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ORIGINAL RESEARCH

The prognostic roles of ALDH1 isoenzymes in gastric cancer

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Abstract: Increased aldehyde dehydrogenase 1 (ALDH1) activity has been determined to be present in the stem cells of several kinds of cancers including gastric cancer (GC). Nevertheless, which ones of ALDH1's isoenzymes are leading to ALDH1 activity remains elusive. In this study, we examined the prognostic value and hazard ratio (HR) of individual ALDH1 isoenzymes in patients with GC using "The Kaplan–Meier plotter" database. mRNA high expression level of *ALDH1A1* was not found to be significantly correlated with the overall survival (OS) of all patients with GC followed for 20 years, HR =0.86 (95% confidence interval [CI]: 0.7–1.05), *P*=0.13. mRNA high expression level of *ALDH1A2* was also not significantly correlated with OS for all patients with GC, HR =1.13 (95% CI: 0.91–1.41), *P*=0.25. mRNA high expression level of *ALDH1A3* was found to be significantly correlated with worsened OS in either intestinal-type patients, HR =2.24 (95% CI: 1.44–3.49), *P*=0.00026, or diffuse-type patients, HR =1.91 (95% CI: 1.02–3.59), *P*=0.04. Interestingly, mRNA high expression level of *ALDH1B1* was found to be significantly correlated with better OS for all patients with GC, HR =0.66 (95% CI: 0.53–0.81), *P*=7.8e–05, and mRNA high expression level of *ALDH1L1* was found to be significantly correlated with worsened OS for all patients with GC, HR =1.23 (95% CI: 1–1.51), *P*=0.048. Furthermore, our results also indicate that ALDH1A3 and ALDH1L1 are potential major contributors to the ALDH1 activity in GC, since mRNA high expression levels of *ALDH1A3* and *ALDH1L1* were found to be significantly correlated with worsened OS for all patients with GC. Based on our study, ALDH1A3 and ALDH1L1 are potential prognostic markers and therapeutic targets for patients with GC.

Keywords: KM plotter, cancer stem cell, ALDH1, hazard ratio, prognosis

Introduction

According to the World Health Organization, gastric cancer (GC), also known as stomach cancer, is the second most common cause of cancer-related death with 800,000 deaths caused by GC each year globally.[1] Despite the progresses in early diagnosis and multimodal therapeutic modalities, at diagnosis, GC remains difficult to cure and prognosis remains poor for advanced disease in Western countries.[2,3] Thus, in order to enhance the clinical consequence of patients with GC, exploration on the molecular mechanism of occurrence and progression of GC, as well as the development of prognostic biomarkers and drug targets, are still demanded and will assist to identify patients with high chances of GC recrudescence and deliver better prognosis and personalized treatments.

The aldehyde dehydrogenase 1 (ALDH1) family is detected at high levels in stem cells (SCs).[4–6] ALDH1 activity has been discovered to be increased in multiple myeloma, myeloid leukemia, and a number of solid cancers.[7–11] Wakamatsu et al[12] first showed that cancer stem cell (CSC) markers, the level of ALDH1 positivity, are significantly higher in metastatic diffuse-type lymph node than in the primary tumor.

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Levi et al[13] also observed that CSC markers, ALDH1, CD166, and LGR5, were detected in very low levels in normal human gastric mucosa, in contrast, significantly increased in gastric adenocarcinomas. Recently, Li et al reported that the expression of ALDH1A1 protein was significantly correlated with depth invasion, lymph node metastasis, stage of disease, as well as recurrence-free survival (RFS) and overall survival (OS).[14] However, the prognostic role of most of the individual ALDH1 isoenzyme in GC has not been determined. In addition, which ones of ALDH1's isoenzymes are causing ALDH1 activity in GC remains elusive.

The "Kaplan–Meier plotter" (KM plotter) was developed from the database of Gene Expression Omnibus.[15] KM plotter can be utilized for the determination of prognostic role of individual genes in patients with cancer.[16,17] Several genes so far have been reported using KM plotter in human breast cancer,[18–26] as well as in ovarian and lung cancer.[27] In the current study, we used KM plotter database and reported the prognostic role of individual ALDH1 isoenzymes in human patients with GC.

Materials and methods

We used an online database[16] to determine the relevance of individual *ALDH1* members' mRNA expression to OS. Currently, breast cancer,[16] lung cancer,[28] ovarian cancer,[29] and GC databases have been generated. All the cancer datasets were selected from Gene Expression Omnibus,[15] Cancer Biomedical Informatics Grid,[30] and The Cancer Genome Atlas.[28,31] The database had a collection of clinical data including sex, perforation history, Lauren classification, differentiation, stage, HER2 status, and treatment. The patients with GC were followed up for 20 years. The database was finally created using gene expression data and survival information of 593 patients with GC. Five *ALDH1* isoenzymes (*ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B1*, and *ALDH1L1*) were entered into the database (http://kmplot.com/analysis/index.php?p=service&cancer=breast)[28] to get KM survival plots. The certain gene mRNA expression above or below the median separates the cases into high expression and low expression. Hazard ratio (HR) and log rank *P* were determined and displayed.

Results

There are six sub-members in the ALDH1 family. We summarized their characteristics and listed them in Table 1. As Wu et al[26] reported, only *ALDH1L2* was not found in www.kmplot.com among all the six ALDH1 isoenzymes, probably due to its low expression.

We first checked the prognostic role of mRNA expression of *ALDH1A1* in the database. The valid gene Affymetrix ID is 212224_at (*ALDH1A1*). For all patients, survival curves are plotted (n=593; Figure 1A), for intestinal type (n=186; Figure 1B), and for diffuse type (n=106; Figure 1C). *ALDH1A1* mRNA high expression was not found to be correlated with the OS for all patients with GC followed for 13 years, HR =0.86 (95% confidence interval [CI]: 0.7–1.05), *P*=0.13. *ALDH1A1* mRNA high expression was also not found to be correlated with OS in intestinal-type patients, HR =0.72 (95% CI: 0.49–1.04), *P*=0.078, and in diffuse-type patients, HR =1.52 (95% CI: 0.87–2.66), *P*=0.13.

Then, we checked the prognostic role of mRNA expression of *ALDH1A2* in the database. The valid gene Affymetrix ID is: 207015_s_at (*ALDH1A2*). *ALDH1A2* mRNA high expression was also not found to be correlated with OS for all patients with GC, HR =1.13 (95% CI: 0.91–1.41), *P*=0.25 (Figure 2A). Interestingly, *ALDH1A2* mRNA high expression was found to be correlated with worsened OS in intestinal-type patients, HR =1.47 (95% CI: 0.99–2.19), *P*=0.057 (Figure 2B). In contrast, *ALDH1A2* mRNA high expression was found to

Table 1 Alternatively spliced variants and characterization of ALDH1 isoenzymes

Isoenzymes	Alternatively spliced variants	Cellular localization	Tissue distribution	Associated diseases
ALDH1A1	ALDH1A1_v2	Cytosol	Brain, breast, Lung, pancreas, kidney, liver, etc	Alcoholism
ALDH1A2	ALDH1A2 v2 ALDH1A2_v3 ALDH1A2_v4	Cytosol	Kidney, liver, testis	Schizophrenia, spina bifida
ALDH1A3	ALDH1A3_v2	Cytosol	Breast, skeletal muscle, lung, kidney, etc	Autosomal recessive anophthalmia/microphthalmia
ALDH1B1	N/A	Mitochondria	Liver, heart, kidney, brain, prostate	Alcohol consumption and diabetes
ALDH1L1	N/A	Cytosol	Kidney, liver, skeletal muscle	Ischemic stroke
ALDH1L2	ALDH1L2 v2 ALDH1L2_v2	Mitochondria	Brain, heart, pancreas	N/A

Abbreviation: N/A, not applicable.

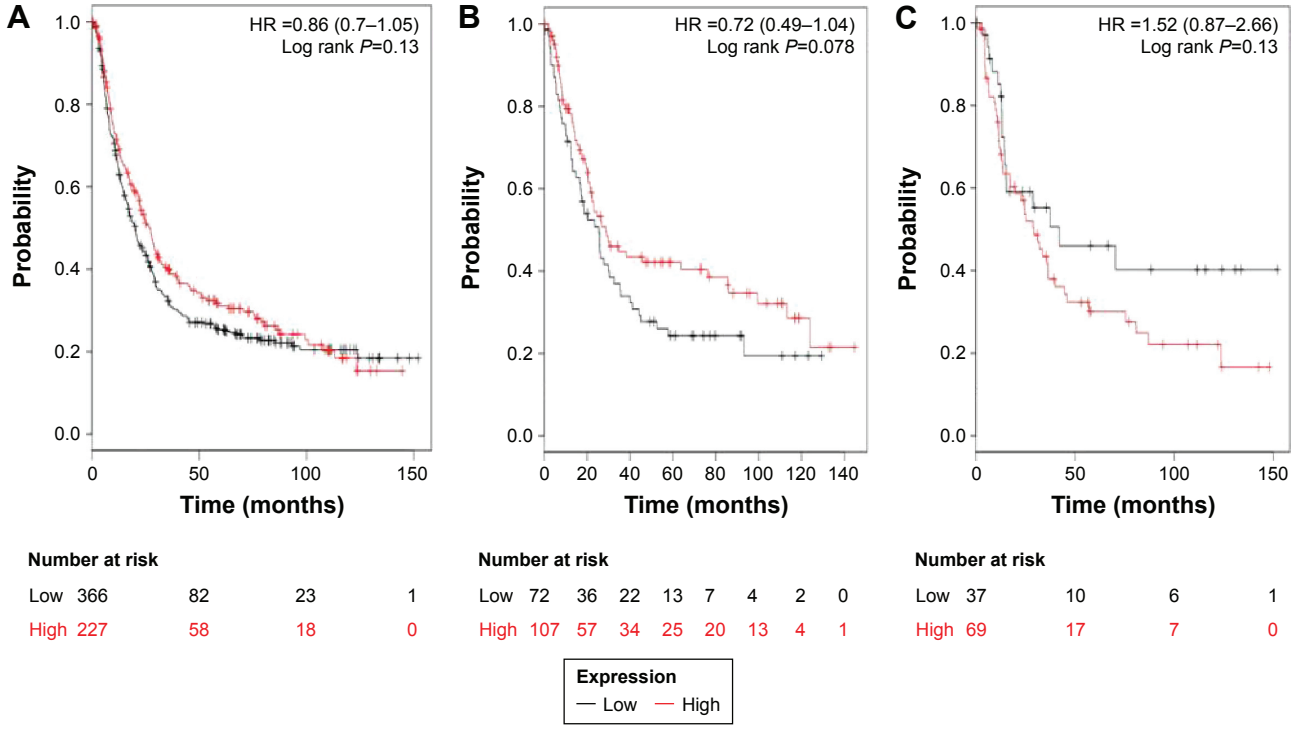

Figure 1 The prognostic value of *ALDH1A1* expression in the database.
Notes: The valid Affymetrix ID is 212224_at (*ALDH1A1*). (**A**) Survival curves are plotted for all patients (n=593), HR =0.86 (95% CI: 0.7–1.05). (**B**) Survival curves are plotted for intestinal type (n=186), HR =0.72 (95% CI: 0.49–1.04). (**C**) Survival curves are plotted for diffuse type (n=106), HR =1.52 (95% CI: 0.87–2.66).
Abbreviations: HR, hazard ratio; CI, confidence interval.

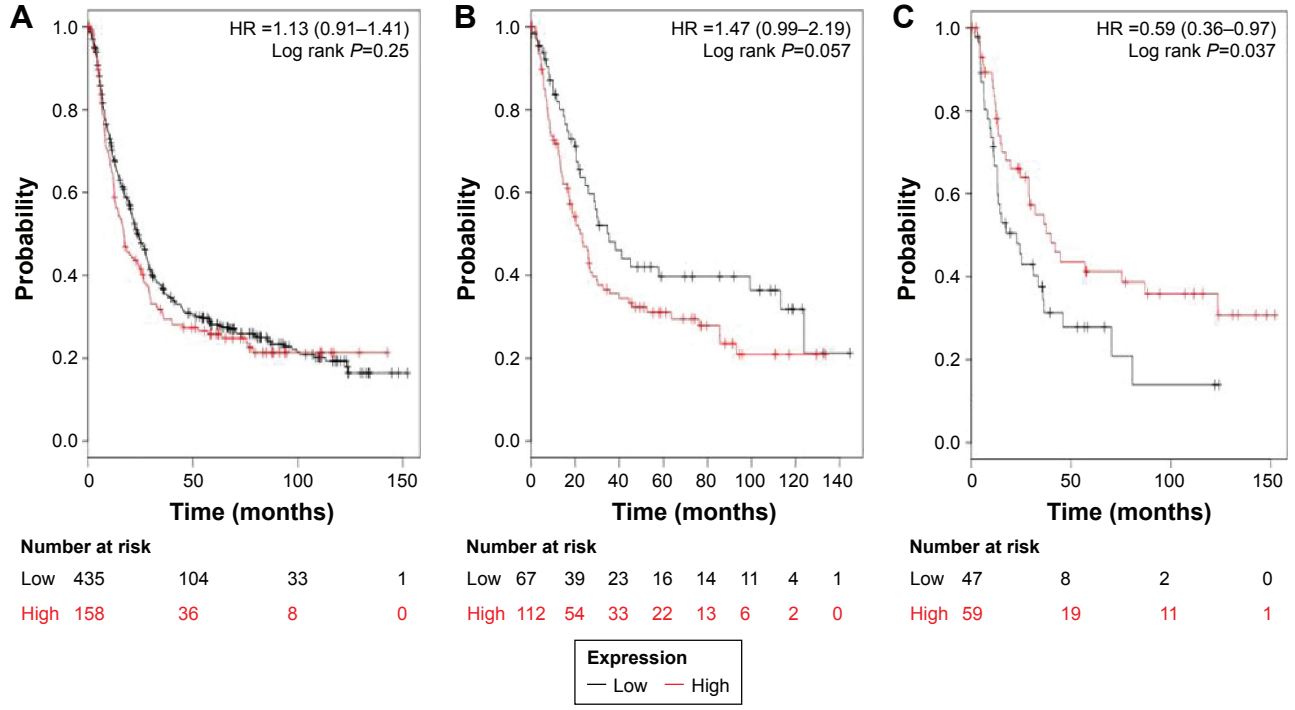

Figure 2 The prognostic value of *ALDH1A2* expression in the database.
Notes: The valid gene Affymetrix ID is: 207015_s_at (*ALDH1A2*). (**A**) Survival curves are plotted for all patients (n=593), HR =1.13 (95% CI: 0.91–1.41). (**B**) Survival curves are plotted for intestinal type (n=186), HR =1.47 (95% CI: 0.99–2.19). (**C**) Survival curves are plotted for diffuse type (n=106), HR =0.59 (95% CI: 0.36–0.97).
Abbreviations: HR, hazard ratio; CI, confidence interval.

be correlated with better OS in diffuse-type patients, HR =0.59 (95% CI: 0.36–0.97), *P*=0.037 (Figure 2C).

Figure 3 shows the prognostic role of mRNA expression of *ALDH1A3* in the database. The valid gene Affymetrix ID is: 203180_at (*ALDH1A3*). The curves show that mRNA expression of *ALDH1A3* above or below the median do not separate the cases into significantly different prognostic groups in all patients with GC, HR =1.19 (95% CI: 0.97–1.46), *P*=0.1 (Figure 3A). However, *ALDH1A3* mRNA high expression was found to be significantly correlated with worsened OS either in intestinal-type patients, HR =2.24 (95% CI: 1.44–3.49), *P*=0.00026 (Figure 3B) or diffuse-type patients, HR =1.91 (95% CI: 1.02–3.59), *P*=0.04 (Figure 3C).

Figure 4 shows the prognostic role of mRNA expression of *ALDH1B1* in the database. The valid gene Affymetrix ID is: 209646_x_at (*ALDH1B1*). *ALDH1B1* mRNA high expression was found to be significantly correlated with better OS for all patients with GC, HR =0.66 (95% CI: 0.53–0.81), *P*=7.8e–05 (Figure 4A). In addition, *ALDH1B1* mRNA high expression was also found to be correlated with better OS in intestinal-type patients, HR =0.7 (95% CI: 0.48–1.02), *P*=0.06 (Figure 4B), but not in diffuse-type patients, HR =1.41 (95% CI: 0.82–2.41), *P*=0.21 (Figure 4C).

Next, we examined the prognostic role of mRNA expression of *ALDH1L1* in the database. The valid gene Affymetrix ID is 205208_at (*ALDH1L1*). *ALDH1L1* mRNA high expression was found to be significantly correlated with worsened OS for all patients with GC, HR =1.23 (95% CI: 1–1.51), *P*=0.048 (Figure 5A). In addition, *ALDH1L1* mRNA high expression was also found to be correlated with worsened OS in intestinal-type patients, HR =1.44 (95% CI: 0.97–2.16), *P*=0.072 (Figure 5B). In contrast, *ALDH1L1* mRNA high expression was found to be significantly correlated with better OS in diffuse-type patients, HR =0.5 (95% CI: 0.31–0.83), *P*=0.0064 (Figure 5C).

For further determination of the correlation of individual ALDH1 isoenzymes with other clinicopathological factors, we determined the correlation with pathological grades (Table 2), clinical grades (Table 3), HER2 status (Table 4), and different choices of treatment (Table 5) of patients with GC. From Table 2, it is found that, except for *ALDH1A1*, other ALDH1 isoenzymes' mRNA high expression is significantly associated with pathological grades. From Table 3, it is found that all the individuals with *ALDH1* mRNA high expression are significantly associated with clinical stages of patients with GC. From Table 4, it is found that *ALDH1A1* and *ALDH1A2* mRNA high expressions are only significantly associated with patients with HER2-negative GC. *ALDH1B1* mRNA high expression is only associated with patients with

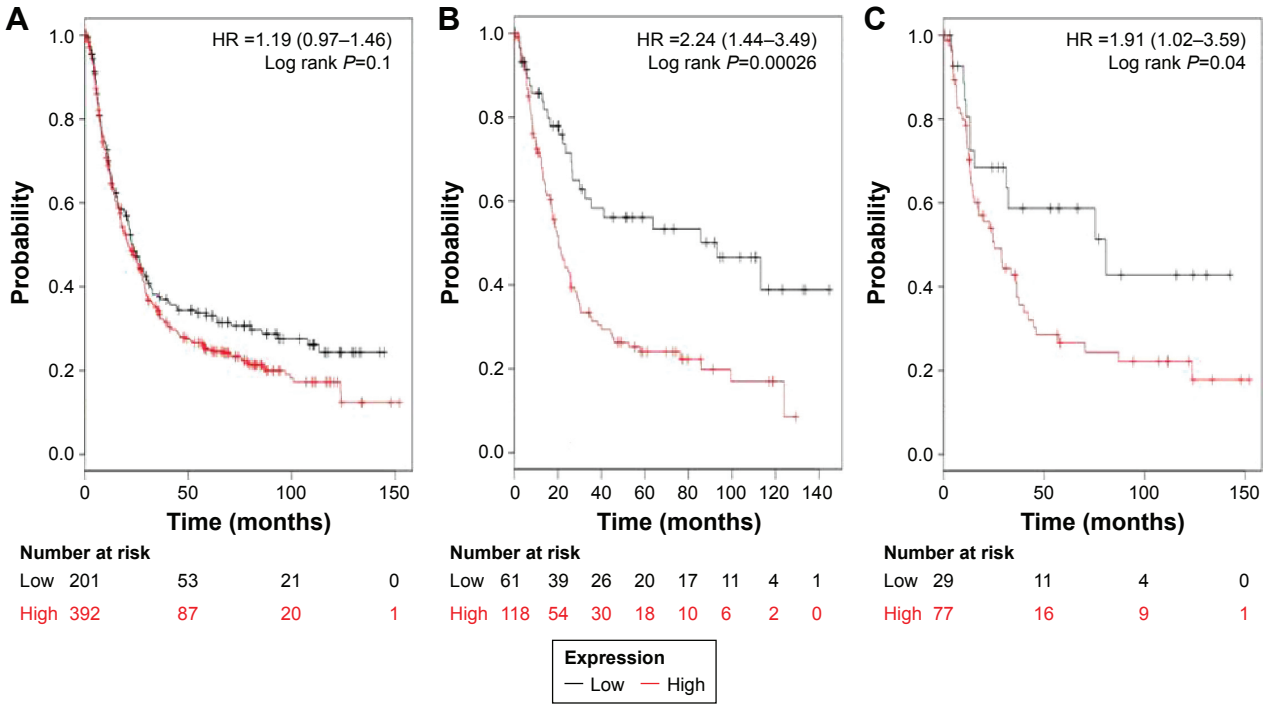

Figure 3 The prognostic value of *ALDH1A3* expression in the database.
Notes: The valid gene Affymetrix ID is: 203180_at (*ALDH1A3*). (**A**) Survival curves are plotted for all patients (n=593), HR =1.19 (95% CI: 0.97–1.46). (**B**) Survival curves are plotted for intestinal type (n=186), HR =2.24 (95% CI: 1.44–3.49). (**C**) Survival curves are plotted for diffuse type (n=106), HR =1.91 (95% CI: 1.02–3.59).
Abbreviations: HR, hazard ratio; CI, confidence interval.

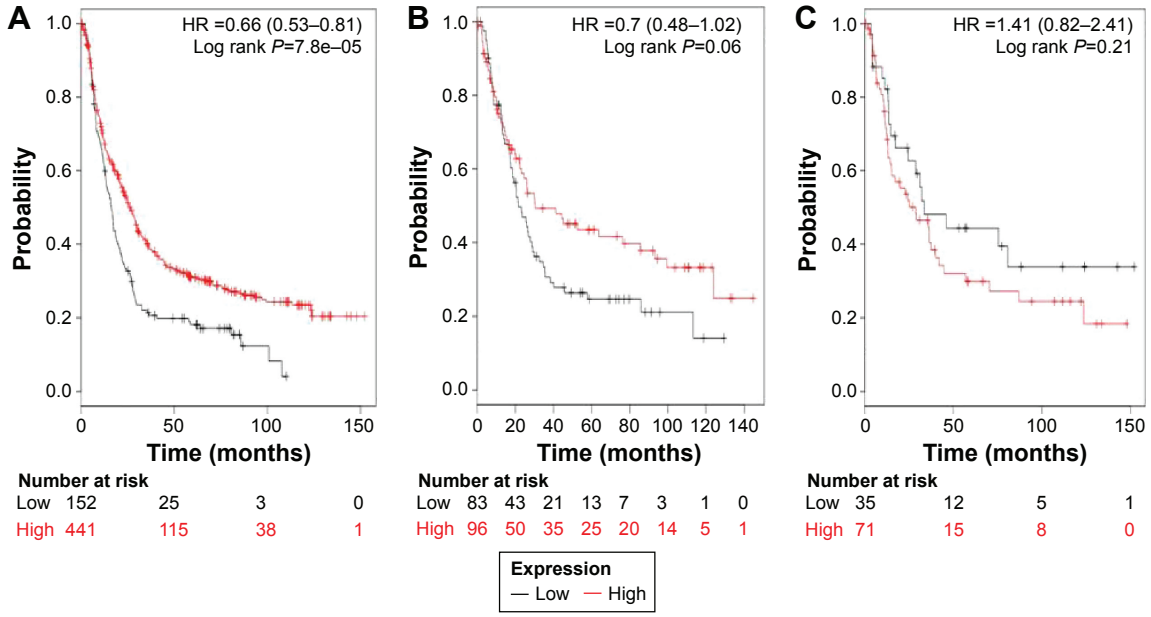

Figure 4 The prognostic value of *ALDH1B1* expression in the database.
Notes: The valid gene Affymetrix ID is: 209646_x_at (*ALDH1B1*). (**A**) Survival curves are plotted for all patients (n=593), HR =0.66 (95% CI: 0.53–0.81). (**B**) Survival curves are plotted for intestinal type (n=186), HR =0.7 (95% CI: 0.48–1.02). (**C**) Survival curves are plotted for diffuse type (n=106), HR =1.41 (95% CI: 0.82–2.41).
Abbreviations: HR, hazard ratio; CI, confidence interval.

HER2-positive GC. *ALDH1A3* and *ALDH1L1* mRNA high expressions are significantly associated with patients with HER2-negative and HER2-positive GC. From Table 5, it is found that with the exception of *ALDH1A2*, other ALDH1 isoenzymes' mRNA high expression is significantly associated with different choice of treatment.

Discussion

Even though ALDH1A1 was first identified as a marker and a characteristic feature of primitive human hematopoietic stem cells isolated from bone marrow[32] and of neural SCs,[33,34] studies have reported that other isoenzymes of ALDH1 (ie, ALDH1A2 and ALDH1A3) are also involved, because

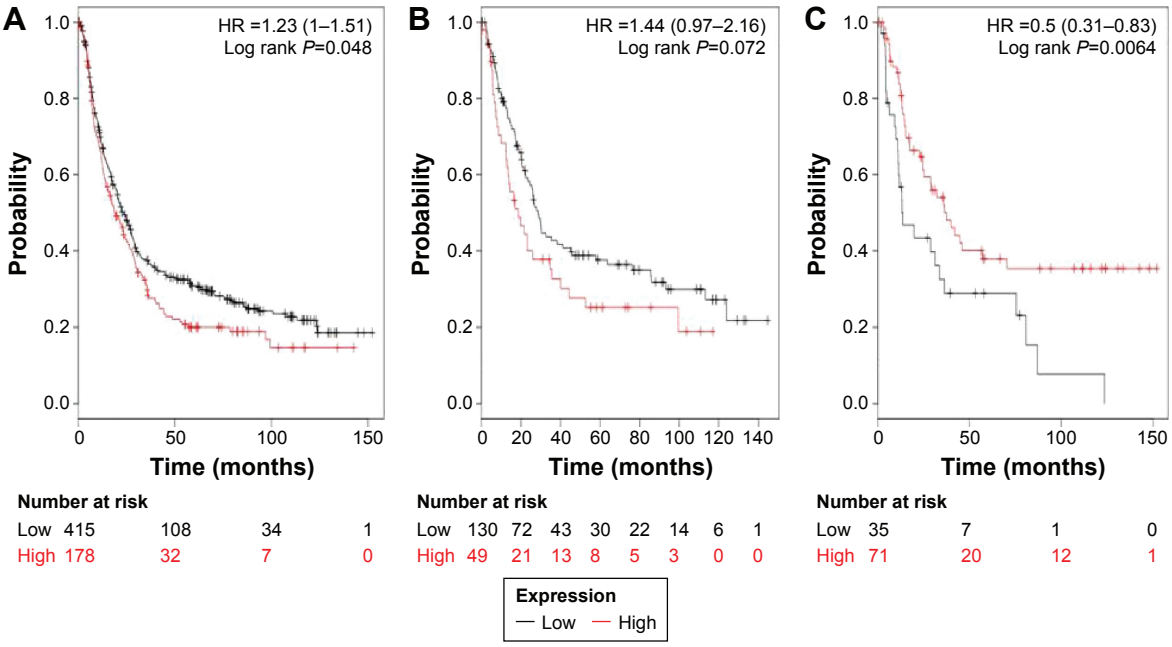

Figure 5 The prognostic value of *ALDH1L1* expression in the database.
Notes: The valid gene Affymetrix ID is: 205208_at (*ALDH1L1*). (**A**) Survival curves are plotted for all patients (n=593), HR =1.23 (95% CI: 1–1.51). (**B**) Survival curves are plotted for intestinal type (n=186), HR =1.44 (95% CI: 0.97–2.16). (**C**) Survival curves are plotted for diffuse type (n=106), HR =0.5 (95% CI: 0.31–0.83).
Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 2 Correlation of ALDH1 isoenzyme mRNA high expression with pathological grades of patients with GC

Isoenzymes	Pathological grades	Cases	HR (95% CI)	*P*-value
ALDH1A1	I	32	0.6 (0.22–1.65)	0.32
	II	67	0.77 (0.4–1.49)	0.43
	III	165	1.49 (1.0–2.23)	0.51
ALDH1A2	I	32	1.53 (0.63–3.69)	0.34
	II	67	1.76 (0.91–3.44)	0.09
	III	165	1.53 (1.03–2.29)	0.035
ALDH1A3	I	32	3.62 (1.48–8.88)	0.0027
	II	67	2.51 (1.3–4.85)	0.0048
	III	165	1.8 (1.2–2.7)	0.0041
ALDH1B1	I	32	0.37 (0.15–0.92)	0.026
	II	67	1.57 (0.74–3.35)	0.24
	III	165	1.29 (0.86–1.93)	0.22
ALDH1L1	I	32	4.55 (1.33–15.57)	0.0082
	II	67	2.19 (0.94–5.09)	0.064
	III	165	0.61 (0.4–0.92)	0.016

Abbreviations: GC, gastric cancer; HR, hazard ratio; CI, confidence interval.

Aldh1A1 deficiency also did not affect Aldefluor staining of hematopoietic cells.[35] It is believed that Aldh1A1 is not a critical regulator of adult SC function or Aldefluor staining in mice, since Aldh1A1 deficiency did not affect the function of SCs from the adult central or peripheral nervous systems. Therefore, this heterogeneity indicates that the other isoforms of ALDH1 are responsible for Aldefluor activity in normal SCs, as well as in CSCs. We hypothesis that individual isoenzymes of ALDH1 may also differently affect the outcome

Table 3 Correlation of ALDH1 isoenzyme mRNA high expression with clinical stages of patients with GC

Isoenzymes	Clinical stages	Cases	HR (95% CI)	*P*-value
ALDH1A1	1	67	0.6 (0.17–2.15)	0.43
	2	140	2.35 (1.12–4.96)	0.021
	3	305	0.63 (0.46–0.86)	0.0032
	4	148	0.48 (0.32–0.73)	0.00043
ALDH1A2	1	67	1.95 (0.44–8.65)	0.37
	2	140	0.7 (0.38–1.29)	0.24
	3	305	1.35 (1–1.83)	0.047
	4	148	1.36 (0.92–1.99)	0.12
ALDH1A3	1	67	4.66 (0.61–35.4)	0.1
	2	140	2.92 (1.6–5.35)	0.00028
	3	305	1.54 (1.16–2.06)	0.0027
	4	148	1.6 (1.08–2.36)	0.018
ALDH1B1	1	67	0.2 (0.08–0.54)	0.00044
	2	140	0.56 (0.28–1.14)	0.11
	3	305	0.57 (0.41–0.77)	0.00029
	4	148	1.96 (1.2–3.19)	0.0062
ALDH1L1	1	67	2.17 (0.49–9.61)	0.3
	2	140	3.17 (1.52–6.61)	0.0012
	3	305	1.79 (1.24–2.56)	0.0014
	4	148	1.58 (1.06–2.37)	0.024

Abbreviations: GC, gastric cancer; HR, hazard ratio; CI, confidence interval.

Table 4 Correlation of ALDH1 isoenzyme mRNA high expression with HER2 status of patients with GC

Isoenzymes	HER2 status	Cases	HR (95% CI)	*P*-value
ALDH1A1	Negative	532	0.7 (0.56–0.88)	0.0018
	Positive	344	0.89 (0.69–1.16)	0.4
ALDH1A2	Negative	532	1.47 (1.13–1.9)	0.0039
	Positive	344	1.23 (0.93–1.65)	0.15
ALDH1A3	Negative	532	1.68 (1.34–2.11)	5.9e–06
	Positive	344	1.6 (1.23–2.07)	0.00038
ALDH1B1	Negative	532	0.8 (0.64–1.01)	0.064
	Positive	344	0.58 (0.44–0.76)	4.6e–05
ALDH1L1	Negative	532	1.92 (1.46–2.51)	1.5e–06
	Positive	344	1.42 (1.1–1.85)	0.0072

Abbreviations: GC, gastric cancer; HR, hazard ratio; CI, confidence interval.

of patients with GC. In the current study, we found that mRNA high expression of *ALDH1A1* was not significantly correlated with OS for all patients with GC followed for 13 years, HR =0.86 (95% CI: 0.7–1.05), *P*=0.13. In addition, *ALDH1A1* mRNA high expression was not found to be correlated with OS in intestinal-type patients, HR =0.72 (95% CI: 0.49–1.04), *P*=0.078, and in diffuse-type patients, HR =1.52 (95% CI: 0.87–2.66), *P*=0.13. Similar to *ALDH1A1* mRNA, *ALDH1A2* mRNA high expression was also not significantly correlated with OS for all patients with GC, HR =1.13 (95% CI: 0.91–1.41), *P*=0.25. However, *ALDH1A2* mRNA high expression was significantly correlated with better OS in diffuse-type patients, HR =0.59 (95% CI: 0.36–0.97), *P*=0.037. In contrast, *ALDH1A3* mRNA high expression was found to be significantly correlated with worsened OS either in intestinal-type patients, HR =2.24 (95% CI: 1.44–3.49), *P*=0.00026 or diffuse-type patients, HR =1.91 (95% CI: 1.02–3.59), *P*=0.04. Interestingly, *ALDH1B1* mRNA high expression was found to be significantly correlated with better

Table 5 Correlation of ALDH1 isoenzyme mRNA high expression with different treatments of patients with GC

Isoenzymes	Treatment	Cases	HR (95% CI)	*P*-value
ALDH1A1	Surgery alone	380	0.81 (0.59–1.11)	0.19
	5-FU-based adjuvant chemotherapy	153	0.7 (0.49–0.99)	0.042
ALDH1A2	Surgery alone	380	1.31 (0.97–1.77)	0.076
	5-FU-based adjuvant chemotherapy	153	0.75 (0.52–1.08)	0.12
ALDH1A3	Surgery alone	380	2.08 (1.54–2.81)	8.9e–07
	5-FU-based adjuvant chemotherapy	153	0.65 (0.44–0.97)	0.033
ALDH1B1	Surgery alone	380	1.26 (0.93–1.71)	0.13
	5-FU-based adjuvant chemotherapy	153	0.56 (0.39–0.81)	0.0015
ALDH1L1	Surgery alone	380	1.75 (1.24–2.46)	0.0013
	5-FU-based adjuvant chemotherapy	153	0.77 (0.54–1.1)	0.15

Abbreviations: 5-FU, 5-fluorouracil; GC, gastric cancer; HR, hazard ratio; CI, confidence interval.

OS for all patients with GC, HR =0.66 (95% CI: 0.53–0.81), *P*=7.8e−05, and mRNA high expression of *ALDH1L1* was found to be significantly correlated with worsened OS for all patients with GC, HR =1.23 (95% CI: 1–1.51), *P*=0.048. ALDH1L2 is expressed in heart, brain, liver, kidney, and pancreas using real-time polymerase chain reaction performed on an array of human tissues, but no information is available for its expression in gastric tissue.[36] No survival information for ALDH1L2 in patients with GC is available, probably due to its low expression in gastric tissue and GC. We also assessed the correlation of individual ALDH1 isoenzymes' mRNA high expression with other clinicopathological features, such as pathological grades, clinical grades, HER2 status, and different choices of treatment of patients with GC. Prognostic values of the ALDH1 in several cancers have been accumulated predominantly by using immunohistochemistry of paraffin-embedded cancer tissues with isotype-specific antibodies, ALDH1A1 or ALDH1A3.[37] Meta-analysis showed that ALDH1 has a poor prognosis in breast cancer,[38,39] colorectal cancer,[40] lung cancer,[41,42] and head and neck cancer.[43] In contrast to the abovementioned reports, ALDH1 is considered a marker of prediction of better prognosis in patients suffering from primary glioblastoma.[44] So far, only a few studies showed the prognostic values of the mRNA expression of ALDH1 isoenzymes in patients with cancer. Liu et al[45] reported that higher ALDH1A1 mRNA level was associated with improved disease-free survival (HR =0.87, 95% CI: 0.80–0.95, per log unit change) and OS (HR =0.85, 95% CI: 0.78–0.93 per log unit change) independent of age at diagnosis, TNM stage, and treatment in triple-negative breast cancer. Chen et al reveal that ALDH1L1 mRNA is significantly reduced in hepatocellular carcinoma tissues and is a new and potential prognostic marker for the survival of patients with hepatocellular carcinoma. However, the exact mechanisms of ALDH1 isoenzymes, in either protein or mRNA levels, which may affect the clinical outcome of patients with cancer are still not clear and need further study. In addition, individual ALDH1 isoenzymes may have interaction among them and finally affect the outcome of patients with GC. Unfortunately, the KM plotter is not able to analyze the correlation between the various isoforms of ALDH1. In addition, the KM plotter cannot be used to determine a positive or negative correlation between isoenzyme expressions.

ALDH1 is able to convert aldehydes into carboxylic acids in several types of normal tissues.[46,47] Recently, accumulating evidence strongly indicates that ALDH1, in particular ALDH1A1, can modulate cell differentiation, proliferation, and survival, as well as the cellular response to oxidative stress in SCs.[37] ALDH1 also has universal markers in CSCs including gastric CSC. However, the specific usefulness of ALDH1 in SCs and CSCs is still unclear. Currently, the activity of ALDH1 in viable cells can be determined by the use of fluorescent substrates and flow cytometry for ALDH1.[10,48,49] Katsuno et al[50] isolated ALDH1+ cells from human diffuse-type gastric carcinoma cells and characterized these cells using an Aldefluor assay. They found that ALDH1+ cells that constituted 5%–8% of the human diffuse-type GC cells were more tumurigenic than ALDH1− cells, and ALDH1+ cells were able to self-renew and generate heterogeneous cell populations. Wakamatsu et al[12] immunohistochemically examined expression and distribution of ALDH1 in primary and metastatic GC and showed that the ALDH1 positivity is significantly higher in diffuse-type lymph node metastasis than in the primary tumor. Levi et al[13] also observed that ALDH1 was expressed in very low levels in normal human gastric mucosa but significantly increased in gastric adenocarcinomas. Until recently, Li et al[14] determined that ALDH1A1 was an independent prognostic factor for both OS and RFS. However, which ones of ALDH1's isoenzymes are causing ALDH1 activity in GC and the prognostic values of most of the individual ALDH1 isoenzyme in GC remains elusive. Our results showed that unlike breast cancer, mRNA expression of *ALDH1A1* in GC is not significantly associated with OS for patients with GC. Additionally, our study results also indicate that ALDH1A3 and ALDH1L1 are potential major contributors to the ALDH1 activity in GC, since *ALDH1A3* and *ALDH1L1* mRNA high expression was found to be significantly correlated with worsened OS for all patients with GC. Based on our results, ALDH1A3 and ALDH1L1 are potential excellent drug targets for patients with GC.

Previous reports have been focusing on the correlation between ALDH1A1 protein and the clinicopathologic parameters. In most types of tumors, such as, breast cancer,[10,51,52] clear cell renal cell carcinoma,[53] colorectal carcinoma,[54] esophageal squamous cell carcinoma,[55] squamous cell carcinoma of the head and neck,[56] and urothelial carcinomas of urinary bladder,[57] ALDH1A1 protein high expression was correlated with tumor metastasis and poor prognosis. In contrast to the abovementioned studies, ALDH1A1 is also identified as a marker of astrocytic differentiation during brain development and of better prognosis in patients suffering from primary glioblastoma.[44] In patients with GC who had ALDH1A1 overexpression, they also had poor OS and shorter RFS.[14] In the current study, *ALDH1A1* mRNA high expression was found to be correlated with worsened OS only in diffuse-type patients with GC, but not in intestinal-type patients with GC.

The two main histologic subtypes of the disease, intestinal and diffuse type, define two distinct entities that have different etiology, pathogenesis, epidemiology, and behavior.[58] In the current study, excerpt for the *ALDH1A3* mRNA high expression that was found to be correlated with worsened OS in both intestinal-type patients and diffuse-type patients, other *ALDH1* isoenzymes had total different OS in these two types of patients with GC. The molecular mechanisms of the regulation of *ALDH1* isoenzymes in intestinal and diffuse type need to be further investigated.

The HER2/*neu* proto-oncogene (also known as *c-erbB-2*) encodes for a 185 kDa transmembrane glycoprotein receptor known as HER2/*neu* or $p185^{HER2}$, partial homology with epidermal growth factor receptor, shares with that receptor-intrinsic tyrosine kinase activity, and has been implicated in cancer with special emphasis on breast cancer.[59,60] HER2 overexpression was detected in 6%–35% of patients with GC and has led to the advent of targeted therapy with anti-HER2 antibody such as Trastuzumab which has improved the OS.[61,62] HER2 and ALDH1 have been identified as potential biomarkers of prognostic significance in patients with GC;[63] however, there are no reports about the association between HER2 and ALDH1 in GC. Interestingly, there are strong evidences showing the correlation between HER2 and ALDH1 in breast cancer. ALDH1 expression was found to be correlated with HER2 overexpression ($P<0.001$) in breast cancer.[64] ALDH1+ breast cancers were also found to be associated with basal-like and HER2-overexpressing subtypes, and the characteristics histologic features were related to these two subtypes.[65] In this study, we found that *ALDH1A1* and *ALDH1A2* mRNA high expression is only significantly associated with patients with HER2-negative GC. *ALDH1B1* mRNA high expression is only associated with patients with HER2-positive GC. *ALDH1A3* and *ALDH1L1* mRNA high expression are significantly associated with patients with HER2-negative and HER2-positive GC.

In patients with breast cancer, only *ALDH1A1* mRNA high expression was found to be significantly correlated with the poor OS, indicating that *ALDH1A1* is potentially a major contributor of ALDH1 activity and a potential drug target of breast cancer.[26] In contrast, in non-small-cell lung cancer, high expression of *ALDH1A2* and *ALDH1B1* mRNA was found to be significantly correlated with the poor OS in patients with non-small-cell lung cancer, indicating that A*LDH1A2* and *ALDH1B1* are potential drug targets for patients with non-small-cell lung cancer.[66] It is not clear about the role of each ALDH1 isoenzyme that contributes to ALDH1 activity in GC cells. It will be helpful to know which ALDH1 isoenzyme contributes to ALDH1 activity, if we measure the changes of ALDH1 activity upon using siRNAs or antibodies of individual ALDH1 isoenzymes in GC cells. Unlike breast and non-small-cell lung cancer, only *ALDH1A3 and ALDH1L1* mRNA high expression was found to be significantly correlated with worsened OS for all patients with GC, indicating that *ALDH1A3 and ALDH1L1* might be potential drug targets for patients with GC. So far, not many specific small molecular inhibitors or other antagonists of the different ALDH1 isozymes have been developed. This lack of selectivity of available individual ALDH1 isozyme inhibitors that have been tested as anticancer agents in the clinical setting has resulted in an unacceptable side-effect profile.[37] Interestingly, Condello et al[67] recently developed A37 ((ethyl-2-((4-oxo-3-(3-(pryrrolidin-1-yl)propyl)-3,4-dihydrobenzo [4,5]thioeno [3,2-d]pyrimidin-2-yl)thio)acetate)), a novel ALDH1A1 small-molecule enzymatic inhibitor for the first time, where it disrupted ovarian cancer cell spheroid formation and cell viability ($P<0.001$). We expect more specific inhibitors target other ALDH1 isozymes, such as ALDH1A3 or ALDH1L1 to be developed and to be validated for their usage in the targeting CSC.

Conclusion

Using KM plotter, we identified the distinct prognostic significances of ALDH1 isoenzymes in patients with GC. Our results indicate that ALDH1A3 and ALDH1L1 are potential major contributors to the ALDH1 activity in GC, since *ALDH1A3 and ALDH1L1* mRNA high expression was found to be significantly correlated with worsened OS for all patients with GC. ALDH1A3 and ALDH1L1 are potential prognostic markers and therapeutic targets for patients with GC.

Disclosure

The authors report no conflicts of interest in this work.

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