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MINI-REVIEW

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Role of Mitochondrial Markers in Improved Detection and Risk-Stratification in Barrett's Esophagus Patients

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Barrett's esophagus (BE†) is the only known precursor of esophageal adenocarcinoma (EAC) and is amenable to treatment. However, more than 90 percent of EAC patients are never diagnosed with antecedent BE. Identification of molecular markers for BE is needed to improve detection of BE through efficient non-endoscopic methods that are cost-effective, sensitive and can be used to cater to a larger group of the population at risk. Alterations in mitochondria and mitochondrial DNA have been shown to be associated with various cancers, including esophageal cancer. Mitochondrial response to oxidative stress, alterations in mitochondrial metabolism, changes in mitochondrial membrane potential and mitochondrial genetic mutations have been found to be associated with BE pathogenesis. This mini-review focuses on the role of mitochondria in the pathogenesis of BE and EAC and the prospects of using that knowledge to develop effective strategies for the improved detection and risk-stratification in BE patients.

INTRODUCTION

The global incidence rate of esophageal carcinoma (EAC) is lower than that of squamous cell carcinoma of the esophagus (0.7 vs 5.2 per 100,000), however, EAC is the most common histologic type in the western world (46 percent of global EAC cases) [1]. Survival is poor in patients with EAC (9 to 15 percent at 5 years) as a significant proportion of them (40 percent) are diagnosed after the disease has metastasized in the body [2,3]. Esophageal cells are known to progress to cancer in a metaplasia-dysplasia-adenocarcinoma sequence (Figure 1). Barrett's esophagus is a pre-malignant condition in which normal squamous mucosa of the distal tubular esophagus is replaced by intestinal mucosa (metaplasia) in the setting of chronic gastroesophageal reflux disease (GERD) [4]. BE is the only known precursor of EAC and is amenable to treatment. However, more than 90 percent of EAC patients are never diagnosed with antecedent BE because currently, the only standard diagnostic modality available is esophagogastroduodenoscopy (EGD), which is a costly invasive procedure and is only recommended for patients

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†Abbreviations: BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; GERD, gastroesophageal reflux disease; EGD, esophagogastroduodenoscopy; mtDNA, Mitochondrial DNA; ROS, reactive oxygen species.

Keywords: Mitochondria, Barrett's Esophagus, Esophageal Adenocarcinoma

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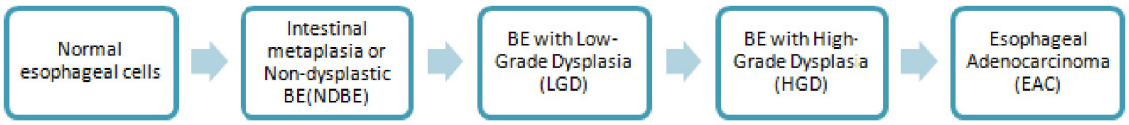

Figure 1. Meta-Dysplasia-Cancer Sequence in the Pathogenesis of Barrett's Esophagus.

with symptomatic GERD having multiple risk factors for BE [5-7]. This strategy causes the exclusion of a majority of patients who go on to develop EAC without any preceding symptoms. Moreover, there is a risk of sampling error associated with EGD and the current standards for histopathological diagnosis of dysplasia are subject to inter-observer variability [8,9]. We believe that identification of molecular markers for BE is needed to improve detection of BE through efficient non-endoscopic methods that are cost-effective, sensitive, and can be used to cater to a larger group of the population at risk [10]. A better understanding of the molecular pathogenesis of BE is vital for developing a model for risk-stratification in these patients. In this mini-review, we discuss the role of mitochondria in the pathogenesis of BE and EAC and the prospects of using that knowledge to develop efficient strategies for the improved detection and risk-stratification in BE patients.

WHY MITOCHONDRIA?

The mitochondrion is an important micro organelle in the cell, which not only plays an important role in cellular metabolism but is also essential for cellular growth and differentiation. Due to its role in ATP synthesis, it is rightly referred to as the "powerhouse of the cell" [11]. Mitochondrial genes are both nuclearly and mitochondrially encoded. Mitochondrial DNA (mtDNA) is maternally inherited and each mitochondrion contains multiple (2 to 10) copies of the same. It encodes 12S and 16S rRNAs, 22 tRNAs, and 13 polypeptides [12]. Overall, mitochondria are the metabolic seat of the cell, carrying out important functions such as energy production, synthesis of phospholipids and heme, calcium signaling, activation of apoptosis, and cell death [13]. Due to the central role played by them in several vital cellular functions, the potential role of mitochondria in carcinogenesis has intrigued scientists of the past few generations.

ATP synthesis leads to the mitochondria being exposed to reactive oxygen species (ROS) which are known to play a role in inducing genetic mutations. NADPH oxidase 1, which is the major source of ROS in the cell and is located inside the mitochondria, is found to be increased in several cancers like breast and ovarian tumors [14]. Apart from the exposure to ROS, the contribution of mitochondria in cellular energy metabolism, aging, and initiation of apoptosis have been linked to the role played by them in tumorigenesis. Close vicinity to ROS and lack of histone proteins put mtDNA at risk of oxidative DNA damage. So much so that, the mutation rate of mtDNA is almost 10 times higher than that of nuclear DNA [15]. An inefficient mtDNA repair system also greatly increases the chances of mitochondria harboring mutations, some of which have been shown to drive carcinogenesis [16]. Alterations in mitochondria and mtDNA have been shown to be associated with various cancers, including esophageal cancer [17]. It has also been postulated that mtDNA content may potentially be used as a biomarker to predict survival and response to chemotherapy in some cancer patients. [18,19]. Although it has been suggested that mtDNA mutations may be identified in the pre-malignant stage [20], the role of mtDNA in pre-malignant conditions like BE is only beginning to be understood better.

Despite the tremendous growth that cellular and molecular biology has witnessed, our knowledge of mitochondrial genetics and biology continues to be very limited [21]. A more elaborate understanding of the role played by mitochondria in the disease progression of premalignant conditions like BE might lead to the identification of molecular targets of diagnostic and prognostic importance, ultimately reducing overall cancer burden [22].

MITOCHONDRIAL OXIDATIVE STRESS AND ITS ROLE IN BE

Reactive oxygen species that are commonly released in inflammatory tissue are known to play a vital role in carcinogenesis. Oxidative stress is also an important component of the pathogenesis of BE [23]. Gastric refluxate has long been known to expose the normal esophageal mucosa to ROS and possibly promote the progression of BE to EAC [24]. However, the molecular basis of the same was not established until the study by Lee *et al.* demonstrated the role of mitochondria in ROS-mediated disease progression and tumorigenesis in BE [23]. They found that ROS level in Barrett's tissue was significantly higher than surrounding normal tissue. In their study, Li *et al.* showed that BE cells have an altered response to

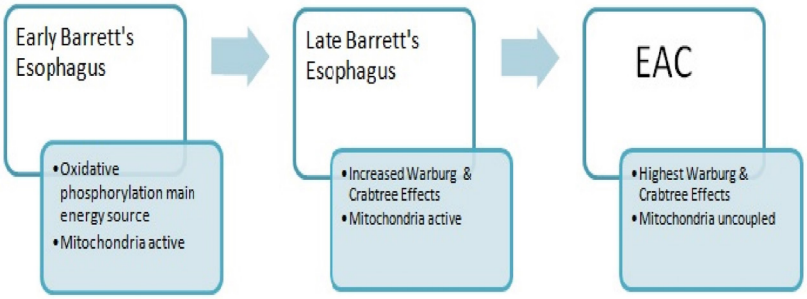

Figure 2. Changes in Mitochondrial Metabolism over the Metaplasia-Dysplasia-Cancer Sequence of Barrett's Esophagus. EAC: esophageal adenocarcinoma. Source: Suchorolski *et al*. [21].

bile-salt induced oxidative stress. They determined that oxidative stress levied upon normal esophageal mucosa by bile exposure leads to increased MnSOD (manganese-dependent superoxide dismutase) expression. They also highlighted the need for further investigation to study the correlation between MnSOD-mediated cellular signaling, oxidative stress induced by bile exposure and progression of BE to EAC [25]. O'Farrell *et al.* showed that oxidative stress and mitochondrial instability were vital for building up an environment that is necessary for the development of intestinal metaplasia. They indicated that these are essentially early changes in the metaplasia-dysplasia-cancer sequence and pre-neoplastic tissue (BE) may be more susceptible to oxidative damage than EAC [26]. Overall, these studies highlight the fact that mitochondria are the seat of pathological changes induced by oxidative stress in Barrett's tissue which ultimately leads to the progression of disease and development of esophageal adenocarcinoma. More translational research projects need to be carried out in order to study the potential role of anti-oxidants in influencing the mitochondrial response to ROS in BE and investigate their effect on pathogenesis and overall disease progression.

MITOCHONDRIAL BIOENERGETICS AND BE

Cancer cells are known to undergo metabolic alteration in order to promote and maintain their growth, survival, and proliferation. In the 1920s, Otto Warburg stated that even in the presence of oxygen and fully functioning mitochondria, tumor cells undergo increased uptake of glucose which they ferment to produce lactate, a process known as aerobic glycolysis. This phenomenon came to be known as the Warburg effect. Later in 1956, Warburg stated that dysfunctional mitochondria were the fundamental cause of aerobic glycolysis in cancer cells. Although this claim has been refuted in several cancers, mitochondria are known to play a crucial role in supporting the survival of cancer cells by undergoing modifications in metabolic and apoptotic functions [27-29]. Herbert Crabtree confirmed the presence of aerobic glycolysis in tumor cells but pointed out that genetic and environmental factors could lead to a significant amount of heterogeneity of glycolysis in different tumor types. In other words, changes in environmental and genetic factors may cause variations in the fermentation of glucose in cancer cells [30].

The role of these metabolic effects observed in malignant cells remains poorly studied in premalignant conditions such as BE. Suchorolski *et al.*, through their study, proposed a model of BE in which metabolism in early BE is largely dependent on oxidative phosphorylation but gradually tends to lean more towards glycolysis (Warburg effect) during disease progression towards EAC. They also proposed that mitochondria maintain their function until an intermediate stage on the metaplasia-dysplasia-cancer sequence is reached; beyond which mitochondria begin to lose function, eventually leading to uncoupling shutdown of oxidative phosphorylation. Increased Crabtree effect in advanced BE also provides a selective advantage to these cells by making them more resilient to changes in glucose and oxygen conditions in the esophagus (Figure 2). They also speculated that TP53 mutation(s) could be a cause of these metabolic alterations in the mitochondria and that the cells with the lowest levels of glycolysis did not eventually progress to cancer [29]. Another study conducted by Phelan *et al.* used a human PCR microarray to identify three genes associated with mitochondrial energy metabolism, namely *ATP12A*, *COX412*, and *COX8C*, which they ultimately found to be expressed differentially across Barrett's sequence. Their study also found that protein markers for glycolysis (PKM2, GAPDH) and oxidative phosphorylation (ATP5B, Hsp60) were significantly altered between BE and EAC cells. They showed that oxidative phosphorylation profiles could be used to segregate BE non-progressors from progressors [22]. This is a very valuable discovery as understanding variations and alterations in cellular metabolism would not only lead to a better under-

standing of the disease progression but also help identify disease patterns and/or types that are more aggressive or bear a relatively poorer prognosis. Polymorphisms in the mitochondrial oxidative phosphorylation chain genes have been shown to possess some prognostic value in colorectal cancer [31].

Overall, it is likely that metabolic changes involving the mitochondria not only offer a survival benefit to BE cells but also promote the progression of the disease from early BE to EAC. Further research on the identification of specific molecular targets that can be used to control and modify these metabolic changes either directly or indirectly could offer some benefit in understanding the pathophysiology of BE better and improve rates of detection and aid effective risk stratification in patients with BE.

MITOCHONDRIAL MEMBRANE POTENTIAL AND BE

An essential part of the process of storage of energy that is generated during oxidative phosphorylation is the mitochondrial membrane potential (MMP). The MMP serves as an intermediate form of energy storage in the cell and results from the redox transformations that are associated with the Krebs cycle. MMP also plays a key role in maintaining mitochondrial homeostasis and overall healthy function of the cell organelle [32]. In their study, Wang *et al.* indicated that alterations in mitochondrial function, especially MMP, are critical in the development of BE. They found that dysplastic cells maintain a higher MMP than metaplastic cells which makes them more energy efficient and resilient to conditions of low oxygen availability, thereby promoting survival. These changes in MMP are also likely responsible for helping dysplastic and neoplastic cells escape apoptosis [33]. Phelan *et al.* identified three mitochondrial genes (bcl-2 homologous antagonistic killer or *BAK1*, Fission 1 or *FIS1*, and stratifin or *SFN*) that were found to be differentially expressed across the metaplastic-dysplastic-EAC disease sequence in BE. Knockout of these genes resulted in lower MMP in Barrett's cells supporting the hypothesis that increased MMP plays an important role in BE pathogenesis [34]. So far, some research has supported the role of proton pump inhibitors in altering the MMP in EAC cells but not BE cells [35]. More research is warranted to identify strategies to target changes in MMP in BE cells and explore the possibilities of using that knowledge to identify strategies of aiding early detection and efficiently stratifying the risk of progression in BE patients.

OTHER MITOCHONDRIAL GENETIC MUTATIONS AS TARGETS AND THE WAY FORWARD

As indicated before, there is a lack of efficient diagnostic and prognostic molecular markers for BE. These markers could be brought to use in the determination of the risk of progression in patients with BE and also determine if particular treatments would work better than the others in preventing the progression to cancer. Although several mitochondrial-associated proteins such as S100 calcium binding protein, peroxisome proliferator-activated receptor-gamma coactivator-1α (PPAR-γ), c-myc, etc., have been shown to play a role in neoplastic progression in inflammatory conditions, their role is poorly understood in BE; which is also a premalignant inflammatory condition [35]. Phelan *et al.* identified three mitochondrial genes (*BAK1*, *FIS1*, and *SFN*) that were found to be differentially expressed between BE and EAC cells. They reported that *BAK1* (associated with apoptosis) could play a role in BE pathogenesis by providing resistance to potential cancer cells which could be targeted to prevent pre-malignant BE cells from developing cancer. *FIS1* (associated with apoptosis and mitochondrial fission) was found to support specific mitochondrial metabolism changes that favor progression of BE to EAC. Similarly, reduced expression of *SFN* (which is already associated with poor outcomes in EAC) was found to be specifically associated with Barrett's mucosa and could potentially be exploited for prognostication in these patients [35]. Through their research, Tan *et al.* indicated that a 4977bp deletion in mtDNA could be a useful biomarker to detect the severity of dysplasia [36]. Mitochondrial signal transducer and activator of transcription 3 (STAT3) expression and a reduced production of ROS was also found to be associated with the progression of BE cells to cancer in the study conducted by Chunhua *et al.* They indicated that targeting STAT3 using specific agents could be useful for chemoprevention in BE patients [37]. Tarnawski *et al.* demonstrated that mtDNA mutations such as with cytochrome c deficiency are an early event in the pathogenesis of BE, preceding the development of dysplasia and cancer. This knowledge could possibly be used to identify non-dysplastic BE more accurately, segregate them from BE with dysplasia non-endoscopically, and personalize their treatment plan. They also reported early growth response protein 1(EGR-1) as a novel biomarker for Barrett's mucosa-related cancers which could be further studied as a potential therapeutic target [38].

The use of mitochondrial markers in early identification of premalignant and malignant conditions is challenging as well. Studying and developing mitochondrial markers for cancer surveillance and screening are technically daunting tasks, as significant variations exist between and within particular disease types, which cannot be generalized [39]. Despite the advancements in our understanding of the role of mitochondrial dysfunction in cancer, it is still mostly unclear as to how exactly

variations and mutations in the mitochondria promote the formation and progression of cancer [40]. In our understanding, more dedicated research targeted at analyzing the detailed molecular mechanisms involving these complex mitochondrial processes is needed in order to generate evidence and information that can eventually be translated into clinical benefit for patients. It is also worth mentioning that, as of now, no biomarkers for BE have been able to replace the gold standard of biopsy for the diagnosis of dysplasia. The validation of biomarkers needs clinical studies that are not only difficult to conduct but also need high expenditure and very large sample sizes [41].

On an encouraging note, however, as highlighted in this mini-review, mitochondrial molecular targets seem to offer good promise in terms of improving the rate of diagnosis of BE and predicting future outcomes in these patients. Cost-effective approaches to sequence whole mitochondrial genomes for hundreds of individuals have been developed, making them promising candidates for use in population-based cancer screening [42]. The development of specific and efficient mitochondrial marker panels and their incorporation into clinical risk-stratification scores may prove to be invaluable in detecting BE early on in more patients and helping clinicians make specific and decisions regarding their management and follow-up. We believe that this will not only curb the incidence and overall burden of esophageal cancer but will also help curtail excessive healthcare costs by avoiding unnecessary surveillance in low-risk populations. Longitudinal research studies are needed to validate and produce stronger evidence to support their use in clinical practice.

CONCLUSION

As evident from the studies summarized in this mini-review, mitochondria play a pivotal role in BE pathogenesis. Mitochondrial oxidative stress and alterations in mitochondrial metabolism are associated with the progression of BE to EAC. Changes in mitochondrial membrane potential and mitochondrial genetic mutations are yet some other ways in which mitochondria could influence the metaplasia-dysplasia-cancer sequence. This review highlights the need for more research focused on developing a better understanding of these mitochondrial mechanisms in BE and EAC. Extensive research to identify clinically relevant mitochondrial molecular targets that could prove to be of diagnostic, prognostic, and therapeutic importance is needed. This knowledge could go a long way in helping us not only improve BE detection and devise better and cost-effective screening tools for esophageal cancer but also in stratifying the risk of progression and tailoring personalized medicine among these patients.

KEY POINTS

-Barrett's esophagus (BE) is the only known precursor of esophageal adenocarcinoma (EAC) and is amenable to treatment. However, more than 90 percent of EAC patients are never diagnosed with antecedent BE.

-Identification of molecular markers for BE is needed to improve detection of BE through efficient non-endoscopic methods that are cost-effective, sensitive, and can be used to cater to a larger group of the population at risk.

-Mitochondrial response to oxidative stress, alterations in mitochondrial metabolism, changes in mitochondrial membrane potential and mitochondrial genetic mutations have been found to be associated with BE pathogenesis.

-The development of specific and efficient mitochondrial marker panels and their incorporation into clinical risk-stratification scores may prove to be invaluable in detecting BE early on in more patients and helping clinicians make specific and decisions regarding their management and follow-up.

-Studying and developing mitochondrial markers for cancer surveillance and screening are technically challenging tasks. The validation of screening biomarkers also needs clinical studies that are difficult to conduct, need high expenditure, and very large sample sizes.

-Extensive research to identify clinically relevant mitochondrial molecular targets that could prove to be of diagnostic, prognostic, and therapeutic importance is needed.

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