Tumor Biology

Distinct clinicopathological significances and potential drug targets of ALDH1 isoenzymes in gastric cancer

--Manuscript Draft--

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Distinct clinicopathological significances and potential drug targets of ALDH1 isoenzymes in gastric cancer

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Abstract

Elevated aldehyde dehydrogenase 1 (ALDH1) activity has been determined in the stem cell populations of several kinds of tumors including gastric cancer (GC). However, which ALDH1's isoenzymes are contributing to ALDH1activity remains elusive. In this study, we examined the prognostic value and hazardous ratio (HR) of individual ALDH1 isoenzymes in GC patients through "The Kaplan-Meier plotter" (KM plotter) database. *ALDH1A1* mRNA high expression was not found to be significantly correlated to overall survival (OS) for all GC patients followed for 13 years, HR 0.86 (0.7-1.05), p=0.13. *ALDH1A2* mRNA high expression was also not significantly correlated to OS for all GC patients, HR 1.13 (0.91-1.41), p=0.25. *ALDH1A3* mRNA high expression was found to be significantly correlated to worsen OS either in intestinal type patients, HR 2.24 (1.44-3.49), p=0.00026 or diffuse type patients, HR 1.91 (1.02-3.59), p=0.04. Interestingly, *ALDH1B1* mRNA high expression was found to be significantly correlated to better OS for all GC patients, HR 0.66 (0.53-0.81), p=7.8e-05 and *ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients, HR 1.23 (1-1.51), p=0.048. In addition, our current study also supports that ALDH1A3 and ALDH1L1 might be major contributors to the ALDH1 activity in GC, since *ALDH1A3 and ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients. Based on our study, ALDH1A3 and ALDH1L1 might be excellent potential drug targets for GC patients.

Key words: Cancer stem cell; ALDH1; Prognosis; Drug target; KM plotter; Hazardous ratio

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 and 800,000 cancer-related deaths are caused by GC each year globally.[1] According to the Lauren classification, GCs are divided into intestinal and diffuse types. Despite the advances in early detection, radical cure operation, and multimodal therapeutic modalities, at diagnosis, GC remains difficult to cure and prognosis remains poor with a median overall survival of 12 months for advanced disease in Western countries.[2-3] Thus, in order to improve the clinical outcome of GC patients, investigation on the mechanism of incidence and progression of GC, as well as identification of prognostic biomarkers and drug targets are still needed and will help to select patients with high chances of GC recurrence and provide better prognosis and individualized treatments.

Aldehyde dehydrogenase 1 (ALDH1) family, are composed of enzymes which contribute to the oxidation of retinol to retinoic acid at high levels in stem cells (SC).[4-6] Increased ALDH1 activity has been reported in multiple myeloma, myeloid leukemia and several types of solid tumors.[7-11] Thus, determination of ALDH1 activity might be served as a common marker for both normal and malignant SC populations. Wakamatsu Y et al first compared cancer stem cell (CSC) markers in primary and metastatic GC and showed ALDH1 positivity to be significantly higher in diffuse-type lymph node metastasis than in the primary tumor.[12] Levi E et al also observed that CSC markers ALDH1, LGR5, and CD166 were expressed in very low levels in normal human gastric mucosa, in contrast, level of expression for all three markers significantly increased in gastric adenocarcinomas.[13] Recently, Li X et al reported that ALDH1A1 protein expression was significantly associated with depth invasion, lymph node metastasis, stage of disease and ALDH1A1 was an independent prognostic factor for both overall survival (OS) and recurrence-free survival (RFS).[14] However, which ALDH1's isoenzymes are contributing to ALDH1activity in GC has not determined. In addition, the prognostic value of most of individual ALDH1 isoenzyme in GC remains elusive. The Kaplan-Meier plotter" (KM plotter)

developed from Gene Expression Omnibus (GEO-www. ncbi. nlm.nih.gov/geo/) database. KM plotter database can be utilized for the analysis of individual genes with clinical results to relapse-free survival and total survival of the patients.[15-16] A number of genes have already been identified and/or validated by KM plotter in breast cancer,[17-25] as well as in ovarian and lung cancer.[26] In this study, we have determined the prognostic value of individual ALDH1 isoenzymes in human GC patients using KM plotter database.

Material and Methods

An online database [15] was used to determine the relevance of individual *ALDH1* members' mRNA expression to relapse free survival. Currently, they established breast cancer,[15] lung cancer, [27] ovarian cancer[28] and GC database. All cancer patients in the database were identified from Cancer Biomedical Informatics Grid (caBIG, http://cabig.cancer.gov/, microarray samples are published in the caArray project), the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA,http://cancergenome.nih.gov) cancer datasets.[27] They collected clinical data including gender, perforation history, Lauren classification, differentiation, stage, HER2 status and treatment. The database was established using gene expression data and survival information of 599 GC patients downloaded from Gene Expression Omnibus (GEO). Briefly, five *ALDH1* sub-members (*ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B1* and *ALDH1L1)* were entered into the database (http://kmplot.com/analysis/index.php?p=service&cancer=breast) to obtain Kaplan-Meier survival plots in which the number-at-risk is indicated below the main plot. Hazard ratio (and 95% confidence intervals) and log rank P were calculated and displayed on the webpage.

Results

There are a total of six sub-members in the ALDH1 family. We summarized their characteristics and listed table 1. Just as previously Wu S reported,[25] among all the six ALDH1 isoenzymes, only *ALDH1L2* was not found in www.kmplot.com, probably due to its low expression.

We first examined the prognostic value of *ALDH1A1* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 212224_at (*ALDH1A1*). Survival curves are plotted for all patients (n =599) (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 to overall survival (OS) for all GC patients followed for 13 years, hazardous ratio (HR) 0.86 (0.7-1.05), *p*=0.13. However, *ALDH1A1* mRNA high expression was found to be correlated to better OS in intestinal type patients, HR 0.72 (0.49-1.04), *p*=0.078, but not in diffuse type patients, HR 1.52 (0.87-2.66), *p*=0.13.

We then examined the prognostic value of *ALDH1A2* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 207015_s_at (*ALDH1A2*). *ALDH1A2* mRNA high expression was also not found to be correlated to OS for all GC patients, HR 1.13 (0.91-1.41), *p*=0.25 (Figure 2A). Interestingly, *ALDH1A2* mRNA high expression was found to be correlated to worsen OS in intestinal type patients, HR 1.47 (0.99-2.19), *p*=0.057 (Figure 2B). In contrast, *ALDH1A2* mRNA high expression was found to be correlated to better OS in diffuse type patients, HR 0.59 (0.36-0.97), *p*=0.037 (Figure 2C).

Figure 3 shows the prognostic value of *ALDH1A3* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 203180_at (*ALDH1A3*). The curves show that *ALDH1A3* expression above or below the median do not separate the cases into significantly different prognostic groups in all GC patients, HR 1.19 (0.97-1.46), *p*=0.1 (Figure 3A). However, *ALDH1A3* mRNA high expression was found to be significantly correlated to worsen OS either in intestinal type patients, HR 2.24 (1.44-3.49), *p*=0.00026 (Figure 3B) or diffuse type patients, HR 1.91 (1.02-3.59), *p*=0.04 (Figure 3C).

Figure 4 shows the prognostic value of *ALDH1B1* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 209646_x_at (*ALDH1B1*). *ALDH1B1* mRNA high expression was found to be significantly correlated to better OS for all GC patients, HR 0.66 (0.53-0.81), *p*=7.8e-05 (Figure 4A). In addition, *ALDH1B1* mRNA high expression was also found to be correlated to better OS in intestinal type

patients, HR 0.7 (0.48-1.02), *p*=0.06 (Figure 4B), but not in diffuse type patients, HR 1.41 (0.82-2.41), *p*=0.21 (Figure 4C).

Finally, we examined the prognostic value of *ALDH1L1* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 205208_at (*ALDH1L1*). *ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients, HR 1.23 (1-1.51), *p*=0.048 (Figure 5A). In addition, *ALDH1L1* mRNA high expression was also found to be correlated to worsen OS in intestinal type patients, HR 1.44 (0.97-2.16), *p*=0.072 (Figure 5B). In contrast, *ALDH1L1* mRNA high expression was found to be significantly correlated to better OS in diffuse type patients, HR 0.5 (0.31-0.83), *p*=0.0064 (Figure 5C).

Discussion

Using KM plotter, we found that *ALDH1A1* mRNA high expression was not significantly correlated to OS for all GC patients followed for 13 years, hazardous ratio (HR) 0.86 (0.7-1.05), *p*=0.13. In addition, *ALDH1A1* mRNA high expression was found to be correlated to better OS in intestinal type patients, HR 0.72 (0.49-1.04), *p*=0.078. In contrast, it was correlated to worsen OS in diffuse type patients, HR 1.52 (0.87-2.66), *p*=0.13. Just like *ALDH1A1* mRNA, *ALDH1A2* mRNA high expression was also not significantly correlated to OS for all GC patients, HR 1.13 (0.91-1.41), *p*=0.25. However, *ALDH1A2* mRNA high expression was significantly correlated to better OS in diffuse type patients, HR 0.59 (0.36-0.97), *p*=0.037. In contrast, *ALDH1A3* mRNA high expression was found to be significantly correlated to worsen OS either in intestinal type patients, HR 2.24 (1.44-3.49), *p*=0.00026 or diffuse type patients, HR 1.91 (1.02-3.59), *p*=0.04. Interestingly, *ALDH1B1* mRNA high expression was found to be significantly correlated to better OS for all GC patients, HR 0.66 (0.53-0.81), *p*=7.8e-05 and *ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients, HR 1.23 (1-1.51), *p*=0.048. Real-time PCR performed on an array of human tissues has shown that ALDH1L2 is expressed in liver, kidney, pancreas,

heart, and brain, no information available for its expression in gastric tissue.[29] No survival information for ALDH1L2 in GC patients is available , probably due to its low expression in gastric tissue and GC.

ALDH1 belongs to a family of detoxifying enzymes that convert aldehydes to their corresponding carboxylic acids and members of this family are present in many types of normal tissues.[30-31] Currently, the "gold standard" of the measurement of the activity of ALDH1 in viable cells has been the use of flow cytometry and fluorescent substrates for ALDH1.[10, 32-33] Katsuno Y et al[34] isolated ALDH1+ cells from human diffuse-type gastric carcinoma cells and characterized these cells using an Aldefluor assay. They found that ALDH1+ cells constituted 5-8% of the human diffuse-type GC cells, were more tumourigenic than ALDH1- cells, and were able to self-renew and generate heterogeneous cell populations. Wakamatsu Y et al immunohistochemically examined expression and distribution of ALDH1 in primary and metastatic GC and showed ALDH1 positivity to be significantly higher in diffuse-type lymph node metastasis than in the primary tumor.[12] Levi E et al also observed that ALDH1 was expressed in very low levels in normal human gastric mucosa, but significantly increased in gastric adenocarcinomas.[13] Until recently, Li X et al determined that ALDH1A1 was an independent prognostic factor for both OS and RFS.[14] However, which ALDH1's isoenzymes are contributing to ALDH1activity in GC and the prognostic value of most of individual ALDH1 isoenzyme in GC remains elusive. Our current study found that unlike breast cancer, *ALDH1A1* mRNA in GC is not significantly associated with OS of GC patients. In addition, our current study also supports that ALDH1A3 and ALDH1L1 might be major contributors to the ALDH1 activity in GC, since *ALDH1A3 and ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients. Based on our study, ALDH1A3 and ALDH1L1 might be excellent potential drug targets for GC patients.

Previous studies have been focused on the relationship between the expression of ALDH1A1 protein and the clinicopathologic parameters, including prognosis of tumor patients. In most types of tumors, such

as, breast cancer,[10, 35-36] clear cell renal cell carcinoma,[37] colorectal carcinoma,[38] esophageal squamous cell carcinoma,[39] squamous cell carcinoma of the head and neck,[40] urothelial carcinomas of urinary bladder,[41] high expression of ALDH1A1 protein was associated with tumor metastasis and poor prognosis. In contrast to above studies, there was also evidence for ALDH1A1 as a marker of astrocytic differentiation during brain development and of better prognosis in patients suffering from primary glioblastoma.[42] In GC patients who had ALDH1A1 overexpression, had poor OS and shorter RFS.[14] In current study, *ALDH1A1* mRNA high expression was found to be correlated to worsen OS only in diffuse type GC patients, but not in intestinal type GC patients.

The two main histologic subtypes of the disease, intestinal and diffuse type, as classified by Lauren, define two distinct entities that have different etiology, pathogenesis, epidemiology, and behavior.[43] In current study, excerpt that *ALDH1A3* mRNA high expression was found to be correlated to worsen OS both in intestinal type patients and diffuse type patients, other *ALDH1* isoenzymes had total different OS in these two types of GC patients. The molecular mechanisms of regulation of *ALDH1* isoenzymes in intestinal and diffuse type need to be further investigation.

In summary, using KM plotter, we identified that distinct prognostic significances of ALDH1 isoenzymes in GC patients. Our results indicate that ALDH1A3 and ALDH1L1 might be major contributors to the ALDH1 activity in GC, since *ALDH1A3 and ALDH1L1* mRNA high expression was found to be significantly correlated to worsen OS for all GC patients. ALDH1A3 and ALDH1L1 might be excellent potential drug targets for GC patients.

Declaration of interest

The authors have no financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in the manuscript.

Figure Legends

Figure 1. The prognostic value of *ALDH1A1* expression in www.kmplot.com. The desired Affymetrix IDs is valid: 212224_at (*ALDH1A1*). A. Survival curves are plotted for all patients (n =599), HR=0.86 (0.7-1.05). B. Survival curves are plotted for intestinal type (n =186), HR=0.72 (0.49-1.04). C. Survival curves are plotted for diffuse type (n =106), HR=1.52 (0.87-2.66).

Figure 2. The prognostic value of *ALDH1A2* expression in www.kmplot.com. The desired Affymetrix IDs is valid: 207015_s_at (*ALDH1A2*). A. Survival curves are plotted for all patients (n =599), HR=1.13 (0.91-1.41). B. Survival curves are plotted for intestinal type (n =186), HR=1.47 (0.99-2.19). C. Survival curves are plotted for diffuse type (n =106), HR=0.59 (0.36-0.97).

Figure 3. The prognostic value of *ALDH1A3* expression in www.kmplot.com. The desired Affymetrix IDs is valid: 203180_at (*ALDH1A3*). A. Survival curves are plotted for all patients (n =599), HR=1.19 (0.97-1.46). B. Survival curves are plotted for intestinal type (n =186), HR=2.24 (1.44-3.49). C. Survival curves are plotted for diffuse type (n =106), HR=1.91 (1.02-3.59).

Figure 4. The prognostic value of *ALDH1B1* expression in www.kmplot.com. The desired Affymetrix IDs is valid: 209646_x_at (*ALDH1B1*). A. Survival curves are plotted for all patients (n =599), HR=0.66 (0.53-0.81). B. Survival curves are plotted for intestinal type (n =186), HR=0.7 (0.48-1.02). C. Survival curves are plotted for diffuse type (n =106), HR=1.41 (0.82-2.41).

Figure 5. The prognostic value of *ALDH1L1* expression in www.kmplot.com. The desired Affymetrix IDs is valid: 205208_at (*ALDH1L1*). A. Survival curves are plotted for all patients (n =599), HR=1.23 (1-1.51). B. Survival curves are plotted for intestinal type (n =186), HR=1.44 (0.97-2.16). C. Survival curves are plotted for diffuse type (n =106), HR=0.5 (0.31-0.83).

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Table
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Table 1. Alternatively spliced variants and characterization of ALDH1 isoenzymes

Isoenzymes	Alternatively spliced variants	Cellular localization	Tissue distribution	Associated diseases
ALDH1A1	ALDH1A1_v2	Cytosol	Lung, breast, brain, pancreas, liver, kidney, etc	Alcoholism
ALDH1A2	ALDH1A2_v2 ALDH1A2_v3 ALDH1A2_v4	Cytosol	Kidney, testis, liver	Schizophrenia; spina bifida
ALDH1A3	ALDH1A3_v2	Cytosol	Skeletal muscle, lung, breast, kidney, etc	Autosomal recessive anophthalmia/microphthalmia
ALDH1B1	N/A	Mitochondria	Liver, heart, kidney, brain, prostate	N/A
ALDH1L1	N/A	Cytosol	Kidney, liver, skeletal muscle	Ischemic stroke
ALDH1L2	ALDH1L2_v2 ALDH1L2_v2	Mitochondria	Pancreas, heart, and brain	N/A

Figure
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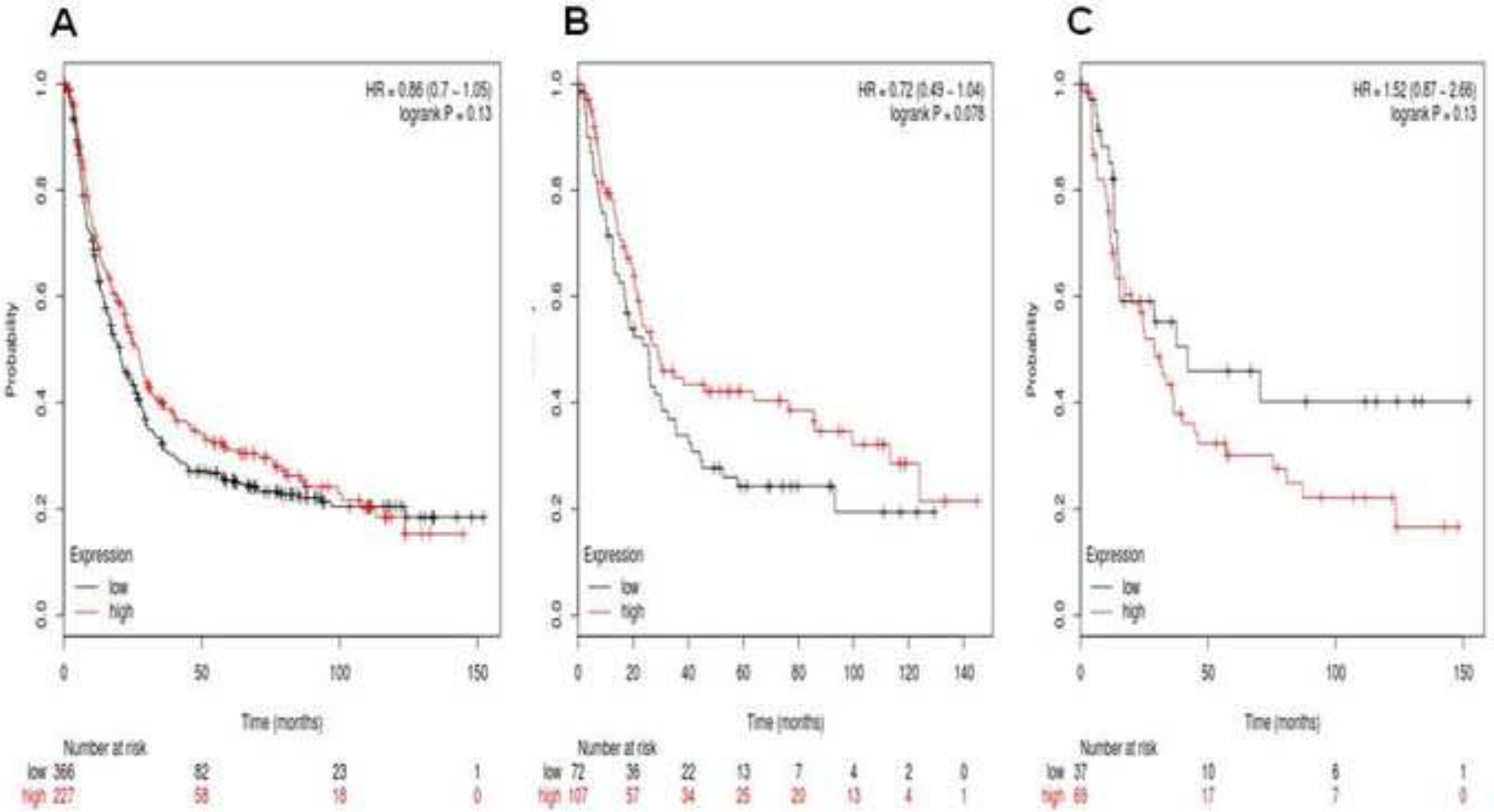
Figure 1



Figure
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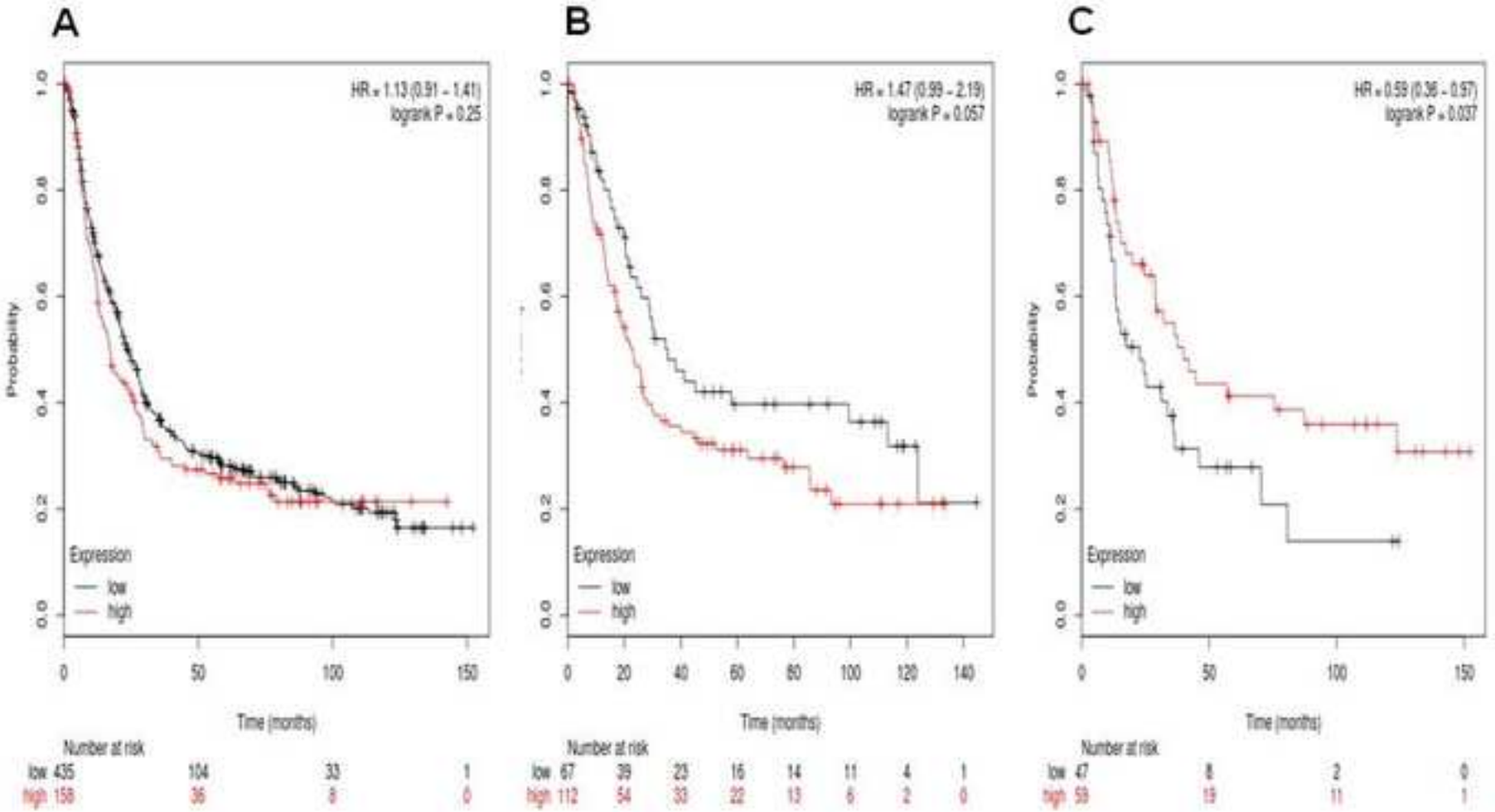
Figure 2



Figure
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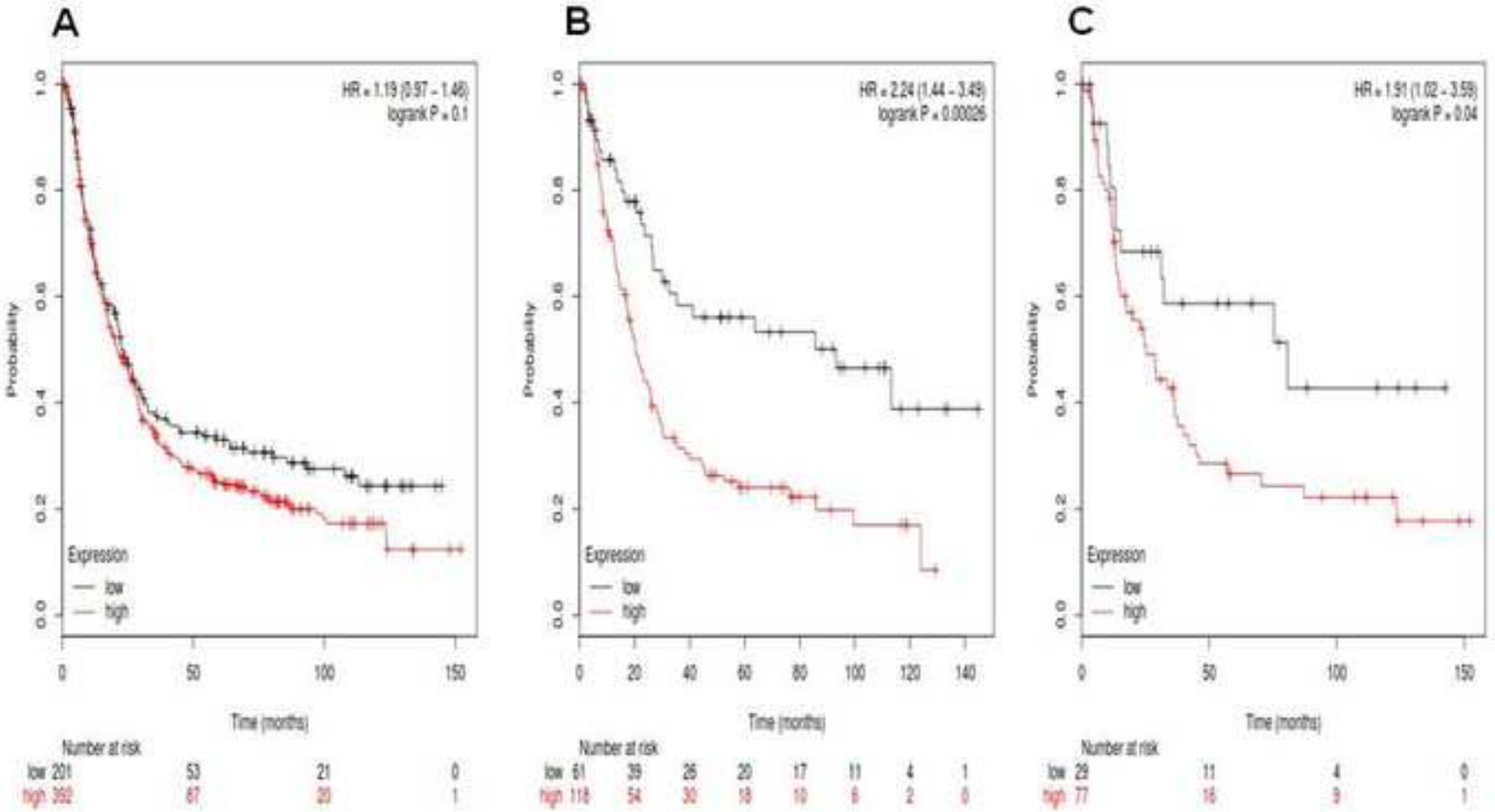
Figure 3



Figure
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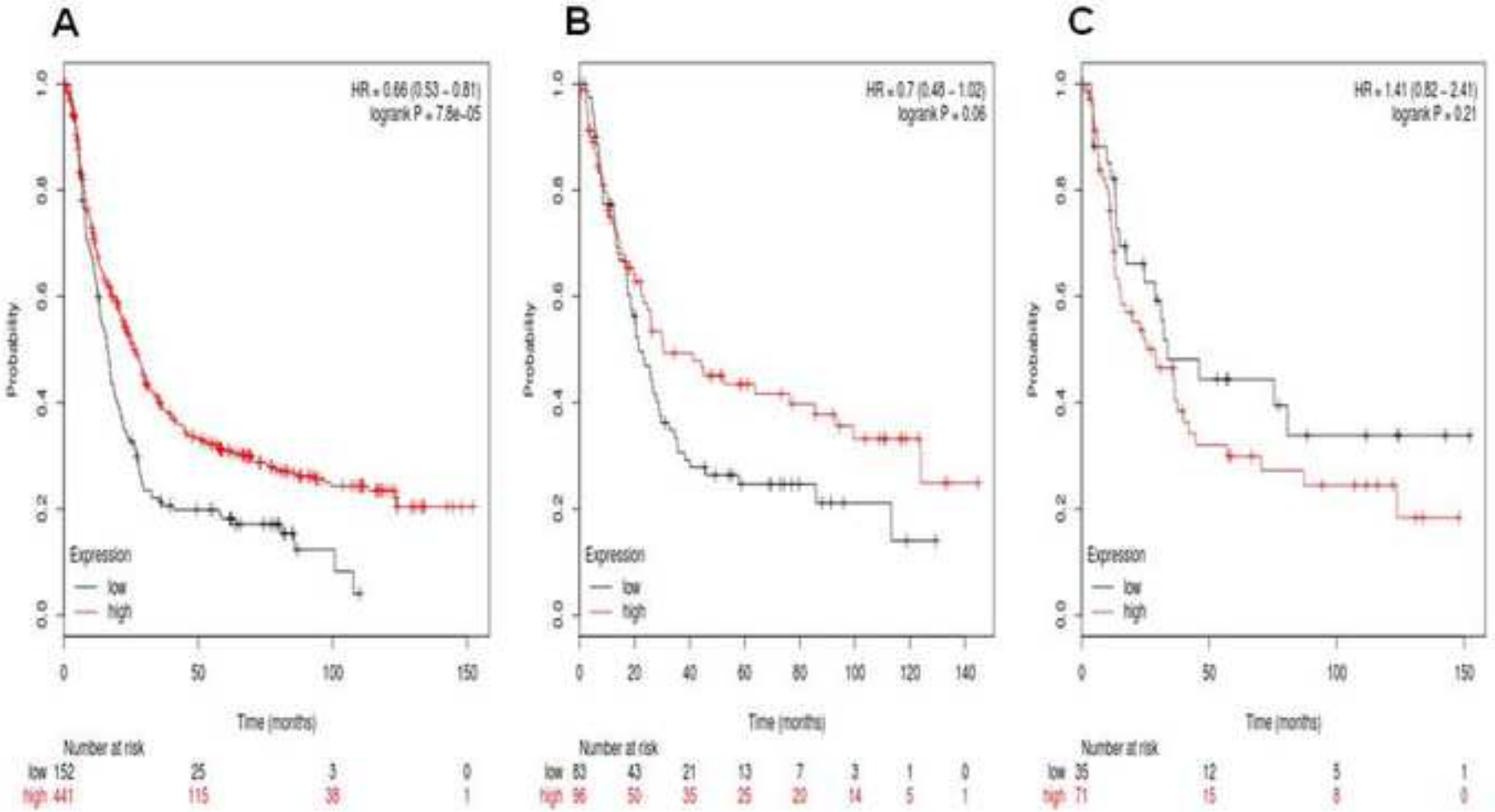
Figure 4



Figure
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Figure 5