impairment of the respiratory chain complex in gastric cancerous tissue, e.g., through promoting mitochondrial ROS production in these cells (reviewed by [42]). However, observation that MKN28 and MKN45 cells, which certainly were not exposed to hypoxia during their growth in the culture medium, exhibited a relative decrease in complex I-dependent respiration; this change being even stronger expressed than in the endobiopsy specimens (Fig. 1) suggests that complex I deficit represents rather an intrinsic property of cancer cells than stems from hypoxic injury. This assumption gains further support by existence of specific molecular mechanisms causal for impairment of complex I. First, mutations in number of subunits of complex I can be accounted for its impaired function. These mutations give rise to excessive ROS production which secondarily affects other complexes of the respiratory chain thereby even rendering cancer cells resistant to apoptotic death [43–45]. Second, activation of K-ras oncogene by its mutations may represent a factor strongly responsible for complex I impairment. Baracca et al. [46] have shown that mouse fibroblasts transformed by an activated form of the K-ras oncogene [47] exhibited a dramatic decreases in the complex I activity, expressed as a lower respiration rate in the presence of glutamate/malate and a suppressed NADH-CoQ oxidoreductase activity in comparison with the control, non-transformed, fibroblasts. Very similar observations were made under K-rasG12V transformation of human embryonic kidney cells (HEK293) [48], demonstrating that conditional activation of K-rasG12V resulted in translocation of that protein to mitochondria, in association with suppressed complex I-dependent respiration, partial loss of 20 kDa component of complex I and decrease in membrane potential. Interestingly, these changes were paralleled by increased glycolytic activity in K-rasG12V transformed cells. The underlying mechanisms included activation of Akt that contributed to upregulation of expression of hexokinase II [48]. Probably, activation of glycolysis serves to compensate the suppressed OXPHOS. Besides the changes described, induction of excessive ROS production appears to be a common phenomenon for ras-oncogene activated cells [46, 48–50]. The ROS may be produced by mitochondria not only due to impairment of respiratory chain [48] but also due to activation of NADPH oxidase [51, 52]. ROS play a pleiotropic role, as they stimulate the growth of the K-ras-induced cells mediated by the ERK MAPK signaling pathway, but, while present in excessive amounts, may damage the complex I proteins [46]. A third mechanism potentially capable to induce dysfunction of complex I may be based on activation of calpains [53]. It has been shown that calpain 10 can translocate into the intermembrane space and the matrix where it cleaves several components of complex I. As this process is activated by increased Ca2+ in mitochondria, it may play a significant role in conditions of induction of apoptosis and, therefore, to underlie the apoptotic loss of gastric cells in the context of our study. Although not addressed in our studies, all these mechanisms described can well underlie the decreased respiration with glutamate/malate observed in gastric mucosa of our patients.

An interesting finding was that the succinate-dependent OXPHOS was better preserved than complex I-dependent process in stomach mucosa of patients with gastric cancer. This is evident from the observation that state 3 respiration during succinate oxidation decreased to the lesser extent than during the use of glutamate/malate in cancer tissue as compared to control (Table 1). As an outcome, RCI succ increased in cancerous mucosa, whereas the RCI Glut did not change, this discrepancy indicating better conversion of oxidative energy released from succinate into ATP. In fact, the RCI succ values reached those registered in MKN45 and MKN28 cells (Fig. 3c). To interpret these data, it is remarkable that ras-dependent transformation leads to up-regulation of the activities and components of SDH [9, 46, 48]. The complex II has been considered as a pH sensor for apoptosis and several of its components appear to serve as tumor suppressors, and, vice versa, mutations of complex II components are associated with tumorigenesis [45]. Thus, alterations in the balance between the functions of complex I and complex II of the mitochondrial respiratory chain in cancer cells may lead to important pathophysiological and clinical consequences. For example, enhanced activity of complex II may support manifestation of cancerous phenotype of the cell by increasing its resistance to hypoxia through preventing loss of membrane potential in hypoxic cells [22]. It has been proposed that whereas normally the complex II operates as a succinate-ubiquinone reductase, in hypoxic conditions it operates in a reverse direction, i.e., as a NADH-fumarate reductase system [12]. This mechanism enables generation of mitochondrial membrane potential and thus drives ATP synthesis in the absence of oxygen, thereby supporting the survival of cancer cells in conditions when the oxygen availability is limited [12]. The observation that the vitamin E analogs such as α-tocopherol succinate that inhibit SDH can induce apoptosis selectively in cancer cells, via acting mainly through rapid generation of ROS (reviewed by [54]), support the suggestion that the cancer cell’s life strongly relies upon complex II function.

In conclusion, gastric cancer is associated by decreased respiratory capacity and relative deficiency of the respiratory complex I but improved coupling of succinate oxidation to ATP generation in mitochondria of the tissues of gastric adenocarcinoma and adjacent atrophic mucosa. Quantitative assessment of these changes by oxygraphy of endobiopsy samples may be of diagnostic importance to detect cancer development in human gastric mucosa.
Acknowledgments This study was supported by Grant no. 7117 of the Estonian Science Foundation and Grant 018254963 of the Estonian Ministry of Education and Research. The authors thank Mrs Ellen Gvozdkova for technical assistance. A.H. is K. Albin Johansson Research Professor of the Foundation for the Finnish Cancer Institute.

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