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## Malic Enzyme 1 (ME1) in the Biology of Cancer: It's not Just Intermediary Metabolism

Frank A. Simmen<sup>1,2</sup>, Iad Alhallak<sup>1</sup>, Rosalia C.M. Simmen<sup>1,2</sup>

<sup>1</sup>Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA.

<sup>2</sup>The Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA.

### Abstract

Malic Enzyme 1 (ME1) is a cytosolic protein that catalyzes the conversion of malate to pyruvate while concomitantly generating NADPH from NADP. Early studies identified ME1 as a mediator of intermediary metabolism primarily through its participatory roles in lipid and cholesterol biosynthesis. ME1 was one of the first identified insulin-regulated genes in liver and adipose and is a transcriptional target of thyroxine. Multiple studies have since documented that ME1 is pro-oncogenic in numerous epithelial cancers. In tumor cells, the reduction of ME1 gene expression or the inhibition of its activity resulted in decreases in proliferation, epithelial-to-mesenchymal transition and *in vitro* migration, and conversely, in promotion of oxidative stress, apoptosis and/or cellular senescence. Here, we integrate recent findings to highlight ME1's role in oncogenesis, provide a rationale for its nexus with metabolic syndrome and diabetes, and raise the prospects of targeting the cytosolic NADPH network to improve therapeutic approaches against multiple cancers.

### Keywords

malic enzyme; glutathione; thioredoxin; NADPH; hyperinsulinemia; cancer

### Introduction

Malic Enzyme 1 (ME1) is a cytosolic protein that catalyzes the conversion of malate to pyruvate while simultaneously regenerating NADPH from NADP. ME1 (along with its cousins, malic enzyme 2 (ME2) and malic enzyme 3 (ME3)) have been well studied for their roles in intermediary metabolism (Fig. 1). ME1 is a major participant in lipid and cholesterol biosynthesis, since it generates NADPH, a required cofactor for fatty acid and cholesterol biosynthesis, and it regulates the reversible oxidative decarboxylation of malate to pyruvate, thus linking the glycolytic and citric acid pathways (Fig. 1). ME1 is also an indirect participant in many other NADPH-dependent metabolic pathways, by virtue of its

**Correspondence:** Frank A Simmen, Ph.D., simmenfranka@uams.edu or Rosalia CM Simmen, Ph.D., simmenrosalia@uams.edu.

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contribution to the cytosol NADPH pool. Recent studies have demonstrated that ME1 is pro-oncogenic in a diverse array of epithelial cancers. This review focuses on this new role of ME1 in cancer and potentially at the interface of cancer, diabetes and obesity (Lega & Lipscombe 2020).

## ME1 and the cytosolic NADPH pool

NADPH is a universal electron donor in all organisms and is a required cofactor in the biosynthesis of major cell components. NADPH is also a required participant in other biological processes such as anti-oxidative stress pathways, detoxification pathways, and the generation of reactive oxygen species (ROS). To help maintain an adequate pool of NADPH, regeneration of the oxidized form (NADP<sup>+</sup>) occurs. Within the cell cytoplasm, there are three major enzymes/pathways that contribute to NADP-NADPH recycling; these are ME1, isocitrate dehydrogenase 1 (IDH1), and two enzymes of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD)). Published work indicates that these enzymes comprise a regulatory network in maintaining the cytosolic NADPH pool, which remains not well understood (Merritt *et al.* 2009, Rzezniczak & Merritt 2012, Goodman *et al.* 2018).

## ME1: the early years

ME1 was an active focus of research during the 1980s and 1990s, serving as a model for nutritional and hormonal regulation of gene expression/transcription and in so doing, contributed to the emergence of the field of molecular endocrinology. Insulin and thyroxine were found to induce ME1 gene expression (transcriptional rate and mRNA abundance) in rat liver and adipose (Drake & Mucenski 1985, McHugh & Drake 1989, Garcia-Jiménez *et al.* 1994). Consistent with ME1 as a lipogenic enzyme, a high carbohydrate diet up-regulated liver and adipose ME1 expression (Mariash *et al.* 1980). ME1 gene expression was increased in the epididymal fat of rats fed a *cafeteria* diet, high in fat and carbohydrates, coincident with increased lipid synthesis and storage (López *et al.* 2003). Dietary effects on ME1 gene expression were not limited to liver and adipose tissues. In C57BL/6J mice, short-term feeding of a high-fat diet led to inductions (~18-fold) in ME1 expression in the small intestine, coincident with increased cell proliferation in the small intestine crypts, resulting in greater villus length and cell number per villus (de Wit *et al.* 2008). Similarly, C57BL/6N mice fed a Western-type diet high in fat had increased (~3-fold) small intestine ME1 transcript levels (Desmarchelier *et al.* 2012). Support for the direct involvement of ME1 in the proliferation of colon stem-progenitor cells came from studies using mice null for ME1 protein (Al-Dwairi *et al.* 2012). In this study, average colon crypt depth of wild-type (WT) C57BL/6J mice was found to be greater than that for counterpart mice that lacked ME1 protein, when both groups were fed a high-fat diet. Conversely, enhancement of gastrointestinal expression of ME1 in C57BL/6J mice (via germline integration of a villin-ME1 cDNA transgene) conferred, in jejunum, a phenotype of increased intracellular NADPH, deeper crypts, and greater incorporation of BrdU into crypt stem-progenitor cells (Al-Dwairi *et al.* 2014). These collective findings led to an emergent picture of ME1 as a nutritionally- and hormonally-regulated metabolic gene/protein with lipogenic actions in liver and adipose and with NADPH-elevating and pro-proliferative actions within the

gastrointestinal tract. These observations likewise support the contribution (direct or indirect) of ME1 in the development of obesity and type 2 diabetes (Yang *et al.* 2009; Zhong *et al.* 2010), via its integrated actions in multiple tissues (Al-Dwairi *et al.* 2014).

## ME1 in cancer biology

Pioneering work in the 1990's showed that ME1 'activity' was relatively high in human tumor cells, and that the tumor-expressed ME1 protein was the same as that expressed in normal liver and adipose (Loeber *et al.* 1994). Many clinical studies of more recent vintage have indeed described the elevated ME1 expression in tumors when compared to adjacent non-tumor tissues (Table 1). These studies serve to highlight the linkage of ME1 over-expression with multiple solid tumors (e.g., gastrointestinal tract, hepatobiliary system and the aerodigestive tract, in particular) and importantly, argue for the potential prognostic value of tumor ME1 expression in adverse patient outcomes (Table 1). From the standpoint of human tumor genomic landscapes, ME1 mutations are relatively rare (1–2% frequency). Exceptions to the latter are prostate and endometrial cancers, where deletions, missense mutations and amplifications occur at frequencies between 3–6%. The underlying molecular basis for cancer-related ME1 over-expression remains an important question, given the limited studies to date. In human colorectal cancers, mutations in TP53 and KRAS elicited induction of steady-state ME1 mRNA levels (Jiang *et al.* 2013, Chakrabarti 2015, Shen *et al.* 2017). The contribution of microRNAs (miRNAs) in the regulation of ME1 expression is gaining ground, with specific miRNAs negatively influencing ME1 expression (Table 2). A decline in abundance of miRNA(s) that interact with ME1 mRNA in tumor cells resulted in an imbalance in redox potential favoring oxidative stress and increased accumulation of intracellular reactive oxygen species (ROS) and consequently, promotion of DNA damage (Table 2). The ME1 gene is richly endowed with diverse transcriptional regulatory motifs as well as larger multipartite regions for transcription factor interactions, including those that mediate its transcriptional regulation by insulin, thyroxine, nuclear receptors (e.g., PPARs) and NRF2, the latter a master regulator of anti-oxidative stress pathways. However, the potential contributions of these cis- and trans-acting motifs/factors to tumor ME1 over-expression remain largely unexplored.

The positive association between ME1 and cell migration (Table 2) is strongly supported by recent studies that directly targeted ME1 in a diverse array of tumor cell models (Table 3). Knock-down of ME1 mRNA or inhibition of ME1 activity, in tumor cell lines and mouse models consistently abrogated proliferation, epithelial-to-mesenchymal transition (EMT) and *in vitro* migration, and promoted oxidative stress, apoptosis and/or senescence (Table 3). While the mechanistic underpinnings of ME1 to these cancer cell phenotypes remain unknown, elevations in intracellular NADPH levels can lead to suppression of apoptosis (Yang *et al.* 2010). However, documentation of this putative association in tumor cells remains lacking. Similarly, increased metabolic fluxes involving the biosynthesis of membrane components such as fatty acids and cholesterol and the pyruvate-malate cycle, provide essential support for cancer cell proliferation and migration (Fig. 1).

Our examination of gene expression databases, established from diverse cancer cell lines, revealed consistent co-expression of ME1 mRNA with mRNAs encoding two cytosolic

NADPH generating enzymes (G6PD and IDH1), other cytosolic enzymes that utilize NADPH as an electron donor (e.g., prostaglandin reductase 1 (PTGR1), NAD(P)H quinone dehydrogenase 1 (NQO1) and proteins that may serve anti-oxidative stress functions (e.g., tripartite motif containing 16 (TRIM16)). Similarly, perusal of expression databases for human tumors show robust co-expression of ME1 mRNA with mRNAs encoding reductases (e.g., PTGR1) that utilize NADPH, as well as those encoding anti-oxidative stress pathway protein components. The expression correlations noted between ME1, G6PD and IDH1 warrant further interest. G6PD is a rate-limiting enzyme of the pentose phosphate pathway and its over-expression has been noted for multiple human tumor types (Chen *et al.* 2018, Lu *et al.* 2018a). Similar to ME1, knock-down of G6PD abrogated tumor cell growth and enhanced intracellular levels of ROS (Chen *et al.* 2018) and inhibited *in vitro* cell migration and invasion (Lu *et al.* 2018a). IDH1 mutations are drivers of several human malignancies (reviewed in Bergaggio & Piva 2019). Overexpression of wild-type IDH1 has also been observed for multiple cancers (e.g., non-small cell lung carcinoma (NSCLC), glioblastoma multiforme (GBM)) whereas its KD abrogated *in vitro* and *in vivo* tumor cell growth, NADPH/NADP ratio, and resistance to oxidative stress (Bergaggio & Piva 2019), similar to that observed for ME1 KD (Table 3).

ME1 stimulates the pentose phosphate pathway (PPP) flux via a physical interaction with and resultant activation of 6-phosphogluconate dehydrogenase (6PGD), the second PPP enzyme that also provides reduced NADP in cytosol (Yao *et al.* 2017). 6PGD itself is implicated in tumorigenesis (Sarfraz *et al.* 2020). Notably, ME1 enhanced 6PGD levels, functioning independent of its enzymatic activity, as evidenced from studies using constructs encoding enzymatically inactive, mutant ME1. Remarkably, when these same mutant ME1 proteins were expressed in cells in which wild-type ME1 was suppressed, augmented cell growth and colony formation *in vitro* were found, supporting the conclusion that ME1's pro-proliferative effects are primarily mediated by interaction with and activation of 6PGD (Yao *et al.* 2017; Table 3). This new mechanism may explain some of the observed effects of KD and over-expression of ME1 in tumor cells (Table 3); however, they are not easily reconciled with the cellular growth-inhibitory effects of ME1 active site-inhibitors (Zhang *et al.* 2006; Saha *et al.* 2014, Fernandes *et al.* 2018). The reciprocal and dynamic regulation of ME1 activity (manifested as increased NADPH/NADP ratio and increased production of fatty acids) by acetylation at amino acid K337 (positive regulation) and by phosphorylation at amino acid S336 (negative regulation) (Zhu *et al.* 2020) raises the important question of whether post-translational modification(s) of ME1 affect its interactions with 6PGD, in differing tumor types and tissue contexts.

Studies of gastrointestinal cancers have illuminated the pro-proliferative role of ME1. Intercrossing of a villin-ME1 transgene into the Apc<sup>Min</sup> mouse background led to increased numbers as well as overall sizes of adenomas within the small intestine (Fernandes *et al.* 2018; Table 3). Also of interest were the noted changes in ME1 expression in the outwardly "normal" mucosa adjacent to adenomas. Fernandes *et al.* 2018 demonstrated that normal-appearing small intestine villi bordering individual adenomas displayed increased ME1 immunoreactivity as well as increased numbers of mucin-producing goblet cells compared to more distant, and presumably more normal, villi. These "transitional" villi, presumably lying within the cancer field, had deeper crypts and were longer than their counterparts more

distant from the adenoma. In this regard, a previous report described an increase in lipid content in the epithelial-like borders of *Apc<sup>Min</sup>* mouse adenomas (Mutoh *et al.* 2009). We speculate that the ME1 phenotype for cancers of the gastrointestinal tract includes a significant lipogenesis component, as well as paracrine communication between tumors and adjacent non-tumor tissue, to induce production of lipids and mucins in the latter for tumor growth.

The ME1-related protein, ME2 also is implicated in tumorigenesis. Recent work has documented the over-expression of ME2 and its functional contributions to tumor phenotypes of erythroleukemia (Ren *et al.* 2010), glioblastoma multiforme (GBM) (Cheng *et al.* 2016), and head and neck squamous cell carcinoma (Woo *et al.* 2016). In the non-small cell lung cancer (NSCLC) cell line A549, ME2 KD resulted in growth cessation, increased cell death, higher levels of ROS, lower ATP and an increased sensitivity to cisplatin (Ren *et al.* 2014). Interestingly, in the same study, KD of ME2 also led to an induction in PTEN levels and a down-regulation of AKT signaling. This reciprocal regulation between ME2 and tumor suppressor (PTEN) *vs.* tumor promoter (AKT) is reminiscent of the inverse relationship found for ME1 and TP53 in colon cancer cells (Jiang *et al.* 2013). Further, the interesting connections between the malic enzymes and major tumor suppressive and tumor promoting pathways suggest their more extensive linkages in various cancers and are worthy of further exploration.

## **ME1 and lung cancer subtypes: a paradigm for parsing ME1-dependent tumor pathways**

Human lung cancers express higher levels of ME1 (gene and protein) than does normal lung tissue, and tumor ME1 over-expression may confer a worse patient prognosis (Table 1). Within lung cancer subtypes, squamous cell carcinomas express more ME1 than do adenocarcinomas (Table 1). Perusal of gene expression databases for lung tumors revealed robust and relatively consistent associations of ME1 mRNA with mRNAs encoding multiple NADPH-dependent reductases (Fig. 2). As a class, NADP/NADPH-dependent reductases are involved in the detoxification or activation of xenobiotics, metabolism of endogenous biochemical pathway intermediates, regeneration of endogenous antioxidants, and overall mediation of cellular redox state. These enzymes also influence susceptibility of cells to exogenous drugs and carcinogens. One example of this is prostaglandin reductase 1 (PTGR1), a cytosolic, NADP-dependent, alkenal/one oxidoreductase that inactivates prostaglandins and related eicosanoids (Sánchez-Rodríguez *et al.* 2017) and which is highly co-expressed with ME1 (Fig. 2). This protein has significant anti-oxidative actions by virtue of its reduction of 4-hydroxy-2-nonenal (4HNE) and fatty acid nitroalkenes. Recent studies have pointed to the potential targeting of PTGR1 in treatment of prostate cancer (Xue *et al.* 2016) and hepatocellular carcinoma (Sánchez-Rodríguez *et al.* 2017). In particular, KD of PTGR1 led to decreased cell proliferation, increased apoptosis and decreased protection against hydrogen peroxide and 4-HNE-induced cell death. Yet another example of an ME1 co-expressed mRNA in lung tumors (Fig. 2) is the mRNA for thioredoxin reductase 1 (TXNRD1), an enzyme which utilizes NADPH to regenerate reduced thioredoxin and thereby, increase cellular reducing capacity and protection against oxidative stress.

TXNRD1 KD in radiation-resistant lung cancer cells sensitized these cells to radiation treatment (Hao *et al.* 2017). A third example (Fig. 2) is the mRNA that encodes aldo-keto reductase family 1 member C1 (AKR1C1), a NADP-dependent cytosolic enzyme involved in intermediary metabolism and for which multiple studies have identified contributory roles in therapy resistance, EMT and metastasis of cancer cells (Tian *et al.* 2016, Zhu *et al.* 2018).

Of interest are the findings that mutations in TP53 and PI3KCA are more frequent in lung squamous cell carcinomas than in lung adenocarcinomas (de Sousa & Carvalho 2018). Inference from other cancers such as those of the colo-rectum suggests that mutations in both genes, along with those of KRAS, constitute potential drivers of enhanced ME1 expression in squamous cell carcinomas. Lung adenocarcinomas exhibit similar, as well as distinct, metabolic characteristics from lung squamous cell carcinomas (Rocha *et al.* 2015). Both lung cancer subtypes manifest respectively, lower glucose and higher lactate levels, when compared to non-tumor tissue; however, squamous carcinomas have a more exaggerated glycolytic phenotype and have greater reliance on glutaminolysis (Rocha *et al.* 2015), highlighting known functional linkages of over-expressed ME1 with increased glutaminolytic flux. Interestingly, squamous adenocarcinomas also have a greater amount of reduced glutathione (GSH,  $\gamma$ -Glu-Cys-Gly) (Rocha *et al.* 2015), consistent with more ME1 available to supply the NADPH used for GSH regeneration. Published work of others (Lee *et al.* 2008, Chakrabarti 2015, Csanadi *et al.* 2015, Hao *et al.* 2017, Yan *et al.* 2019) support the concept of targeting ME1 and downstream NADPH-dependent pathways as an adjunct approach to lung cancer treatment.

## **ME1 at the nexus of hyperinsulinemia and cancer initiation and progression: NOX/DUOX family**

Obesity and type 2 diabetes are associated with elevated risks for numerous cancers (Lega & Lipscombe 2020). Sequela of obesity and diabetes that are likely contributory to this elevated cancer risk include hyperinsulinemia, hyperglycemia, inflammation and oxidative stress. Based on the current literature, oxidative stress is a probable point of convergence of ME1, obesity and type 2 diabetes, and cancer development. Perhaps, increased oxidative stress (via induced ME1) provides one mode by which hyperinsulinemia increases cancer risk.

Reactive oxygen species (e.g.,  $\cdot$ NO,  $\text{H}_2\text{O}_2$ , OH and  $\text{O}_2^-$ ) are chemically reactive molecules that contain oxygen and are important participants in extracellular and intracellular signaling elicited by various hormones and growth factors (Bauer 2014). Depending on their steady-state levels and cell/tissue context, they can act as inducers of cell proliferation and of cell death. At low levels, ROS may act as signaling molecules that activate proliferation. ROS at intermediate levels can promote DNA damage and mutagenesis while at high levels, they can cause oxidative stress that may result in apoptosis or senescence. Since cancer cells, which are highly proliferative, produce relatively high levels of ROS, they must buffer excess ROS to maintain proliferation, while avoiding apoptosis and senescence. This intracellular redox state is maintained via the buffering capacity of the reduced glutathione

(GSH)-oxidized glutathione (GSSG) system and the thioredoxin system, in support of proliferation.

One mechanism by which NADPH (and indirectly ME1) can contribute to oxidative damage is via its direct coupling to NADPH oxidases (NOX/DUOX family members) that catalyze the formation of ROS. This pathway also provides a plausible means by which insulin can induce DNA damage in cells during pre-neoplastic and neoplastic stages. Studies have implicated NOX/DUOX family members in the pathway(s) by which elevations in circulating insulin lead to inductions in ROS and ROS-mediated DNA damage (Xiao *et al.* 2008, Meng *et al.* 2012, Othman *et al.* 2014, Mahmoud *et al.* 2017, Steinhorn *et al.* 2017). Moreover, suppression of ROS generation by inhibition of NOX/DUOX member(s) can inhibit insulin signaling (Sypowska & Burgering 2011).

One tissue whose cancer risk is known to be positively associated with obesity, diabetes and hyperinsulinemia is the colo-rectum. Certain members of the NOX/DUOX family were found to be over-expressed and likely growth promoting in tumors of the human colon and rectum (Juhasz *et al.* 2009, Juhasz *et al.* 2017, Lin *et al.* 2017, Cho *et al.* 2018, Kang *et al.* 2018). NOX1 overexpression (similar to that of ME1) in human colorectal cancers is associated with activating mutations in KRAS (Laurent *et al.* 2008). Interestingly, individual NOX/DUOX members differ with respect to their apparent contributions to clinical outlook and tumor therapeutic response (Lin *et al.* 2017, Cho *et al.* 2018). Cancer cells, compared to normal cells, are generally more susceptible to the cytotoxic effects of glucose deprivation, effects that are mediated, in part, by their increased production of ROS via NOX/DUOX (Aykin-Burns *et al.* 2009). In this regard, human colon cancer cells (HCT116) grown in a glucose-restricted medium were extra-sensitive to the growth-inhibition elicited by ME1 KD (Murai *et al.* 2017). Screening of human colon and rectal cancers for KRAS mutations coupled with determination of relative co-expression of ME1 and NOX/DUOX genes/proteins may allow dissection of these potential functional linkages and if confirmed, enable therapeutic targeting of tumor cells via ME1 and NOX/DUOX inhibitors.

## Future directions

The work to date suggests several avenues to refine our understanding of ME1 as pro-oncogenic in multiple tissues and to apply this emerging knowledge to future cancer treatments. Foremost to consider is that ME1 is a participant in a network of cancer promoters, rather than as a single, independent entity (Fig. 3). We infer that ME1 is a major contributor to: a) cancer cell biosynthesis of lipids and cholesterol, b) generation of ROS *via* NOX/DUOX proteins for pro-proliferative (and perhaps for pro-migration/EMT) signaling, and c) buffering of ROS *via* NADPH-dependent recycling of glutathione and thioredoxin pathways, working in concert with other NADP-recycling cytosolic enzymes G6PD, PGD and IDH1 (Fig. 3). We further hypothesize that insulin (i.e., hyperinsulinemia) via its coincident induction of ME1 expression and of NOX/DUOX activity in pre-neoplastic cells, can tip the balance towards oxidative stress/genotoxic state while countering ROS-induced apoptosis and senescence (Fig. 3).

In *Drosophila* flies and human cancer cells, the major cytosolic NADP-reducing enzymes (ME1, G6PD, PGD, IDH1) behave as a network, with changes of some members occurring in response to diminution in expression/activity of another (Merritt *et al.* 2009, Rzezniczak & Merritt 2012, Chen *et al.* 2019). Interestingly, not all such changes in this network are compensatory, suggesting an unappreciated degree of complexity. A key to understanding the nature of these relationships and their cooperation during tumor genesis will require further discernment of their network regulation, of which little is presently known. Additional knowledge of these enzyme's interactions, both physical and temporal, within normal, pre-cancerous and cancerous cells is clearly of interest. Further molecular dissection of the NADP/NADPH flux as well as identification of the functioning NADP/NADPHome (Goodman *et al.* 2018) in tumor cells will be important for the selective targeting of NADPH regeneration leading to tumor progression. In this regard, ROS-induced signaling can occur in defined signaling complexes (Xi *et al.* 2013, Bauer 2014). Thus, a defined molecular picture of the spatiotemporal organization of ME1, G6PD, PGD, IDH1, other NADPHome partners, and NADPH is necessary to understand how these proteins form complex tumor-promoting networks. The role of ME1 and its immediate network partners (and the expanded NADPHome) in oxidative stress during aging (Hecker *et al.* 2014), and how these pathways influence cancer development in older individuals is ripe for further exploration.

Glutathione and thioredoxin anti-oxidant pathways buffer oxidative stress in cancer cells and appear to be synergistic with respect to tumor initiation and progression (Harris *et al.* 2015, Lien *et al.* 2016, Yan *et al.* 2019). Combined inhibition of both anti-oxidant pathways leads to cancer cell death *in vitro* and *in vivo* (Palomares *et al.* 2009). Targeting ME1 and its network of associated NADPH-regenerating enzymes may phenocopy or may be additive or even synergistic with the co-targeting of the glutathione and thioredoxin pathways to counter cancer cell growth, survival, metastasis and therapy resistance.

The nature (and the counter balance) of downstream oncogenic and tumor suppressive proteins/pathways that are activated by NOX/DUOX-elicited ROS in different tissues and tumors is an important question in cancer biology (Xi *et al.* 2013, Chen *et al.* 2018). Review of literature suggests that NADPH/NOX/DUOX signaling may be particularly relevant to initiation, progression and treatment of cancers of the colon and rectum. In this regard, Komiya *et al.* 2015 documented a suppressive effect of apocynin, an NADPH oxidase-inhibitor, on adenoma occurrence in the Apc<sup>Min</sup> mouse model.

Pyruvate, the oxidative product of ME1, is also consequential to any discussion of ME1 in cancer biology. One route that ME1-derived pyruvate can follow is its conversion to lactate by the enzyme lactate dehydrogenase A (LDH-A) during the process of anaerobic glycolysis (Warbug effect) (Fig. 1). LDH-A is itself a molecular target for cancer therapies (Miao *et al.* 2013).

While the studies summarized in Tables 2 and 3 have implicated ME1 in epithelial-mesenchymal transition (EMT) and tumor metastasis, many of the molecular details of how these actions are promoted by ME1 (and/or its network) are missing. However, one candidate pathway may involve the induction of EMT by DUOX2-generated ROS in 5-fluorouracil-resistant human colon cancer cells *in vitro* (Kang *et al.* 2018).



## Conclusions

Malic Enzyme 1 is an old enzyme, both figuratively and evolutionarily, with an illustrious history. It is an important player in intermediary metabolism in animals and plants where it is subject to complex multifactorial transcriptional and post-transcriptional regulation. As a multifunctional enzyme, ME1 contributes to adipogenesis, lipogenesis and steroidogenesis. Variations in human ME1 sequence/expression have been linked to propensity for developing type 2 diabetes and for fat deposition (Yang *et al.* 2009, Zhong *et al.* 2010). In the plant kingdom, ME1 has been explored as a marker/tool to increase stress resistance of plants. In its newest fascinating iteration, ME1 and its interacting network may constitute important drivers of cancer development and metastasis, in part, by inducing oxidative stress resistance, somewhat akin to its role in plant physiology. Further development and refinement of small molecule inhibitors of ME1 (Zhang *et al.* 2006, Saha *et al.* 2014, Fernandes *et al.* 2018) may prove useful as adjuncts to therapies for primary and metastatic cancers and particularly for cancers associated with obesity, type 2 diabetes and hyperinsulinemia.

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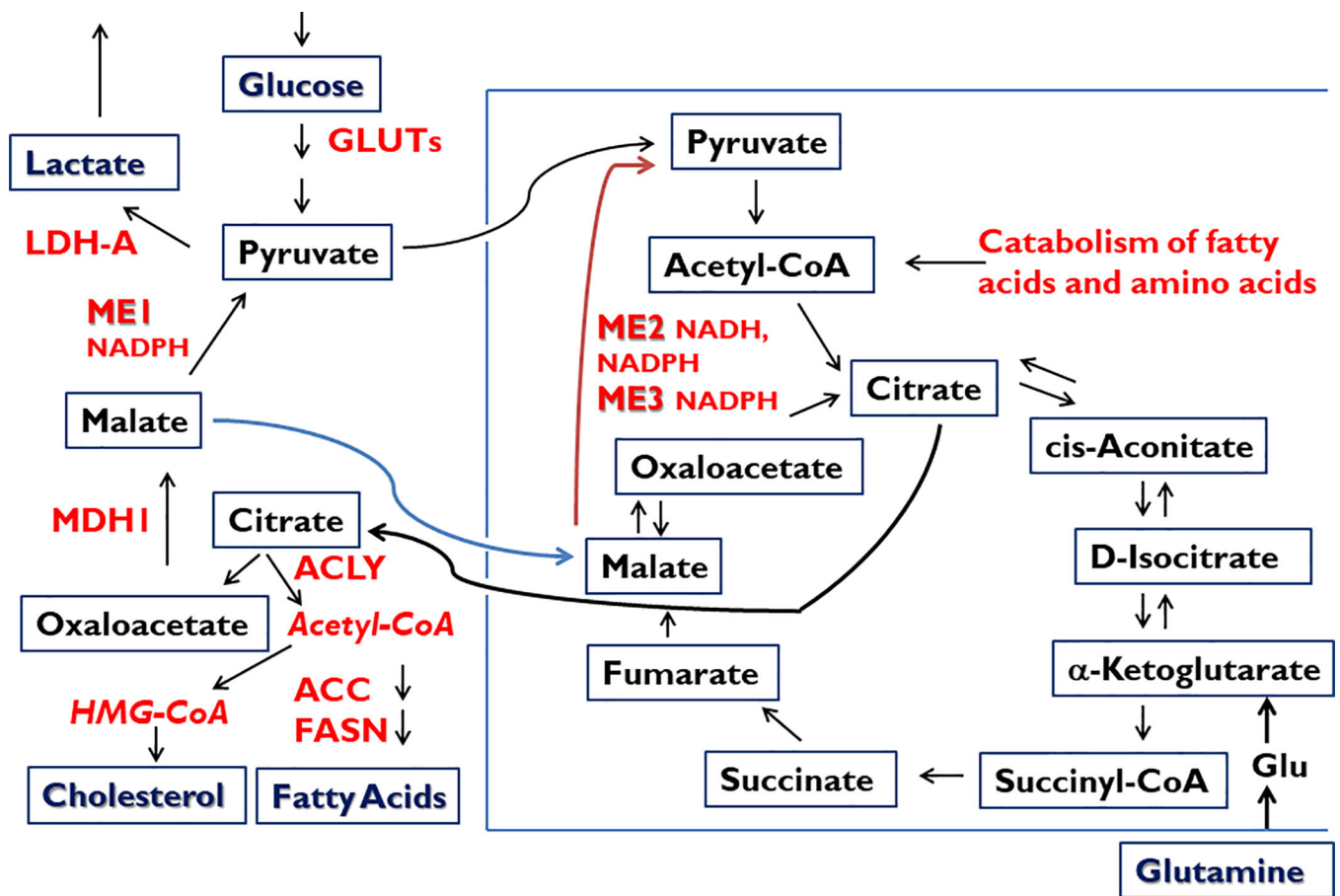
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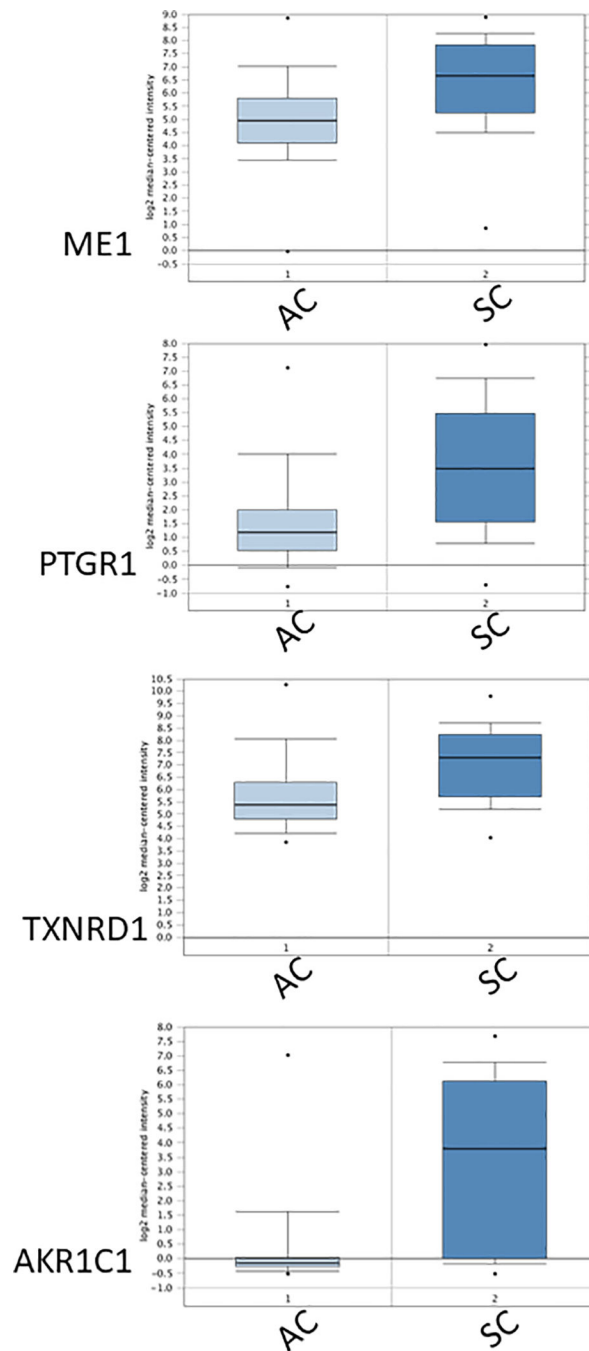
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**Figure 1.**

Central role of malic enzymes (ME) in normal and cancer cell metabolism. Malic enzymes located within cytoplasmic (ME1) and mitochondrial (ME2, ME3) compartments catalyze the oxidative decarboxylation of malate to pyruvate, while concomitantly storing reducing equivalents in the form of NADPH (ME1, ME2, ME3) or NADH (ME2). NADPH serves in multiple biochemical capacities such as in countering ROS (via glutathione and thioredoxin pathways), contributing to ROS signaling (via NOX/DUOX family members), and providing reductant capacity for the biosynthesis of fatty acids and cholesterol, among many other metabolic pathways (Goodman *et al.* 2018). Besides being a major source of cytosolic NADPH, ME1 participates in a pathway by which excess mitochondrial Acetyl-CoA is transferred to cytosol (in the form of citrate; via the pyruvate-malate cycle) for incorporation into cholesterol and fatty acids. ME1 also participates in the Warburg effect (aerobic glycolysis, secretion of lactate) that is characteristic of many cancer cells and tumors. Lactate may serve as a paracrine energy source for tumor cells. ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; FASN, fatty acid synthase; Glu, Glutamate; GLUTs, glucose transporters; LDH-A, lactate dehydrogenase A; MDH1, malate dehydrogenase 1.

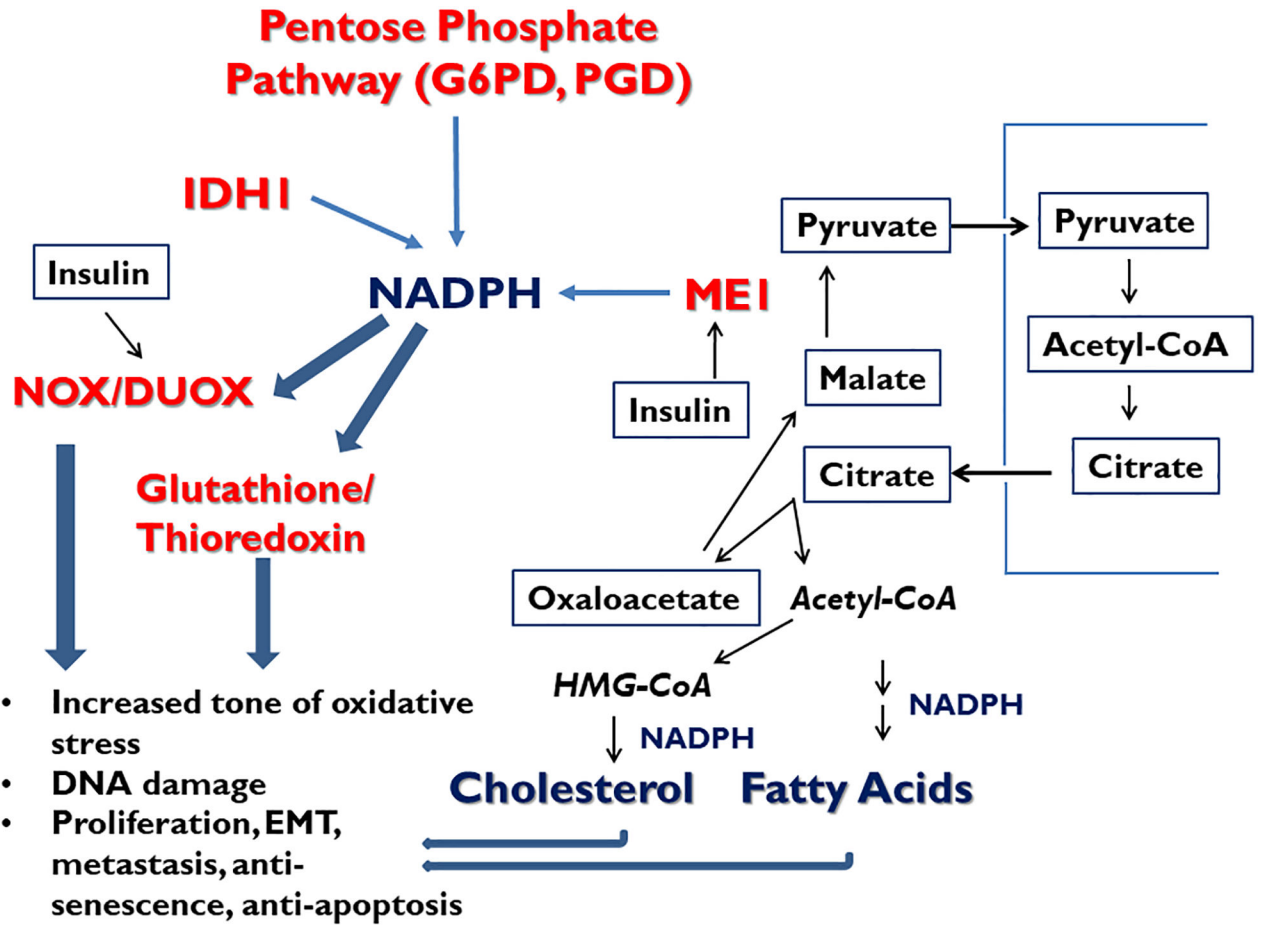


**Figure 2.**

Elevated abundance and significant co-expression of mRNAs encoding ME1 and the NADPH-dependent proteins PTGR1, TXNRD1 and AKR1C1 in human lung squamous cell carcinomas (SC) compared to human lung adenocarcinomas (AC). Co-expression analysis identified many NADPH-dependent reductases that are highly co-expressed with ME1 in lung tumors; shown are expression data for three such genes. Box plots present normalized expression data for 63 individual AC and 75 individual SC (obtained from OncoPrint Research Edition (<http://www.oncoPrint.org>; Rhodes *et al.* 2004); gene expression data are



originally from Lee *et al.* 2008). Dots represent maximum and minimum values; error bars represent 10<sup>th</sup> and 90<sup>th</sup> percentiles; boxes span 25<sup>th</sup> to 75<sup>th</sup> percentiles; thick line is the median. Fold changes (SC compared to AC) were ME1, 2.677; PTGR1, 3.818; TXNRD1, 2.405; AKR1C1, 8.188. All fold-differences were highly significant ( $P < 0.01$ ).



**Figure 3.** Integrative model for involvement of ME1 and the other cytosolic NADP-reducing enzymes in the initiation and progression of cancer. The model posits that ME1, IDH1, and pentose phosphate pathway function collaboratively within a network to maintain required levels of NADPH. In pre-cancerous and tumor cells, one or more of these enzymes/pathways becomes over-expressed, or augmented in activity, thereby favoring increased steady state levels of NADPH. This elevation in NADPH promotes NOX/DUOX signaling leading to increased oxidative stress and DNA damage, and increased glutathione and thioredoxin recycling; these latter pathways protect tumor cells from the apoptosis-inducing effects of increased oxidative stress. The NADPH-dependent pathways favor proliferation, DNA damage, epithelial-mesenchymal transition (EMT), cell migration and metastasis, anti-senescence and anti-apoptosis. We further posit that hyperinsulinemia drives a) the increased expression of ME1 favoring increased activity of the pyruvate-malate cycle and thereby, leading to increased synthesis of fatty acids and cholesterol in support of biosynthesis, proliferation and anti-apoptosis (Xiao *et al.* 2008), and b) the increased activity of NOX/DUOX members. The collective actions contribute to a heightened genotoxic and proliferative state, ultimately contributing to tumor genesis.

**Table 1:**

## ME1 as a Human Cancer Biomarker

Cancer (tumor type)	Approach	Reported Associations/Findings	References
<b>Human gastric cancer (adenocarcinoma)</b>	Immunohistochemistry (IHC) of tumors.	ME1 was more highly expressed in tumors than adjacent normal tissue; ME1 was more highly expressed in metastases than primary tumor; tumor ME1 protein abundance (IHC signal) was correlated with shorter overall survival and disease-free survival of patients.	Lu <i>et al.</i> (2018b)
<b>Human gastric cancer (adenocarcinoma)</b>	IHC of tissue multi array (TMA); independent scoring by several pathologists.	No difference in IHC staining score between tumor and adjacent normal (differs from Lu <i>et al.</i> 2018); no significant correlation between ME1 expression and clinico-pathological parameters; however, moderate or high ME1 expression predicted lower cumulative patient survival.	Shi <i>et al.</i> (2019)
<b>Human breast cancer</b>	Mining of gene expression databases; Western blots of tumor lysates.	ME1 was more highly expressed in basal-like breast cancer (BLBC) than luminal breast cancer. ME1 copy number amplification was evident for many BLBCs; larger tumors expressed more ME1; Grade 3 tumors expressed more ME1 than Grade 1 and 2 tumors; patients with tumors with higher ME1-expression had worse overall and relapse-free survival and higher frequency of chemotherapy resistance.	Liao <i>et al.</i> (2018)
<b>Human colon (mucinous adenocarcinoma)</b>	Mining of gene expression databases.	ME1 mRNA expression was greater in tumor than adjacent non-tumor tissue.	Jiang <i>et al.</i> (2013)
<b>Human colon and rectal cancers (adenocarcinoma)</b>	IHC of TMA.	ME1 protein content was positively correlated with tumor pathological stage and invasion.	Gdynia <i>et al.</i> (2016)
<b>Human colon and rectal cancers (adenocarcinoma)</b>	Quantitative reverse transcriptase-PCR (qRT-PCR), Western blot analysis.	ME1 expression was higher in tumor than adjacent non-tumor tissue; KRAS-mutant tumors had more ME1 than KRAS <sup>wt</sup> tumors.	Shen <i>et al.</i> (2017)
<b>Human colon and rectal cancers (adenocarcinoma)</b>	IHC of TMA.	ME1 expression was robust in tumor epithelial but not stromal cells; however, not all tumors were of the high ME1-expressing type.	Fernandes <i>et al.</i> (2018)
<b>Human hepatocellular carcinoma (HCC) (adenocarcinoma)</b>	IHC of tumor and paired adjacent non-tumor; segregation of patients/tumors into ME1 high vs. ME1 low-expressing types.	HCC had more ME1 staining than did adjacent non-tumor tissue; patients with an “ME1 high” tumor had lower overall survival and progression-free survival than did those with an “ME1 low” tumor.	Wen <i>et al.</i> (2015)
<b>Human hepatocellular cholangiocarcinoma, subtype with stem cell features, intermediate-cell type (INT)</b>	Microarrays and IHC.	INT tumor had greater expression of ME1 mRNA and protein than did HCC or intrahepatic cholangiocarcinoma tumors.	Mihara <i>et al.</i> (2019)
<b>Human non-small cell lung cancer (NSCLC)</b>	IHC of TMA (tumors and including some non-tumor tissues).	ME1 protein abundance was greater in NSCLC than non-tumor lung tissue; within NSCLC, ME1 expression was greater for squamous cell carcinomas than for adenocarcinomas; ME1 expression was greater in tumors of smokers than non-smokers; ME1 expression in primary tumor was positively associated with metastasis to lymph nodes; tumor ME1 expression predicted longer overall survival and shorter overall survival in younger and older patients, respectively (median cut-off of 65 years of age).	Csanadi <i>et al.</i> (2015)
<b>Human non-small cell lung cancer (NSCLC)</b>	Mining of gene expression databases.	Patients with elevated tumoral ME1 expression had worse overall survival after radiotherapy, but this effect was not observed with chemotherapy alone.	Chakrabarti (2015)
<b>Human hereditary clear cell renal cell carcinoma</b>	Mining of gene expression databases.	ME1 expression was greater in tumor than non-tumor tissue.	Jiang <i>et al.</i> (2013)
<b>Human oral squamous cell carcinoma</b>	IHC of TMA.	ME1 expression was greater in tumor than non-tumor tissue; ME1 expression was positively associated with	Nakashima <i>et al.</i> (2018)

Cancer (tumor type)	Approach	Reported Associations/Findings	References
		cancer progression; patients with moderate to high tumor ME1 had worse prognosis.	
<b>Human laryngeal squamous cell carcinoma</b>	Microarrays of tumors and paired nonmalignant mucosa.	ME1 mRNA expression was greater in tumor than non-tumor tissue.	Nicolau-Neto <i>et al.</i> (2020)

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**Table 2.**

## Functional Identification of ME1 mRNA-interacting microRNAs

MicroRNA	Cells/tumors	Associations/effects	References
<b>miR-30a</b>	KRAS-mutant colorectal cancers; HCT116 and DLD1 human colorectal cancer cell lines	Over-expression (OE) of miR-30a suppressed ME1 mRNA and protein; miR-30a was down regulated in KRAS-mutant colorectal tumors compared to KRAS <sup>wt</sup> tumors; miR-30a inhibited the growth of HCT116 and DLD1 cells <i>in vitro</i> ; miR-30 attenuated proliferation, migration and invasion of HCT116 cells <i>in vitro</i> , as well as growth of HCT116 xenografts in nude mice.	Shen <i>et al.</i> (2017)
<b>miR-612</b>	Human T24 bladder cancer cell line	miR-612 expression was decreased with bladder tumor progression; miR-612 depressed the expression of ME1; OE of miR-612 inhibited bladder cancer cell growth, colony formation, migration, invasion and epithelial-mesenchymal transition.	Liu <i>et al.</i> (2018)
<b>miR-30c-5p</b>	Mouse aorta smooth muscle cells	OE of miR-30c-5p caused down-regulation of ME1 and increased ROS <i>in vitro</i> ; <i>in vivo</i> silencing of miR-30c-5p led to increase in ME1 and reduction in ROS.	Zhu <i>et al.</i> (2018)

**Table 3.**

Effects of Knock-down (KD), Knock-out (KO) or Over-expression (OE) of ME1 in Human and Mouse Tumor Cells

Cell line(s)/Models	Reagent/Approach	Major Effect(s) of ME1 Perturbation	References
<b>HCT116 human colorectal cancer</b>	siRNA KD	Decreased cell proliferation, decreased colony formation in agar, decreased xenograft growth in nude mice, reduced glutamine consumption, increased number of senescent cells, no effect on apoptosis or intracellular ROS level.	Jiang <i>et al.</i> (2013)
<b>HCT116 human colorectal cancer</b>	siRNA KD	Increased number of senescent cells, decreased cell growth, decreased colony formation <i>in vitro</i> , increased rate of glycolysis, increased flux through the pentose phosphate pathway, oxidative stress state, decreased extracellular glucose levels, increased extracellular lactate levels.	Murai <i>et al.</i> (2017)
<b>HCT116, DLD1 human colorectal cancer</b>	shRNA KD	Decreased colony formation <i>in vitro</i> , decreased content of intracellular NADPH and triglycerides in KRAS-mutant but not KRAS <sup>wt</sup> cell lines, decreased xenograft growth in nude mice.	Shen <i>et al.</i> (2017)
<b>HCT116, HT29 human colorectal cancer</b>	Small molecule inhibitor of ME1	Suppressed growth (fewer cell numbers) <i>in vitro</i> , decreased cell viability, decreased colony-formation <i>in vitro</i> .	Fernandes <i>et al.</i> (2018)
<b>HCT116 human colorectal cancer</b>	CRISPR deletions	ME1 KO: no effect on <i>in vitro</i> growth or fatty acid synthesis. ME1/G6PD KO: major cell growth inhibition.	Chen <i>et al.</i> (2019)
<b>Apc<sup>Min</sup> mouse model of Familial Adenomatous Polyposis</b>	ME1 OE (villin promoter-enhancer driving rat ME1 transgene)	Increased number (and overall size distribution) of adenomas in the small intestine, increased expression of Sp5, a Wnt-inducible transcription factor of intestinal stem-progenitor cells, no effect on EMT-related gene expression, trend to decreased apoptosis.	Fernandes <i>et al.</i> (2018)
<b>AOM/DSS-induced mouse colorectal cancer model</b>	ME1 OE (adenoviral ME1 construct delivered intraperitoneal)	Mice that received adenoviral ME1 exhibited increased number and size of carcinomas in the colo-rectum.	Zhu <i>et al.</i> (2020)
<b>SGC7901, MGC803 human gastric cancer</b>	shRNA KD, low glucose medium	ME1 KD: decreased NADPH level, increased apoptosis, increased intracellular level of ROS.	Lu <i>et al.</i> (2018b)
<b>SGC7901, MGC803 human gastric cancer</b>	shRNA KD, anoikis condition	ME1 KD: decreased NADPH level, increased intracellular H <sub>2</sub> O <sub>2</sub> , decreased colony-formation, increased apoptosis.	Lu <i>et al.</i> (2018b)
<b>SGC7901 human gastric cancer</b>	shRNA KD	ME1 KD: less cell growth, cell cycle arrest (in G2), decreased cell migration (transwell assay).	Shi <i>et al.</i> (2019)
<b>MHCC97H, HCCLM3 human hepatocellular carcinoma</b>	shRNA KD	ME1 KD: decreased NADPH level, increased ROS, decreased cell migration and invasion (transwell assay), reversion of EMT.	Wen <i>et al.</i> (2015)
<b>SUM159, T47D human breast cancer</b>	shRNA KD, OE	ME1 enhanced glucose uptake and lactate production by cells, depressed OXPHOS, and promoted aerobic glycolysis; perturbations in ME1 levels caused minimal growth effects under normoxic conditions, however, KD of ME1 under hypoxic conditions caused inhibition of proliferation, ME1 promoted colony formation under normoxic conditions.	Liao <i>et al.</i> (2018)
<b>BT474 human ERBB2-positive breast cancer</b>	shRNA KD	ME1 KD: decreased cell viability.	Kourtidis <i>et al.</i> (2010)
<b>H522 (KRAS<sup>wt</sup>), HCC44 (KRAS<sup>mut</sup>) human non-small cell lung cancer (NSCLC) cell lines</b>	siRNA KD	ME1 KD had no effect on clonogenic survival (colony number) after radiation treatment; whereas KD of HCC44 cells eliminated colony formation after radiation treatment.	Chakrabarti (2015)
<b>A549 human lung cancer U2OS Human osteosarcoma</b>	siRNA KD of wild-type ME1 coupled with OE of enzyme-inactive (mutant) ME1	OE of enzymatically dead ME1 augmented proliferation and colony formation of tumor cells (A549, U2OS) that were devoid of wild-type ME1.	Yao <i>et al.</i> (2017)

Cell line(s)/Models	Reagent/Approach	Major Effect(s) of ME1 Perturbation	References
<b>T24 human bladder cancer</b>	siRNA KD	ME1 KD: decreases in cell proliferation, colony formation, migration, invasion, reversion of EMT.	Liu <i>et al.</i> (2018)
<b>CNE-2 human nasopharyngeal cancer</b>	shRNA KD	ME1 KD: inductions in expression of the PPP enzymes G6PD and PGD, NADPH level was suppressed only in a low glucose medium, reduced level of GSH, increased intracellular ROS, decreased levels of FASN and SNAIL, decreased cell migration and invasion <i>in vitro</i> .	Zheng <i>et al.</i> (2012)
<b>HN31 human head and neck squamous cell carcinoma (HNSCC)</b>	shRNA KD	ME1 KD: no effect on NADPH level, increased cell sensitivity to metformin or ionizing radiation in the colony formation assay, enhanced senescence with metformin treatment, increased intracellular ROS level.	Woo <i>et al.</i> (2016)
<b>HSC3 human tongue squamous cell carcinoma</b>	ME1 antisense oligonucleotide	ME1 KD: decreased cell proliferation, reversion of EMT, less "stemness", decreased lactate production, decreased GSH content, increased oxidative stress state.	Nakashima <i>et al.</i> (2018)