Unlikely role of glycolytic enzyme α-enolase in cancer metastasis and its potential as a prognostic biomarker

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Abstract

Reliance on glycolysis for energy production is considered a hallmark of cancer and the glycolytic enzyme α-enolase is overexpressed in a range of cancer types. However, recent studies have revealed that α-enolase is involved in a variety of unrelated physiological processes and can be found in multiple unexpected cellular locations. This review focuses on the unlikely role of α-enolase as an extracellular plasminogen-binding receptor localised to the plasma membrane. Conversion of plasminogen to plasmin on the surface of cancer cells enhances their ability to invade through stroma by activating collagenases and degrading fibrin as well as extracellular matrix proteins. Increased expression of α-enolase is associated with increased migration and invasion of cancer cells, and decreased metastasis-free survival in patients with several cancer types, including non-small cell lung, pancreatic, breast and colorectal cancers. Due to its overexpression in a range of cancer types and multi-functional roles in key areas of tumour metabolism and metastasis, α-enolase may be useful as a universal cancer prognostic biomarker or therapeutic target.

Keywords: Alpha-enolase, ENO1, metastasis, migration, invasion, proliferation, plasminogen-binding receptor
INTRODUCTION

All cancer cells have a high energy demand due to their increased rate of proliferation\(^1\). Increased glycolysis is considered a hallmark of cancer\(^2\) and investigating glycolytic enzymes may yield new therapeutic approaches for cancer treatment. Enolases are key glycolytic enzymes\(^3\), and increased expression of one isoform, \(\alpha\)-enolase, has been identified in several cancer types\(^4-22\). This review provides an overview of the expression of \(\alpha\)-enolase and key functions it controls in cancer cells, with a focus on the potential role of \(\alpha\)-enolase as a cancer prognostic biomarker or therapeutic target.

ENOLASE IS A GLYCOLYTIC ENZYME THAT HAS THREE ISOFORMS

Enolases (EC 4.2.1.11) are metalloenzymes that catalyse the dehydration of 2-phospho-\(D\)-glycerate to phosphoenolpyruvate in the glycolysis pathway [Figure 1], and catalyse the hydration of phosphoenolpyruvate to 2-phospho-\(D\)-glycerate in the reverse anabolic pathway during gluconeogenesis\(^3\). In mammals, the three genes \(ENO1\), \(ENO2\), \(ENO3\) encode three isoforms, with expression being regulated in a tissue-specific manner. Alpha-enolase (\(ENO1\)) is ubiquitously expressed, whereas \(\gamma\)-enolase (\(ENO2\)) is primarily expressed in neurons and neuroendocrine tissues, and \(\beta\)-enolase (\(ENO3\)) in muscle tissues\(^23\). Active enolase consists of a dimer in which two subunits face each other in an antiparallel formation\(^24\), and requires two non-covalently bound magnesium ions as cofactors for enzyme activity\(^25\).

ALPHA-ENOLASE IS A MULTI-FUNCTIONAL PROTEIN

Although many glycolytic enzymes are considered to be housekeeping proteins, \(\alpha\)-enolase expression can vary dramatically depending on the stress, metabolic, or pathological state of the cell. A retrospective proteomic meta-analysis identified that \(\alpha\)-enolase was the most differentially expressed protein in humans and rodents irrespective of tissue type and pathological condition\(^26\). Disrupted expression and/or activity of \(\alpha\)-enolase has been reported in several pathologies with distinct aetiologies, including Alzheimer's disease, systemic sclerosis, rheumatoid arthritis, bacterial infections and hepatic fibrosis\(^27-38\).

Apart from its role in the glycolytic pathway, recent studies have revealed that \(\alpha\)-enolase is a multi-functional protein that controls a variety of cellular processes, including proliferation, survival, migration and invasion. Additionally, using an alternative transcription start codon, the \(ENO1\) gene can produce a 37 kDa protein, \(c\)-myc promoter-binding protein (MBP-1). MBP-1 localises to the nucleus, where it acts as a transcription repressor by binding to the \(c\)-myc P2 promoter\(^39\), helping regulate and maintain the function of the glycolysis pathway.

ALPHA-ENOLASE EXPRESSION IS ALTERED IN TUMOURS AND VARIES WITH CANCER TYPE

The overexpression of \(\alpha\)-enolase is associated with tumour development via a process known as aerobic glycolysis or the Warburg effect. The Warburg effect has been hypothesised to be an adaptation mechanism in cancer cells to support the biosynthetic requirements of rapid proliferation. Alpha-enolase expression has been shown to be altered at the mRNA and/or protein level in a range of tumours [Table 1], and generally upregulated in most, including acute myeloid leukaemia (AML), glioma, melanoma, lymphoma, and colorectal, endometrial, gastric, head and neck, liver, ovarian and pancreatic cancer\(^4-22\).

ALPHA-ENOLASE CAN SHUTTLE BETWEEN CELLULAR COMPARTMENTS

Alpha-enolase can be localised to the cytoplasm and plasma membrane, as well as secreted in exosomes, and its location varies with cancer type. For example, in pancreatic, breast and lung tumours, \(\alpha\)-enolase is localised to the plasma membrane\(^22,44,45\), whereas in melanoma, mesothelioma, non-small cell lung, colorectal and prostate cancer \(\alpha\)-enolase is also secreted and found in exosomes\(^46-49\). Alpha-enolase can
shuttle between compartments, performing different functions when in different subcellular locations, such as surface membrane plasminogen binding, controlling the overall metabolic state of the cell, stress-related or acting as a heat-shock protein, RNA transport, mitochondrial membrane stability, and cell cycle control.

INCREASED ALPHA-ENOLASE EXPRESSION ENHANCES CELL PROLIFERATION IN A VARIETY OF CANCERS

In most solid tumours, the Warburg effect causes an increase in total glycolysis under both hypoxic and normoxic conditions [Figure 2]. Enhanced cell proliferation leads to increased anabolic needs, and cancer cells remodel metabolic processes by diverting nutrients to anabolic pathways to satisfy increased cellular energy demands. Therefore, the Warburg effect may provide cancer cells with an advantage when competing with non-cancerous tissues for nutrients. This suggests that increased $\alpha$-enolase expression will contribute to enhanced proliferation commonly observed in cancer cells.

Indeed, upregulated $\alpha$-enolase expression has been shown to regulate cell proliferation in various solid tumours in vitro, and to increase tumour growth in a HCT116 colorectal cancer xenograft model in vivo [Table 2]. Conversely, silencing of $\alpha$-enolase in glioma, pancreatic, lung, endometrial, colorectal and breast cancer cells was found to induce cell cycle arrest and senescence, and also to reduce tumour volume in CFPAC-1 pancreatic, MDA-MB-231 breast and U-87MG glioma xenograft models in vivo. Furthermore, $\alpha$-enolase is also implicated in the control of apoptosis and sensitivity to chemotherapeutic agents, as silencing of $ENO1$ in cancer cells induced apoptosis and increased sensitivity to cisplatin and 5-fluorouracil in vitro. Unexpectedly, cells respond to $\alpha$-enolase silencing by inducing catabolic adaptations that lead to restoration of pyruvate, acetyl-CoA bulk and oxidative phosphorylation.

![Diagram of the glycolysis pathway](image1)

Figure 1. The glycolysis pathway. Enolases catalyse the dehydration of 2-phospho-D-glycerate (2-P-glycerate) to phosphoenolpyruvate (P-enolpyruvate) in the glycolysis pathway.
and exhibit an increased expression of proteins involved in both oxidative stress- and sirtuin-induced autophagy. Taken together, these studies demonstrate that α-enolase is an important regulator of tumour cell metabolism, proliferation and survival, which by definition make it a perfect target for anticancer therapy.

**Table 1. The expression of α-enolase is altered in cancer**

| Cancer                   | Sample type                                                                 | Detection method                        | Alteration (frequency of alteration) | Ref.  |
|--------------------------|------------------------------------------------------------------------------|-----------------------------------------|--------------------------------------|-------|
| AML                      | Peripheral blood and bone marrow from AML patients (n = 41) and healthy patients (n = 20) | Microarray                              | Increased                            | [4]   |
| Breast cancer            | Breast cancer tissue with adjacent matched normal tissue (n = 24)            | Western blot                            | Increased (100%)                     | [9]   |
| Cervical cancer          | Squamous cell carcinoma (n = 33) and normal cervical tissue (n = 17)         | Quantitative real-time PCR              | Decreased                            | [10]  |
| Colorectal cancer        | SW620 cell line (lymph node metastasis) compared to SW480 (primary lesion)  | 2D-DIGE                                 | Increased                            | [41]  |
| Endometrial carcinoma    | Endometrial cancer (n = 100), endometrial atypical hyperplasia (n = 22) and normal endometrium tissues (n = 20) | IHC                                      | Increased in endometrial cancer (52%) and atypical hyperplasia (31.8%) compared to normal | [12]  |
| Gastric cancer           | Gastric cancer and adjacent normal tissue (n = 94)                          | IHC                                      | Increased                            | [13]  |
| Glioma                   | Primary glioblastoma tissue (n = 24)                                        | IHC, Quantitative real-time PCR         | Increased (48%)                      | [14]  |
| Glioma                   | Glioma (n = 136) and normal brain (n = 15)                                   | IHC, Quantitative real-time PCR and IHC | Increased (68%)                      | [5]   |
| Head and neck cancer     | Head and neck cancer and adjacent normal tissue (n = 44)                     | Real-time PCR                           | Increased (50%)                      | [15]  |
| HCC                      | HCC (n = 374 and n = 1309), adjacent matched tissue (n = 50), and normal tissue (n = 1442) from TCGA and GEO data source; tissue microarray (93 HCC and 87 normal liver tissues) | Microarray and IHC                      | Increased                            | [16]  |
| Lymphoma                 | Pretherapeutic tumour biopsies from peripheral T-cell lymphoma not otherwise classified (n = 87) | IHC                                      | Increased                            | [8]   |
| Melanoma                 | A375, MeWo, MEL-HQ, Colo-800, Colo-853 melanoma cell lines and a normal melanocyte cell line | 2D-DIGE and nano-HPLC-chip ion trap mass spectrometry | Increased                            | [7]   |
| NSCL                     | Primary NSCLC tissue and matched normal lung (n = 26) from RNA and primary NSCLC tissue (n = 55) and normal lung tissue (n = 17) | Real-time PCR and IHC                   | Increased                            | [17]  |
| Ovarian cancer           | Ovarian cancer (n = 4) and ovarian tissue from endometriosis (n = 1)         | 2D-DIGE and LC-MS/MS                   | Increased (16%)                      | [18]  |
| Pancreatic cancer        | Pancreatic cancer and adjacent normal tissue (n = 31)                        | IHC                                      | Increased                            | [20]  |
| Primary pancreatic and adjacent normal tissue (n = 3) | Western blot                        | Increased (48%)                      | [21]  |
| Prostate cancer          | Exosomes from prostate cancer cell lines                                     | Western blot and mass spectrometry      | Decreased                            | [43]  |

2D-DIGE: two-dimensional differential in gel electrophoresis; HCC: hepatocellular carcinoma; HPLC: high performance liquid chromatography; IHC: immunohistochemistry; LC-MS/MS: liquid chromatography tandem mass spectrometry; MALDI-TOF: matrix assisted laser desorption/ionization time of flight; NSCLC: non-small cell lung cancer; PCR: polymerase chain reaction; AML: acute myeloid leukaemia; TCGA: the cancer genome atlas; GEO: gene expression omnibus
ALPHA-ENOLASE IS A SURFACE PLASMINOGEN-BINDING RECEPTOR

In addition to a role in cell proliferation and survival, \( \alpha \)-enolase located on the plasma membrane acts as a plasminogen-binding receptor\(^{[55]} \) [Figure 3]. Plasminogen is a zymogen, which is converted to plasmin in the presence of the activators tissue plasminogen activator or urokinase-type plasminogen activator (uPA)\(^{[65]} \). This cell surface interaction concentrates protease activity in the tissue surrounding the cell, protecting plasmin from inactivation by circulating \( \alpha \)-2-antiplasmin\(^{[66]} \). Plasmin activates collagenases and degrades fibrin and other matrix proteins, resulting in cell migration and invasion into tissue, ultimately underpinning cancer metastasis and relapse.

Role in invasion and migration

Overexpression of \( \alpha \)-enolase has been shown to increase the migration and invasion of hepatocellular carcinoma, colorectal and gastric cancer cells \textit{in vitro}\(^{[11,58,60,61,67]} \) and to enhance colorectal cancer metastasis \textit{in vivo}\(^{[11]} \), demonstrating that it is an important driver of metastasis in multiple cancer types [Table 3]. Conversely, knockdown or pharmacological inhibition of \( \alpha \)-enolase decreased the migration and invasion of glioma, colorectal, pancreatic and endometrial carcinoma \textit{in vitro}\(^{[6,12,63,68,69]} \), and decreased tumourigenesis and metastasis of endometrial carcinoma \textit{in vivo}\(^{[12]} \). Furthermore, binding of recombinant \( \alpha \)-enolase to the surface of prostate cancer cells was shown to promote cell migration via its plasminogen receptor activity\(^{[70]} \). By contrast, anti-\( \alpha \)-enolase monoclonal antibodies inhibited plasminogen-dependent invasion of human pancreatic cancer cells \textit{in vitro} and metastasis formation \textit{in vivo}\(^{[23]} \) and also lung cancer cell invasion \textit{in vitro} and growth \textit{in vivo}\(^{[22]} \) [Table 3].
Table 2. Alpha-enolase controls cancer cell proliferation and survival

| Cancer               | Experimental model                                                                 | Effect of modulation of ENO1 expression                                                                 | Ref. |
|----------------------|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|------|
| Bladder cancer       | Overexpression and knockdown in T253 and 5637 cells                                | Knockdown decreased cell proliferation and colony formation. Overexpression increased cell proliferation and colony formation | [59] |
| Breast cancer        | Downregulation in MDA-MB-231 cells                                                 | Downregulation decreased cell proliferation and survival in vitro and reduced tumour growth in vivo     | [62] |
| Colorectal cancer    | Overexpression and knockdown in HCT116 cells                                       | Overexpression promoted cell proliferation and tumour growth in vivo; Decreased expression decreased cell proliferation and tumour growth in vivo | [11] |
| Endometrial carcinoma| Knockdown in HEC-1B and Ishikawa                                                 | Decreased expression reduced cell proliferation in vitro and tumourigenesis in vivo                     | [12] |
| Gastric cancer       | Knockdown in MGC-803 and MKN45 cells                                              | Knockdown led to cell cycle arrest at the G1 phase and promoted apoptosis, and repressed the rate of cell proliferation and colony formation | [13] |
|                      | Knockdown in MKN45 cells                                                          | Knockdown decreased cell proliferation, induced apoptosis and increased sensitivity to chemotherapeutics | [64] |
|                      | Knockdown in AGS cells and overexpression in SGC7901 cells                        | Knockdown decreased proliferation and colony formation, whereas overexpression increased cell proliferation and colony formation | [60] |
|                      | Overexpression in AGS cells                                                      | Overexpression increased cell proliferation and colony formation                                       | [61] |
| Glioma               | Knockdown in U-87MG cells                                                         | Knockdown suppressed cell proliferation and colony formation in vitro and tumour growth in vivo         | [6]  |
| HCC                  | Knockdown in HCC cells                                                           | Knockdown inhibited cell growth                                                                       | [58] |
| Lung cancer          | Knockdown in NCI-H441 cells                                                       | Knockdown decreased cell proliferation and survival in vitro                                           | [62] |
| Pancreatic cancer    | Knockdown in CFPAC-1 cells                                                       | Downregulation decreased cell proliferation and survival in vitro and reduced tumour growth in vivo     | [62] |
| Retinoblastoma       | Knockdown in Y79 cells and overexpression in Meri-RB1 cells                      | Knockdown led to cell cycle arrest at the G1 phase, decreased cell proliferation and increased apoptosis. Overexpression increased cell proliferation. | [57] |

HCC: hepatocellular carcinoma

Figure 3. Alpha-enolase acts as a surface plasminogen-binding receptor to mediate cancer cell invasion and metastasis formation. PLG binds to its receptors and is subsequently converted to PLIN by plasminogen activators (e.g., uPA). Cell surface-associated plasmin facilitates degradation of the ECM, allowing tumour cells to invade and metastasise into other tissues. PLG: plasminogen; PLIN: plasmin; ECM: extracellular matrix; uPA: urokinase-type plasminogen activator
ALPHA-ENOLASE IS A TUMOUR-ASSOCIATED ANTIGEN

Externalisation of $\alpha$-enolase by cancer cells exposes it to the immune system as a tumour-associated antigen that has been found to induce autoantibody production in cancer patients, including those with acute and chronic leukaemias, melanoma, and lung, breast, gastric and pancreatic cancers [22,45,73-79]. In pancreatic cancer, T cells activated by $\alpha$-enolase-pulsed dendritic cells lysed pancreatic cancer cells, but not normal human keratinocytes in vitro and inhibited CF-PAC-1 tumour growth in vivo [22]. In oral squamous cell carcinoma, an HLA-DR8-restricted human $\alpha$-enolase peptide was recognised by CD4+ T cells and produced a cytotoxic response against OSC-20 cells [80]. Additionally, vaccination with ENO1 in Kras G12D/Cre and Kras G12D/Trp53R172H mice prior to development of pancreatic carcinoma delayed tumour growth and increased survival [81]. Taken together, these studies suggest that immune responses directed against $\alpha$-enolase may be immunostimulatory and ultimately beneficial to patients.

ALPHA-ENOLASE IS A PROGNOSTIC FACTOR FOR MULTIPLE CANCER TYPES

In addition to being overexpressed in many cancers, $\alpha$-enolase has been identified as a putative prognostic biomarker in a range of tumour types [Table 4]. Whilst ENO1 expression was not associated with tumour stage in colorectal cancers, it was significantly correlated with tumour size and presence of distant metastases [11]. Alpha-enolase expression was positively correlated with lymph node status in endometrial and gastric cancer patients [12,13,60], and increased $\alpha$-enolase expression in endometrial, gastric, lung, lymphoma and hepatocellular cancer patients was associated with worse overall survival [16,12,15,14,77,82]. Furthermore, increased $\alpha$-enolase expression was correlated with worse distant metastasis-free survival in breast cancer patients [9], worse disease-free survival in hepatocellular carcinoma and chordoma [8,83] and worse progression-free survival in lung cancer patients [72]. By contrast, downregulation of $\alpha$-enolase is a predictor of poor prognosis in clear cell renal cell carcinoma (ccRCC) [84], demonstrating that $\alpha$-enolase may control different cellular functions in ccRCC when compared to other cancers.

Autoantibodies generated against $\alpha$-enolase in its capacity as a tumour-associated antigen represent an additional type of prognostic biomarker that may be assayed in serum. The presence of autoantibodies against $\alpha$-enolase correlated with longer disease-free survival and overall survival in pancreatic and lung cancer patients [43,45-47] [Table 4]. Furthermore, compared with healthy individuals, $\alpha$-enolase antibodies...
are decreased in stage IV lung and breast cancers\textsuperscript{[69]}, and are lower in stage III/IV than in stage I/II lung cancer patients\textsuperscript{[70]}. By contrast, the presence of anti-α-enolase antibodies in sera from chronic lymphocytic leukaemia (CLL) patients is predictive of a shorter time to first treatment\textsuperscript{[71]}, indicating that the presence of α-enolase antibodies are indicative of a disrupted immune system in CLL. Taken together, these studies suggest that autoantibodies against α-enolase are a good prognostic factor in pancreatic, lung and breast cancers, and provide further evidence that targeting α-enolase may be beneficial in solid tumours.

### ENOLASE INHIBITORS ARE POTENTIAL ANTICANCER AGENTS

Due to its important cancer-related roles, enolase is one of several glycolytic enzymes being examined as a potential anticancer therapeutic target. Polyamine sulphonamide analogues have proven particularly effective at inhibiting α-enolase activity. Two such compounds have been further developed and shown to

| Cancer Type | Sample Type | Patient Outcome | Ref. |
|-------------|-------------|-----------------|------|
| Breast cancer | Kaplan-Meier Plotter database (n = 5143 breast cancer patients) | Increased mRNA expression correlated with worse DMFS | [9] |
| Chordoma | Cervical or sacral spine chordomas (n = 39) | Increased protein expression was associated with worse disease-free survival | [83] |
| CLL | Sera from CLL patients (n = 86) | Presence of anti-α-enolase antibodies was predictive of a shorter time to first treatment | [90] |
| Colorectal cancer | Colorectal tumour tissues (n = 41) | Protein expression correlated with tumour size and distant metastasis | [11] |
| Endometrial cancer | Endometrial cancer tissue (n = 100) | Protein expression correlated with lymph node status and depth of myometrial invasion; patients with high expression had worse OS | [89] |
| Gastric cancer | Gastric cancer tissue (n = 76) | Protein expression correlated with lymph node metastasis and TNM stage | [60] |
| | TCGA dataset (n = 410 gastric cancer patients); | Protein expression correlated with high TNM stage and metastasis; Increased mRNA was associated with poor OS | [13] |
| HCC | TCGA dataset (n = 374 HCC tissues); meta-analysis of 12 cohorts in GEO database | Increased mRNA was associated with poor OS and disease-free survival; Protein expression correlated with high TNM stage and was negatively correlated with OS | [16] |
| | Sera from HCC patients (n = 61) | Anti-α-enolase antibodies were lower in patients without microvascular invasion compared to those with microvascular invasion | [91] |
| Lung cancer | Kaplan-Meier Plotter database (n = 348 lung cancer patients); Lung adenocarcinoma tissue (n = 37) | Increased mRNA and protein was associated with poor OS; Increased expression was associated with bone metastasis incidence | [82] |
| | Malignant pleural effusion samples (n = 54) | High protein was associated with poor OS and PFS | [73] |
| | Plasma from non-small lung carcinoma patients (n = 85) | Patients with a higher increase in anti-α-enolase had a lower hazard ratio and better PFS | [85] |
| | Sera from patients with lung cancer (n = 72), benign lung diseases (n = 69), and healthy individuals (n = 70) | Autoantibodies were higher in lung cancer sera compared with sera from normal and benign lung disease patients; Autoantibodies were higher in stage I/II than in stage III/IV | [89] |
| Lymphoma | Peripheral T-cell lymphoma not otherwise classified tissue (n = 87) | Increased protein correlated with worse OS | [8] |
| Pancreatic cancer | Sera from pancreatic ductal adenocarcinoma patients (n = 120) | Presence of auto-antibodies correlated with a better clinical outcome | [86] |
| | Sera and PBMCs from pancreatic ductal adenocarcinoma patients (n = 15) | Patients with > 20% peripheral α-enolase-specific T cells or anti-α-enolase antibodies showed a better OS | [87] |
| ccRCC | Primary ccRCC tissue (n = 360) and TCGA dataset (n = 428) | Negative correlation between protein expression, tumour stage and grade. Patients with higher mRNA had lower hazard ratio of recurrence and longer OS | [84] |

ccRCC: clear cell renal cell carcinoma; CLL: chronic lymphoblastic leukaemia; DMFS: distant metastasis-free survival; GEO: gene expression omnibus; HCC: hepatocellular carcinoma; OS: overall survival; PBMC: peripheral blood mononuclear cell; PFS: progression-free survival; TCGA: the cancer genome atlas; TNM: tumour node metastasis
be cytotoxic to KG-1 (AML) cells and to the AML leukaemic stem cell fraction, with minimal effects on normal healthy stem cells. This report highlights that $\alpha$-enolase is an actionable therapeutic target that may be useful in the treatment of cancer, particularly AML.

CONCLUSION

Alpha-enolase plays a supportive role in cancer progression and has been implicated in three of the hallmarks of cancer: cellular energetics and metabolism; cell proliferation; and invasion and migration. In cancer cells, $\alpha$-enolase is overexpressed and localised on the surface, where it acts as a key promotor of metastasis, driving invasion through plasminogen activation and extracellular matrix degradation. In several cancer types, patients develop an immune response against $\alpha$-enolase, and anti-$\alpha$-enolase antibodies can be detected in their sera. Increased expression of $\alpha$-enolase mRNA, proteins or autoantibodies are associated with decreased metastasis-free survival in several cancer types, including non-small cell lung, pancreatic, breast and colorectal cancers. Future examination of the expression and function of $\alpha$-enolase in cancers may ultimately result in $\alpha$-enolase becoming a therapeutic target and prognostic biomarker for a range of cancer types.

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