Differential expression of mitochondrial energy metabolism profiles across the metaplasia-dysplasia-adenocarcinoma disease sequence in Barrett’s oesophagus

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ABSTRACT

Contemporary clinical management of Barrett’s oesophagus has highlighted the lack of accurate predictive markers of disease progression to oesophageal cancer. This study aims to examine alterations in mitochondrial energy metabolism profiles across the entire disease progression sequence in Barrett’s oesophagus. An in-vitro model was used to screen 84 genes associated with mitochondrial energy metabolism. Three energy metabolism genes (ATP12A, COX4I2, COX8C) were significantly altered across the in-vitro Barrett’s disease sequence. In-vivo validations across the Barrett’s sequence demonstrated differential expression of these genes. Tissue microarrays demonstrated significant alterations in both epithelial and stromal oxidative phosphorylation (ATP5B and Hsp60) and glycolytic (PKM2 and GAPDH) protein markers across the in-vivo Barrett’s sequence. Levels of ATP5B in sequential follow up surveillance biopsy material segregated Barrett’s non-progressors and progressors to HGC and cancer. Utilising the Seahorse XF24 flux analyser, in-vitro Barrett’s and adenocarcinoma cells exhibited altered levels of various oxidative parameters. We show for the first time that mitochondrial energy metabolism is differentially altered across the metaplasia-dysplasia-adenocarcinoma sequence and that oxidative phosphorylation profiles have predictive value in segregating Barrett’s non-progressors and progressors to adenocarcinoma.

Introduction

Barrett’s oesophagus, defined pathologically as specialised intestinal metaplasia of the distal oesophagus, is a common premalignant lesion in the Western world [1]. Barrett’s oesophagus arises from chronic gastrooesophageal reflux disease (GORD) of acid and bile, and can progress to oesophageal adenocarcinoma (OAC) through low-grade dysplasia (LGD) and high-grade dysplasia (HGD) [2,3]. The measured annual risk of OAC in individuals with Barrett’s metaplasia varies worldwide and is currently approximately 0.12% [2]. Barrett’s oesophagus lacks any proven therapeutic strategy and despite numerous multi-modality therapies, the prognosis for individuals with OAC still remains modest, with a commonly quoted 9–15% survival rate [4]. Some cancer centres, however, report survival in patients treated with curative intent to be approximately between 35% and 50% [5]. In addition, contemporary clinical management of Barrett’s oesophagus through surveillance programs has highlighted the lack of accurate predictors of disease progression.

First reported by Otto Warburg in 1926, and thus coined the Warburg effect, cancer cells produce the majority of their ATP through aerobic glycolysis to support the extensive transformation, differentiation and aggressive proliferation of malignant cells [6]. These transformed cells convert the majority of incoming glucose to lactate rather than metabolising it through oxidative phosphorylation. Furthermore, despite aerobic glycolysis being more rapid at ATP production, it is far less efficient in terms of adenosine triphosphate (ATP) produced per molecule of glucose and therefore glucose uptake demands can function at abnormally high rates [6].

Studies investigating cancer metabolism have documented metabolic shifts from oxidative phosphorylation to aerobic glycolysis to generate energy. In human breast, gastric, squamous oesophageal and lung carcinomas, expression of mitochondrial and glycolytic protein markers varied significantly in carcinomas when
compared with paired normal tissues [7]. In the colonic mucosa of ulcerative colitis patients, mitochondrial complex activity is decreased between 50% and 60% compared with healthy controls [8]. This profile has also been documented in human kidney, liver and colonic carcinomas in tandem with increases in key glycolytic enzymes [9]. No study to date has investigated differential gene and protein expression associated with mitochondrial energy metabolism in Barrett’s patients in such a sequential, retrospective and longitudinal manner.

The focus on the mitochondria as primary instigators of tumour progression is of great interest. It is speculated that the metabolic shift occurs as an adaptation to defects in oxidative phosphorylation in the mitochondria since mitochondria lack DNA repair enzymes and are adjacent to cancer-causing free radicals [10]. The production of lactate during glycolysis may also facilitate tumour invasion and metastasis [11]. Other studies report that mitochondrial function is crucial for transformation in some tumour progression systems [12]. Moreover, a recent study has found that the fidelity of the mitochondrial genome is increased in human colorectal cancer in conjunction with a shift in glucose metabolism from oxidative phosphorylation to glycolysis [13].

Despite innovative strategies in tumour detection and monitoring, for example, in fluorescent-glucose positive emission tomography imaging and glycolytic pathway inhibitors, a thorough understanding of the molecular mechanisms mediating tumour progression, particularly in Barrett’s oesophagus and its progression to oesophageal adenocarcinoma, is warranted [14]. Accordingly, more accurate predictors of disease progression should be validated, thus allowing early preneoplastic detection of such molecular mechanisms offering a greater insight into the risk of disease progression.

Therefore, the aim of this study was to examine mitochondrial energy metabolism gene and protein changes across the disease sequence in-vitro and in-vivo to assess glucose metabolism across the disease sequence utilising two oxidative phosphorylation and two glycolytic protein markers. We have shown that genes associated with mitochondrial energy metabolism are differentially expressed across the metabolism–dysplasia–oesophageal adenocarcinoma sequence both in-vitro and in-vivo. Furthermore, the Barrett’s disease sequence exhibits significant alterations in the expression of oxidative phosphorylation and glycolytic proteins. Interestingly, levels of the oxidative phosphorylation protein ATP5B is sequentially downregulated with progression to cancer.

**Material and methods**

Mitochondrial dysplastic and adenocarcinogenic cell line models

QH (Barrett’s), GO (dysplasia) and OE33 (oesophageal adenocarcinoma) cell lines, representing stages of the Barrett’s disease sequence, were grown to 70% confluence in RPMI medium (OE33 in RPMI, 2 mM glucose; QH in RPMI, 2 mM glucose, 100 mM FBS, 1% penicillin-streptomycin-l-ascorbate; supplemented with B27 SingleQuots (2 mL B27, 0.5 mL insulin, 0.5 mL HCl, 0.5 mL Ca-1000, 0.5 mL retinoic acid, 0.5 mL transferrin, 0.5 mL triiodothyronine, 0.5 mL adiponectin and 0.5 mL MGF per 500 mL media). QH and GO cell lines were obtained from the American Type Culture Collection (ATCC) (LGC, Standards, Middlesex, UK). The OE33 cell line was sourced from the European Collection of Cell Cultures (Salisbury, UK). Cell RNA extractions were subsequently performed using RNeasy Mini Kit (Qiagen) following manufacturer’s instructions. RNA quality and quantity was quantified and assessed respectively and RNA reverse transcribed using the Kapa RT-PCR array first strand kit (Kapa Biosciences).

Screening of RT-PCR microrna analysis

A catalogue of human mitochondrial energy metabolism PCR gene microarray (Qiagen) was used to simultaneously quantify the expression of 84 mitochondrial genes across the in-vitro sequence using GeneChip as the endogenous control. PCR was performed using the Kapa Realtime Sybr Green PCR Master Mix (Kapa Biosciences) following manufacturer’s instructions on a 7900HT Fast Real-Time PCR Light-Cycler System (Applied Biosystems). Data were analysed utilising the 2-Δ ΔCt method. First threshold cycle (Ct) values were converted to 2 in order to be proportional to the amount of transcripts in all samples. Next, 2-ΔΔCt values were calculated by normalising the data to a housekeeping gene, beta-2-microglobulin (B2M), as follows: 2-ΔΔCt = 2-ΔCt (sample) / 2-ΔCt (B2M). In order to compare the data between cell line models, 2-ΔΔCt values were calculated by normalising the experimental data by reference data. For example, data from the calibrator QH cell line was normalised to the GO cell line as follows: 2-ΔΔCt = 2-ΔCt (QH) / 2-ΔCt (GO). Differentially expressed genes were defined as those that changed by ≥4-fold.

**In vitro validation of gene targets**

Cell lines were cultured and RNA extracted as described above. RNA was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad). Gene primer probes for COX1, COX2, ATP7A and IRS (Applied Biosystems) were purchased and realtime PCR was performed using human mammarian ABI7500 (Applied Biosystems). Data were analysed utilising the 2-ΔΔCt method.

**In vivo validation of gene targets**

The expression of these three genes was validated in adjacent normal mucosa and tumours across the Barrett’s sequence. Ethical approval to conduct all aspects of this work was granted by the Medical and Health Research Ethics Committee in Ireland (University College Dublin) and all patients attending the clinic were informed (written consent was obtained) of the study. Institutional review boards were obtained from all locations in which the study was conducted. Patients involved in the study were at low risk for colorectal cancer.

**Results**

Expression levels of COX1, COX2 and IRS were investigated in tissue microarrays (TMAs) of Barrett’s oesophagus using a panel of antibodies (Abcam; Cambridge UK). Immunohistochemistry was performed using the Vectastain AEC Kit (Vectorlabs, Burlingame, CA) as per manufacturer’s instructions. All sections were counterstained with Mayer’s haematoxylin. Immunohistochemical images were acquired on a Nikon Eclipse E800 microscope. Between 200 and 500 high power fields were scored.

**Conclusion**

This study has demonstrated the potential of using mitochondrial gene expression analysis as a diagnostic tool for Barrett’s oesophagus. The next step is to validate this approach in a larger cohort of Barrett’s patients.
Characterising the metabolic plasticity of Barrett's and adenocarcinoma cell lines utilising the Seahorse XF24 analyser

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), reflecting oxidative phosphorylation and glycolysis respectively, were measured before and subsequent to treatment with oligomycin (2 μg/mL, Seahorse Biosciences), dichloro-dicyclohexyl-carbodiimide (DCCD) (5 μM, Seahorse Biosciences) and antimycin A (A2,6-M, Seahorse Biosciences) using the Seahorse XFe24 analyser (Seahorse Biosciences). QH and O3E3 cells were seeded at 20,000 cells per well in a 24-well cell culture XFplate (Seahorse Biosciences) and allowed to adhere for 24 hours. Cells were rinsed with assay medium (subtracted DMEM supplemented with 10 mM glucose and 20 mM sodium pyruvate, pH 7.4) before incubation with assay medium for 1 hour at 37 °C in a non-CO₂ incubator. Four baseline OCR and ECAR measurements were obtained over 2 minutes before injection of specific metabolic inhibitors. Three OCR and ECAR measurements were obtained over 1.5 minutes following injection of oligomycin, FCCP and antimycin A. Percentage ATP synthesis was calculated by subtracting the OCR post oligomycin injection from baseline OCR prior to oligomycin addition and expressing residual OCR as a percentage of baseline OCR. Proton leak was calculated by subtracting percentage OCR versus baseline post antimycin A addition from percentage ATP synthesis. The experiment was repeated five times (n = 5) with adequate technical replicates. All measurements were normalised to cell number using the crystal violet assay.

Statistical analysis

Data were analysed using GraphPad Prism (GraphPad Software Inc, San Diego, CA, USA) and SPSS [MSW] (Predictive Analytics Software, version 13) [IBM, Armonk, New York, USA] software. qRT-PCR data were normalised using the ΔΔCt method and statistically analysed (GraphPad Prism) in a matched pair test. The significance of expression differences was determined using Student’s t-test when comparing Seahorse metabolites of two groups of normally distributed independent groups. Differences of P < 0.05 (*), P < 0.01 (**), and P < 0.001 (***), were considered statistically significant.

Results

In-vitro screening using human ICR gene microarrays

To analyse expression of human 84 mitochondrial energy metabolism genes across the disease sequence in-vitro, fold expression of all target genes was normalised relative to the Barrett's metaplastic cell line model, QH. The cut-off for differential gene expression was defined by either a relative fold change or p-value. Three of the 84 mitochondrial energy metabolism genes targets (ATPI2A, COX412 and COX6C) were differentially expressed across the metaplastic-dysplastic-OAC cell line sequence. These three gene targets were subsequently validated in-vitro and in-vivo using patient samples. Figure 1 shows the in-vitro PCR gene microarray screen between the Barrett's dysplastic and OAC cells and the subsequent in-vitro validation of the three mitochondrial energy metabolism gene targets. Using a fourfold cut-off, six gene targets were found to be differentially expressed between QH and OAC cells (Fig. 1A). Using a fourfold cut-off, two gene targets were found to be differentially expressed between QH and O3E3 cells (Fig. 1B). Using a fourfold cut-off, six gene targets were found to be differentially expressed between the QH and OCE3 cells (Fig. 1C). Table 1 summarises the relative expression of all 84 genes screened between QH and O3E3 cell lines (see Supplementary material Table S1 for differences in expression). ATPI2A (P < 0.05) expression significantly increased across the Barrett’s sequence (Fig. 1D). COX412 expression decreased; however, this was not statistically significant (Fig. 1E). COX6C (P < 0.05) expression significantly increased across Barrett’s and dysplastic cell lines (P < 0.05) but subsequently decreased between dysplastic and OAC cell lines; however, this was not statistically significant (Fig. 1F).

In-vivo validation of gene targets

Figure 2 illustrates mitochondrial energy metabolism gene expression of the three gene targets across the disease sequence in diseased and matched normal adjacent tissue samples. ATPI2A (Fig. 2A) (P < 0.001), COX412 (Fig. 2C) (P < 0.01) and COX6C (Fig. 2E) (P < 0.05) were found to be differentially expressed across the Barrett’s sequence. Field effect changes in gene expression of these targets in diseased versus matched normal adjacent biopsies were examined. ATPI2A (Fig. 2B) (P < 0.001), COX412 (Fig. 2D) (P < 0.01) and COX6C (Fig. 2F) (P < 0.01) were found to be differentially expressed across the Barrett’s disease sequence suggesting this effect was specific to the pathological disease tissue (Barrett’s, LGD, HGD and OAC), compared with the matched surrounding normal mucosa.

Oxidative phosphorylation and glycolytic activity across Barrett’s sequence

To assess oxidative phosphorylation at the protein level, levels of ATP5B and HSP60 were assessed as no reliable antibodies to ATP7A, COX412 and COX6C have yet been developed. Even though ATPI7A is a direct component of the electron transport chain and thus an effective determinant of oxidative phosphorylation, HSP60 is increasingly being used as a surrogate marker for mitochondrial function and for the assessment of oxidative mitochondrial metabolism due to its role as an active mitochondrial chaperone. Figure 3 shows immunohistochemical expression of these oxidative phosphorylation protein biomarkers across the metaplasia-dysplasia-OAC disease sequence. Figure 3A and 3B shows representative images of ATP5B expression in oesophagitis and OAC tissue respectively. Figure 3C and 3D shows representative images of HSP60 expression in oesophagitis and HGD tissue respectively. Epithelial ATP5B positivity across the Barrett’s disease sequence was increased significantly (Fig. 3E) (P = 0.0003). Moreover, epithelial HSP60 positivity

Table 1

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** Relative expression, e.g. expression of ATPI4A (6.32 times greater in O3E3 than QH cells).
across the Barrett’s disease sequence was increased significantly (Fig. 3F) \( P < 0.0001 \). Interestingly, no significant changes in the levels of both oxidative phosphorylation markers ATP5B and HSP60 were detected in stromal tissue across the Barrett’s sequence. Moreover, Figure 3G and 3H respectively shows that epithelial expression levels of ATP5B \( P < 0.0001 \) and HSP60 \( P < 0.05 \) were significantly altered across the Barrett’s disease sequence in matched normal adjacent.

Figure 4 illustrates epithelial and stromal tissue expression of the glycolytic protein markers, FKN2 and GAPDH, across the metaplastic–dysplastic–OAC disease sequence. Figure 4A and 4B shows representative images of epithelial FKN2 expression in oesophagitis and HCD tissue respectively. Figure 4C and 4D shows representative images of epithelial GAPDH expression in Barrett’s and OAC tissue respectively. Epithelial FKN2 positivity across the Barrett’s disease sequence was shown to increase significantly (Fig. 4E) \( P < 0.0003 \). Epithelial GAPDH positivity also increased significantly across the Barrett’s disease sequence (Fig. 4F) \( P < 0.0002 \). In contrast to the markers of oxidative phosphorylation in the stromal compartment, stromal tissue expression of the glycolytic protein
markers. PKM2 (Fig. 4G) (P = 0.03) and GAPDH (Fig. 4H) (P = 0.0007), decreased significantly across the metaplastic-dysplastic-OAC disease sequence.

In addition to analysing the expression of the protein markers ATP5B and HSP60 in independent groups of tissue across the Barrett's sequence, we assessed their expression in sequential longitudinal material from Barrett's non-progressors and progressors to investigate a potential predictive biomarker of OAC. Focusing on patients with a primary diagnosis of intestinal metaplasia on first surveillance endoscopy, Barrett's non-progressors (n = 15) and progressors (n = 11) were separated with the primary end-point being progression to OAC. The median age of patients with intestinal metaplasia was 58 years and there was a 3.3-fold male predominance. There was no significant difference in age between progressors and non-progressors (P = 0.6404). Median time of progression to cancer was 2.6 years. TNM staging for progressors was as follows: 20% T1 N0 M0, 20% T1 N1 M0, 20% T2 N0 M0, 10% T2 N1 M0, 10% T3 N0 M0, and 10% T3 N1 M0. The non-progressor group was followed for a median of 5.4 years and had no evidence of conversion to HGD and/or OAC. Figure 5 illustrates the longitudinal tissue microarray expression of the metabolic biomarkers ATP5B, HSP60, PKM2 and GAPDH between Barrett's patients who did and did not progress using initial first-time surveillance biopsies from these patients. Interestingly, there was a significant increase in mean
percentage positivity of stromal cytoplasmic ATP5B in Barrett’s patients who prospectively progressed to OAC (Fig. 5A) (P < 0.01). This predictive protein was specific to ATP5B and not the other metabolomic markers (Fig. 5B-D).

Characterisation of oxidative metabolic plasticity in the in-vitro Barrett’s sequence

As ATP5B, the marker of oxidative phosphorylation, was predictive in segregating Barrett’s non-progressors and progressors, we examined how metaplastic and OAC cells would behave when challenged with mitochondrial inhibitors known to alter metabolic reprogramming. Figure 6 illustrates different metabolic parameters examined subsequent to challenging QH and OE33 cell lines with oligomycin, FCCP and antimycin-A using the Seahorse XF24 flux analyser. Figure 5A-F illustrates relative mitochondrial respiration in QH and OE33s cells, ATP synthesis, maximal respiratory capacity, non electron transport chain respiration and proton leak between the QH and OE33 cell lines, respectively. Approximately 80% of total OCR accounted for mitochondrial respiration in the QH cell line (Fig. 6A) and 64.7% of total OCR accounted for mitochondrial respiration in the OE33 cell line (Fig. 6B). Levels of oxygen consumed by the electron transport chain in both cell lines was significantly higher versus oxygen consumed for non electron transport chain purposes (Fig. 6A–B) (P < 0.001). QH cells (63%) exhibited higher levels of ATP synthesis compared with the OE33 (50.6%) cells (Fig. 6C) (P < 0.05). Upon mitochondrial uncoupling with FCCP, OE33 cells exhibited a significantly greater spare respiratory capacity of 33% versus QH cells (Fig. 6D) (P < 0.01). OE33 cells demonstrated 15.2% higher levels of OCR, attributed to non electron transport chain processes, compared with QH cells (Fig. 6E) (P < 0.05).
Fig.4. Epithelial and stromal immunohistochemical tissue expression of the glycolytic protein markers, PKM2 (A, B, E and G) and GAPDH (C, D, F, H), across the metaplasia-dysplasia—adenocarcinoma disease sequence. (A) Tissue section from an esophagectomy patient negative for levels of epithelial PKM2 staining. (B) Tissue section from a HGD patient positive for levels of PKM2 staining in epithelium. (C) Tissue section from a Barrett’s oesophagus patient exhibiting minimal baseline levels of epithelial GAPDH staining. (D) Tissue section from a OAC patient exhibiting strong positive levels of GAPDH staining in epithelium. (E) Epithelial PKM2 positivity across the Barrett’s disease sequence was shown to increase significantly (P < 0.01, Mann–Whitney U) (ANOVA; P = 0.0003). (F) Epithelial GAPDH positivity increased significantly across the Barrett’s disease sequence (P < 0.01, Mann–Whitney U) (ANOVA; P = 0.0002). (G) Stromal PKM2 positivity across the Barrett’s disease sequence was shown to decrease significantly (P = 0.01, Mann–Whitney U) (ANOVA; P = 0.0007). (H) Stromal GAPDH positivity decreased significantly across the Barrett’s disease sequence (P < 0.01, Mann–Whitney U) (ANOVA; P = 0.0007).

Discussion

Understanding the underlying molecular mechanisms that support the progression of Barrett’s oesophagus to cancer would significantly affect the clinical management of these patients. We have shown for the first time that mitochondrial energy metabolism is altered across the normal—metaplasia—dysplasia—OAC sequence of events in Barrett’s oesophagus and that early changes in metabolic alterations, specifically oxidative phosphorylation, are associated with an increased risk of disease progression from Barrett’s metaplasia to OAC.

In this study, a human PCR gene microarray identified three genes associated with mitochondrial energy metabolism differentially expressed between Barrett’s and OAC cells. Few studies have associated COX412, COX8C, and ATP7A with cancer progression and their role in energy metabolism. We have shown a significant increase in COX8C expression in Barrett’s patients compared with normal squamous tissues. Interestingly, the increase in COX8C expression in Barrett’s tissue was subsequently followed by a significant decrease in COX8C expression in LGD and HGD/cancer tissue. This increase in COX8C expression was specific to Barrett’s tissue compared with matched surrounding mucosa. No studies to date have reported a role for
In tumourigenesis, therefore, we hypothesise that 

**COX8C** may play an important role in Barrett’s patients by increasing basal oxidative phosphorylation levels, altering energy metabolism and subsequently promoting neoplastic progression.

We have also shown in-vivo a significant increase in COX412 expression across the Barrett’s sequence. COX8C and COX412 pertain to the one protein complex and both have different expression patterns - COX8C increases and subsequently decreases while COX412 increases and maintains its expression pattern. Interestingly, both complex IV genes are upregulated despite a significant downregulation in a subunit of their downstream associate complex, ATP synthase. This downregulation of ATP12A was also shown to be specific to Barrett’s tissue compared with the matched surrounding mucosa.

COX412 and ATP12A have been ascribed to few pathologies: A single base mutation in the ATP12A subunit results in complex V deficiency [15]. Immunological ATP12A expression in normal and benign prostate hyperplasia and cancerous prostate tissue have been shown to be altered in luminal cells of the glandular epithelium [16]. More recently, in a study proposing a protective role for decreased ATP levels analysing the function of lung-specific COX412 in-vitro and in COX412 knockout mice in-vivo, it was found that cytochrome c oxidase activity and ATP levels were significantly reduced in knockout mice [17]. In addition, decreased oxidative phosphorylation in cancer development is commonly associated with a parallel increase in glycolysis [18-22]. This decrease in oxidative phosphorylation is frequently linked with defects in complex I and III, for example, in renal, leukaemia and fibroblast cell lines [18,20,22]. One possible explanation for the dysregulation of these three genes is in the microenvironment they reside. Mitochondrial DNA is highly prone to oxidative damage as it is situated on the inner mitochondrial membrane [23]. Moreover, it is in close proximity to the electron transport chain and the levels of oxidised bases are estimated to be two to three times greater than nuclear DNA [23]. The increased oxidative microenvironment demonstrated in this study may strengthen this hypothesis in Barrett’s oesophagus.

Next, we investigated the expression of different oxidative phosphorylation and glycolytic proteins across the Barrett’s sequence and investigated if a metabolic marker could segregate Barrett’s non-progressors and progressors to cancer using established markers of oxidative phosphorylation (ATP5B and HSP60) and glycolysis (PKM2 and GAPDH) [13,24,25].

We have shown that both epithelial ATP5B and HSP60 positivity significantly increased across the Barrett’s sequence, consistent with a metabolic shift in glucose metabolism to a more actively oxidative metabolic level. Moreover, the expression levels of ATP5B and HSP60 were significantly altered between Barrett’s disease tissue and the matched surrounding mucosa. This increase in oxidative phosphorylation is analogous to COX412 and COX8C discussed above.

One recent study showed a similar trend in glucose metabolism whereby human breast tumours demonstrated increased epithelial enzymatic activity in various complexes [25]. To recall, HSP60 positivity significantly increased across the Barrett’s sequence. HSP60 expression has been previously shown to be increased in various cancer types [27-29]. This increase in oxidative phosphorylation complex IV genes and associated proteins highlighted may be a com-
perspiratory mechanism to counteract the significant downregulation of complex IV’s main downstream protein complex, complex V, namely attributed to ATP12A as this loss can lead to mitochondrial dysfunction [15]. Therefore, we hypothesise that cancer cells may act as ‘metabolic parasites’, secreting hydrogen peroxide into the local microenvironment, inducing oxidative stress in normal host cells resulting in autophagy, mitophagy and aerobic glycolysis. As a result, high-energy glycolytic nutrients such as ketones, L-lactate and glutamine may fuel the anabolic growth of tumour cells through oxidative phosphorylation.

The epithelial expression of both glycolytic biomarkers, PKM2 and GAPDH, were significantly increased across the Barrett’s sequence and concur with an elevation in epithelial glycolysis, a common entity shown in other cancers [15,20,31]. PKM2 is more abundant during aerobic glycolysis in many tumour types, including the Barrett’s sequence in non-sequential tissue [32]. Interestingly, in contrast to the increased epithelial expression of both glycolytic markers, the expression of these markers is significantly decreased in matched stromal tissue. However, the proliferation status of the epithelium may in part exacerbate the degree of aerobic glycolysis. Interestingly, our longitudinal analysis demonstrates that Barrett’s patients with increased levels of the oxidative phosphorylation marker ATP5B are more likely to progress to OAC. This novel finding indicates a crucial and pivotal role for oxidative phosphorylation in OAC progression in the Barrett’s disease sequence. Increased levels of ATP5B, and thus oxidative phosphorylation, are fueled by the greater availability of high-energy nutrients through increased glycolysis. As a result, the subsequent oxidative state may
make local tissue more amenable to the anabolic growth of tumour cells, tumour differentiation and progression. Subsequent validation of ATP5B in different patient cohorts from different clinical institutions would potentially strengthen the applicability of this marker in the clinical setting as this assessment could be undertaken efficiently on formalin fixed paraffin embedded tissue. It is evident from this study that oxidative phosphorylation at both the gene and protein level play a vital role in exacerbating disease progression in Barrett’s oesophagus. It was necessary, therefore, to decipher elements of oxidative phosphorylation, specifically the complexes of the electron transport chain that play an important role. Using the Seahorse technology, we challenged the mitochondria to gain insight into the oxidative capacities in both cell types as this could not be performed in vivo. We investigated basal oxidative phosphorylation, basal glycolysis, mitochondrial respiration, ATP synthesis, spare respiratory capacity, non electron transport chain respiration and proton leak in both QH and OEC3 cell lines. We demonstrate that metaplastic cells are more energy demanding compared with oesophageal cancer cells suggesting an early metabolic advantage for differentiation. Metastatic transformation and subsequent proliferation due to an increased capacity to generate ATP for anabolic purposes. When oxidative capacity of the QH and OEC3 cells was challenged, mitochondrial respiration differed between the two cell types and the degree of ATP synthesis and proton leak attributed to mitochondrial respiration was also substantially different. The OEC3 cell line maintains an equilibrium between both metabolic pathways thereby demonstrating metabolic plasticity while the QH cell line favours a more detrimental oxidative phenotype that may be selected during early stages of disease progression. Overall, we have shown in this study that metabolic reprogramming is active during disease progression in Barrett’s and specifically, markers of oxidative phosphorylation can segregate Barrett’s non-progressors from progressors to cancer. This needs to be further examined using different patient cohorts from multicentres to establish clinical utility.

Conflict of Interest

The authors declare no conflicts of interest.

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Appendix: Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.canlet.2014.07.035.

References