Effects of mRNA expression of five Notch ligands on prognosis of gastric carcinoma

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Article

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Abstract

Objectives Notch ligands are expression changes in a great many malignancies including gastric cancer (GC) frequently. The prognostic value of each Notch ligands in GC patients remains lack of large sample data results. In present research, we researched the prognostic value of Notch ligands in GC patients in order to fill the shortage areas.

Methods We used an online database (http://kmplot.com/analysis/index.php?p= service & cancer gastric) to identify the relationship between mRNA expression of each Notch ligand and overall survival (OS) in GC. We analyze the relevance of overall survival and clinical data which includes gender, Lauren's classification, differentiation, clinical stage and treatment.

Results The study found that high DLL1, DLL3, DLL4 and JAG2 mRNA expression were tied to worse OS in all GC patients followed up for 10 years. There is no significant relevance to the expression of JAG1 mRNA and OS in patients with GC. We also did a survey of each Notch ligands in different clinical and pathological features present different prognosis.

Conclusion The information will help to better understand the biology of gastric cancer heterogeneity, provide more accurate prognostic evaluation tools and provide new targets for targeted drug development besides.

Introduction

Worldwide, the latest statistics show that the cancer-related death for gastric cancer (GC) was the third-highest percentage (Around 8.2% of cancer-related deaths each year globally) [1]. Early detection is still a worldwide problem and the diagnosis of GC generally is locally advanced. Patients with advanced GC still present poor prognosis despite the advances in radical cure operation such as Laparoscopic surgery or Da Vinci Robotic surgery and multimodal therapeutic modalities after surgery [2–3]. Therefore, to clarify the molecular mechanism of the occurrence and development of GC, finding prognostic biomarkers and targets will help provide individualized treatment, to cut down the chance of postoperative recurrence and enhance clinical outcome. According to research findings, the Notch signaling pathway was one of the crucial factors therapeutic targets for cancers. The Notch affects patient outcome by regulating cell proliferation, apoptosis, invasion and angiogenesis [4–5]. The Notch includes four receptors (Notch 1–4) and five ligands (DLL1, -3, -4 and JAG1, -2). New research has found that once the Notch signal is activated, it plays a pivotal role in the occurrence and development of GC as well as interact with other intercellular signaling pathways, participating in inhibition of apoptosis, metastasis and chemotherapy resistance[6–7]. So far, the effect of Notch receptor mRNA level on OS of patients with non-small cell lung cancer and GC has been reported[8–10].

Nevertheless, the effect of individual Notch ligand mRNA level on prognosis in patients with GC hasn't been reported. We used "Kaplan-Meier Plotter" (KM Plotter) to analyze the expression of Notch ligand in

GC patients and its effect on prognosis. The KM plotter can analyze survival of 876 GC patients combined with clinical data and gene expression (http://kmplot.com/analysis/index.php?p=service&cancer=ovar), dealt with by a PostgreSQL server. Up to now, four receptors of Notch have been reviewed and analyzed by KM plotter in GC [11–12]. The objective of the study was: to analyze the prognostic effects of mRNA expression of 5 Notch ligand in 876 patients with GC using the KM plotter database.

Materials And Methods

We used an online database to identify the correlation factor of individual Notch ligands expression to the OS in GC, and analyze the relevance of OS and clinical data, which is included in gender, clinical stage, differentiation, Lauren's classification and treatment. A database was set up using ten-year follow-up data and gene expression data of 876 carcinoma of stomach downloaded from Gene Expression Omnibus (GEO). In another word, five Notch ligands were entered into the database (http://kmplot.com/analysis/index.php?p=service&cancer gastric) to acquire Kaplan-Meier survival plots. In general, p values < 0.05 were considered statistically significant.

Results

Kaplan-meier survival data of GC patients with differential expressions of Notch ligand can be discovered at www.kmplot.com. We were the first to analyze the prognostic value of DLL1 expression. The Affymetrix ID is: 224215_s_at. Figure 1A shows the survival curve of GC patients based on DLL1 mRNA expression level(n = 631). The ten-year survival rate of all GC patients with high expression level of DLL1 mRNA was worse, HR 1.27 (1.02–1.57), p = 0.032. The Affymetrix ID is: 227938_s_at. OS curves were plotted for carcinoma of stomach patients(n = 631) (Fig. 1B). The ten-year survival rate of all GC with high expression level of DLL1 was also worse, HR 1.75 (1.35–2.28), p = 2.1e-05.

For DLL3, the Affymetrix ID is: 219537_x_at. The OS of GC patients with high expression level of DLL3 mRNA was worse, HR 1.46 (1.23–1.73), p = 1.3e-05 (Fig. 2A), the same as the Affymetrix ID is: 222898_s_at, HR 1.49 (1.19–1.87), p = 0.00051 (Fig. 2B).

For DLL4, the Affymetrix ID is: 223525_at. High expression of DLL4 was significantly tied to worsening OS in GC patients, HR 1.35 (1.08–1.68), p = 0.0091 (Fig. 3).

For JAG1, the Affymetrix ID is: 216268_s_at. High expression of JAG1 was insignificant correlation with worsening OS in GC patients, HR 1.2 (0.99–1.46), p = 0.06 (Fig. 4A). Also, the same as the Affymetrix ID: 209098_s_at(Fig. 4B), the Affymetrix ID is: 209099_x_at(Fig. 4C) and the Affymetrix ID is: 216268_s_at(Fig. 4D).

For JAG2, the Affymetrix ID is: 209784_s_at. The OS of GC patients with high expression level of JAG2 mRNA was worse, HR 2.08 (1.69–2.55), p = 1.6e-12 (Fig. 5A). The same as Affymetrix ID is: 32137_at. HR 1.89 (1.57–2.29), p = 1.6e-11 (Fig. 5B).

Table-1 Correlation between expression of Notch ligands mRNA and gender in patients with GC

Notch ligands	Gender	Cases	HR 95% CI	P value
DLL1	Male	567	1.26 (0.94–1.70)	0.123
	Female	244	0.64 (0.38–1.07)	0.083
DLL3	Male	567	1.71 (1.38–2.14)	1.2e-6*
	Female	244	0.81 (0.55–1.20)	0.298
DLL4	Male	567	1.43(1.06–1.95)	0.020*
	Female	244	1.29(0.84–2.00)	0.245
JAG1	Male	567	0.86 (0.69–1.07)	0.167
	Female	244	0.75(0.53–1.06)	0.103
JAG2	Male	567	2.12(1.70–2.64)	7.3e-12*
	Female	244	1.77 (1.24–2.52)	0.002*

Table-2 Correlation between expression of Notch ligands mRNA and clinical stages with GC

Notch ligands	clinical stages	Cases	HR 95% CI	P value
DLL1		69	3.27 (0.87–11.7)	0.073
		145	0.51 (0.22–1.16)	0.101
		319	0.70 (0.48–1.02)	0.062
		152	0.82 (0.55–1.23)	0.338
DLL3		69	0.29 (0.11–0.79)	0.010*
		145	2.37 (1.23–4.55)	0.008*
		319	1.56 (1.12–2.19)	0.009*
		152	0.75 (0.51–1.12)	0.157
DLL4		69	0.65(0.22–1.94)	0.434
		145	1.45(0.77–2.72)	0.243
		319	1.4(0.91–2.17)	0.128
		152	1.49(1.01–2.22)	0.045*
JAG1		69	0.33(0.12–0.91)	0.025*
		145	0.59(0.32–1.07)	0.081
		319	0.75(0.56–0.10)	0.052
		152	1.19(0.81–1.75)	0.370
JAG2		69	3.58(1.32–9.69)	0.007*
		145	1.78(0.95–3.31)	0.066
		319	2.09(1.53–2.84)	1.8e-6*
		152	1.68(1.14–2.47)	0.009*

Table-3 Correlation between expression of Notch ligands mRNA and Lauren classification in patients with GC

Notch ligands	Lauren classification	Cases	HR 95% CI	P value
DLL1	intestinal	336	1.6 (1.02–2.57)	0.048*
	diffuse	248	0.67 (0.45–1.00)	0.048
	mixed	33	3.21 (0.71–14.54)	0.110
DLL3	intestinal	336	1.88 (1.34–2.64)	0.0002*
	diffuse	248	0.72 (0.48–1.09)	0.125
	mixed	33	0.43 (0.14–1.27)	0.114
DLL4	intestinal	336	0.81 (0.56–1.16)	0.253
	diffuse	248	1.5 (1.06–2.13)	0.021*
	mixed	33	4.18 (0.91–19.29)	0.047
JAG1	intestinal	336	0.63 (0.46–0.86)	0.004*
	diffuse	248	1.38 (0.95–2.02)	0.089
	mixed	33	0.16 (0.03–0.73)	0.008*
JAG2	intestinal	336	2.72 (1.97–3.74)	1.8e-10*
	diffuse	248	1.66 (1.16–2.38)	0.005*
	mixed	33	2.54 (0.89–7.26)	0.071

Table 4 Correlation between expression of Notch ligands mRNA and different differentiation in patients with GC

Notch ligands	differentiation	Cases	HR 95% CI	P value
DLL1	low differentiation adenocarcinoma	166	0.67 (0.38–1.17)	0.158
	median differentiation adenocarcinoma	67	1.47 (0.71–3.04)	0.299
	high differentiation adenocarcinoma	32	none	0.221
DLL3	low differentiation adenocarcinoma	166	0.61 (0.39–0.94)	0.024*
	median differentiation adenocarcinoma	67	1.93 (0.98–3.77)	0.052
	high differentiation adenocarcinoma	32	2.57 (1.03–6.39)	0.036*
DLL4	low differentiation adenocarcinoma	166	1.31 (0.78–2.19)	0.310
	median differentiation adenocarcinoma	67	0.78 (0.41–1.5)	0.463
	high differentiation adenocarcinoma	32	none	0.221
JAG1	low differentiation adenocarcinoma	166	0.59 (0.38–0.91)	0.017*
	median differentiation adenocarcinoma	67	0.62 (0.28–1.35)	0.224
	high differentiation adenocarcinoma	32	0.18 (0.04–0.79)	0.011*
JAG2	low differentiation adenocarcinoma	166	1.57 (0.95–2.6)	0.073
	median differentiation adenocarcinoma	67	2.06 (0.98–4.32)	0.051
	high differentiation adenocarcinoma	32	3.66 (1.32–10.15)	0.008*

Table-5 Correlation between expression of Notch ligands mRNA and different treatment of GC

Notch ligands	Treatment	Cases	HR 95% CI	P value
DLL1	Surgery	393	1.30 (0.91–1.85)	0.147
	surgery combined with 5-FU chemotherapy	158	0.63 (0.23–1.69)	0.354
DLL3	Surgery	393	1.25 (0.93–1.66)	0.135
	surgery combined with 5-FU chemotherapy	158	1.86 (1.30–2.67)	0.0006*
DLL4	Surgery	393	1.47 (1.06–2.04)	0.019*
	surgery combined with 5-FU chemotherapy	158	2.92 (1.17–7.28)	0.016*
JAG1	Surgery	393	1.35 (1.01–1.8)	0.042*
	surgery combined with 5-FU chemotherapy	158	0.77 (0.54–1.08)	0.128
JAG2	Surgery	393	1.54 (1.15–2.05)	0.003*
	surgery combined with 5-FU chemotherapy	158	1.92 (1.34–2.76)	0.0003*

We filled the gaps in the association between individual Notch ligands and clinicopathological features, we separately associated gender (Table-1), clinical stages (Table-2), Lauren's classification (Table-3), different differentiation of GC patients (Table-4) and differences in treatment methods (Table-5). Just as Table 1, DLL3 high expression was so clearly tied to worsening OS in male, HR 1.71 (1.38–2.14), p = 1.2e-6. DLL4 mRNA expression was so clearly tied to worsening OS in male, HR 1.43(1.06–1.95), p = 0.02. JAG2 mRNA expression was so clearly tied to worsening OS in male, HR 2.12(1.70–2.64), p =7.3e-12 and female as well, HR 1.77 (1.24–2.52), p=0.002. According to Table 2, high expression of DLL3 was tied to worsen OS in advanced GC, such as stage Ⅲ, HR 2.37 (1.23–4.55), p=0.008 and stage Ⅳ, HR 1.56 (1.12–2.19), p =0.009. Nevertheless, DLL3 expression was linked with a better OS in grade Ⅰ, HR 0.29 (0.11–0.79), p = 0.01. DLL4 expression was only tied to worsening OS in stage Ⅳ, HR 1.49(1.01–2.22), p =0.045. JAG1 mRNA expression was so clearly tied to a better OS in stageⅠ, HR 0.33(0.12–0.91), p =0.025. JAG2 expression was so clearly tied to worsening OS in stageⅠ, HR 3.58(1.32–9.69), p= 0.007, stage Ⅲ, HR 2.09(1.53–2.84), p = 1.8e-6 and stage Ⅳ, HR 1.68(1.14–2.47), p=0.009. According to Table 3, DLL1 and DLL3 mRNA expressions were tied to worsening OS in intestinal, HR 1.6 (1.02–2.57), p = 0.048. HR 1.88 (1.34–2.64), p = 0.0002. DLL4 expression was so clearly tied to worsening OS in diffuse, HR 1.5 (1.06–2.13), p = 0.021. High expression of JAG1 was linked to improving OS in intestinal, HR 0.63 (0.46–0.86), p = 0.004 and mixed, HR 0.16 (0.03–0.73), p = 0.008. JAG2 mRNA expression was so clearly tied to worsening OS in intestinal, HR 2.72 (1.97–3.74), p = 1.8e-10 as well as diffuse, HR 1.66 (1.16–2.38), p = 0.005. According to Table-4, DLL3 expression was tied to a better OS in low differentiation adenocarcinoma, HR 0.61 (0.39–0.94), p = 0.024. However, with worsen OS in high differentiation adenocarcinoma, HR 2.57 (1.03–6.39), p = 0.036. JAG1 expression was tied to a better OS in low differentiation adenocarcinoma, HR 0.59 (0.38–0.91), p = 0.017 and high differentiation adenocarcinoma, HR 0.18 (0.04–0.79), p = 0.011. JAG2 mRNA expression was tied to worsening OS in high differentiation adenocarcinoma, HR 3.66 (1.32–10.15), p = 0.008. From Table 5, DLL3 mRNA expression was tied to worsening OS in surgery combined with 5-FU chemotherapy, HR 1.86 (1.30–2.67), p = 0.0006. DLL4 and JAG2 mRNA expression was so clearly tied to worsening OS in Surgery, same as surgery combined with 5-FU chemotherapy, p<0.05. JAG1 expression was just tied to worsening OS in Surgery, HR 1.35 (1.01–1.8), p = 0.042.

Discussion

λ The four receptors and five ligands constituted the Notch signaling pathway, a kind of intercellular communication system which is vitally important for embryogenesis and tissue homeostasis[13]. Current research has discovered that DLL1 clearly has a vital effect on tumorigenesis and development of plenty of cancer. The most widely studied and relatively mature is breast cancer. DLL1 was a high expression in breast cancer which exerts carcinogenic effects contribute to breast cancer through various mechanisms[14–15]. And at the same time, DLL1 overexpression in ER + breast cancer patients leads to poor prognosis through cell proliferation, maintenance of tumor stem cells and angiogenesis[16]. It has been reported that clustered DLL1 treatment results in significant enhancement of tumor antigen-specific T cell immune responses and memory, decreased proportion of regulatory T cells, remarkably increased

tumor infiltration by T cells, attenuated tumor vascularization, and suppression of tumor growth and produced remarkably improved lung cancer progression-free survival[17]. Recently, Xie et al[18] reported that downregulation of DLL1 can reverse the activation of Notch signaling. The expression of miR-34a can directly bind to the 3'UTR of DLL1 mRNA, and negatively regulate the expression of DLL1 to inhibit the classical Notch signaling pathway, thereby reducing chemotherapy resistance in colon cancer. However, Notch ligands have little research on the mechanism and prognosis of gastrointestinal tumors. How five Notch ligands mRNA expression affects the prognosis of GC patients remains unclear. Angiogenesis in GC is formed under pathological conditions, and it is still regulated by molecular signals similar to the formation of blood vessels under physiological conditions. Moreover, DLL1 takes a crucial role in blood vessels formation. During the formation of new blood vessels, arterial endothelial cells induce a large amount of DLL1 expression. In this report, by analyzing 10-year follow-up data of GC, we were pleasantly surprised found that patients with high expression of DLL1 had a worsening prognosis. Therefore, it also indirectly proved that DLL1 has great potential in tumor therapy of the formation of blood vessels. High expression of DLL1 leads to poor prognosis in patients with intestinal type of GC. In terms of 10 years survival rate, gender, differentiation, clinical stage and treatment were not statistically different from DLL1 mRNA expression.

λ DLL3 is part of the Notch five transmembrane ligands family and probably a crucial predictive biomarker and therapeutic target[19]. Vadim's first study found that high expression of DLL3 and CD56 in patients with small cell bladder cancer predicted poor prognosis and could serve as independent biomarkers of negative prognosis[20]. It has been reported that the achaete-scute complex-like 1 (ASCL1)-DLL3-Notch1 pathway is critical for promoting the growth of lung neuroendocrine cells, and DLL3 is a downstream target of ASCL1 transcription factors[21]. Hopefully, the high expression of DLL3 in LCNEC stage IV testifies to it could be a potential target for LCNEC targeted therapy in the immediate future[22]. It has been reported that the high-grade neuroendocrine carcinomas were negative for DLL3, the five-year OS and PFS were significantly improved in patients who received adjuvant chemotherapy. However, the patients with positive for DLL3 were no difference[23]. In recent years, the literature we found only a few data have analyzed the relationship between DLL3 and prognosis in gastrointestinal tumors, and the expression of DLL3 in GC and its effect on prognosis have not been reported. The research we analyzed the DLL3 expression in patients with GC. The expression of DLL3 was tied to worsening OS for GC patients followed for ten-year, as well as in male patients, in intestinal and surgery combined with 5-FU chemotherapy. The results could be similar to the high-grade neuroendocrine carcinomas[23]. DLL3 mRNA overexpression was associated with worsen OS in stage Ⅱ and stage Ⅲ but better OS in stage Ⅳ. Clinical validation of hu et al. 's conclusion that overexpression of DLL3 promotes the proliferation of GC cells, and that downregulation of DLL3 inhibits the proliferation of GC cells[24]. These results could herald DLL3 as a negative regulator and an independent biomarker of negative prognosis in advanced GC. DLL3 high expression was tied to better prognosis in poorly differentiated than well differentiated.

The expression of DLL4 ligand in Notch signaling pathway is most significant in tumor endothelial cells. In the tumor microenvironment, tumor secreted angiogenic factors such as VEGF can induce the

expression of DLL4 in endothelial cells. Dll4-notch signaling in turn inhibits the transcriptional expression of VEGFR-2 and its co-receptor NRP-1 in signal receiving cells, making DLL4 a negative feedback regulatory molecule to inhibit hyperplasia of blood vessels[25]. DLL4 has significantly more GC research than DLL1 and DLL3. Miao et al. reported that DLL4 silencing restrained the self-renewal ability of GC stem cells and promoted their differentiation, significantly inhibiting their tumorigenic ability. DLL4 overexpression activates the Notch-1 signaling pathway, improving tumor invasion and remarkably increasing the resistance to 5-FU chemotherapy. In the analysis of 383 patients with GC, DLL4 overexpression was meaningfully relevant to an increased risk of lymph node metastasis and distant metastasis, with poor prognosis[26]. This study verifies that DLL4 expression was meaningfully tied to worsening OS in GC patients for ten years, as well as male patients, stage Ⅲ and diffuse cancer patients. Unfortunately, the overexpression of DLL4 was tied to worsen OS in not only surgery but 5-FU based adjuvant. These results are fully in line with Miao et al's conclusion. Recently, Kim Y reported that about 19.9% of GC patients have high expressions of DLL4, and the probability of recurrence and metastasis after radical surgery is high[27]. In conclusion, perhaps DLL4 is a potential novel prognostic indicator for GC to guide clinical practice.

JAG1 is one of the five ligands of Notch gene. Jag1-notch interaction leads to proteolytic cleavage of cascade, which promotes the transport of the Intracellular domain of Notch into the nucleus, thereby activating transcription of downstream target genes[28]. There is now compelling evidence that JAG1 exerts pro-oncogenic functions in breast, cervical and ovarian cancer. Overexpression of JAG1 can promote the metastasis of colorectal carcinoma by inducing epithelial-mesenchymal transition, so JAG1 can be used as a target for anti-tumor therapy[29–30]. In contrast, JAG1 has been deemed a tumor suppressor gene in acute myeloid leukemia, which cancer cell growth can be reduced and patients with JAG1 overexpression also have a better prognosis[31]. In recent years, studies on the regulatory mechanism of JAG1 in GC have been limited, especially those that can guide prognosis. Xiao et al. reported that when miR-124 overexpression in GC the expression of the JAG1 and EZH2 was downregulated, and silencing of JAG1 or EZH2 by RNA interference also suppressed GC cell growth and metastasis. It has been certified that the JAG1 mRNA overexpressions were found in low differentiation adenocarcinoma, samples with lymph node metastasis, and samples at stage II, II and IV in GC tissue[32]. In our data, we found JAG1 high expression was tied to worsening OS for GC patients' follow-up of 10 years. JAG1 overexpression was tied to better OS in stage Ⅲ, intestinal, poorly differentiated and well differentiated cancer presents. Nevertheless, patients treated with surgery alone had a worse prognosis. Our findings complement Xiao's results, but there were some notable differences.

The latest research reported one of Notch ligand, JAG2 correlated to several carcinogenesis, endometrial cancer, myeloma, childhood medulloblastoma, glioblastoma and breast cancer. In GC, Kang et al. has been reported that JAG2 was meaningfully higher in GC tissues than in pericarcinomatous tissue. High relative expression of JAG2 was tied to better OS, the high expression in univariate analysis[33]. Our study figures out JAG2 mRNA overexpression was found to be significantly tied to worsening OS for GC patients for ten-year. It was inconsistent with the findings of Kang's. The reason may be due to the

difference in sample size. Although this study is the largest sample size reported to date. With the continuous updating and improvement of the database, we believe that there will be a larger sample size to verify our views in the future. JAG2 mRNA overexpression was tied to worsen OS in all clinical stages except stage Ⅱ. In addition, JAG2 mRNA overexpression was tied to worsen OS in intestinal, diffuse as well as well differentiated.

In conclusion, by exploiting the KM plotter database, we clear and definite the prognostic roles of five Notch ligands in GC, and update gene expression data and survival information from the largest sample library to date of 876 GC patients. There is insignificant correlation between the expression of only JAG1 mRNA and OS in all patients with GC. Our study preliminarily explored the prognostic role of Notch ligand in diverse clinicopathological characteristics. The results of the research will useful for understanding the biology of GC heterogeneity, provide more accurate prognostic evaluation tools and provide new potential targets for targeted drug development.

Declarations

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Data Availability: The datasets generated during and/or analysed during the current study are available in the [Kaplan-Meier plotter] repository, [http://kmplot.com/analysis/index.php?p= service & cancer gastric].

Statement: This research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants and/or their legal guardians. This Research have been performed in accordance with the Declaration of Helsinki.

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Figures

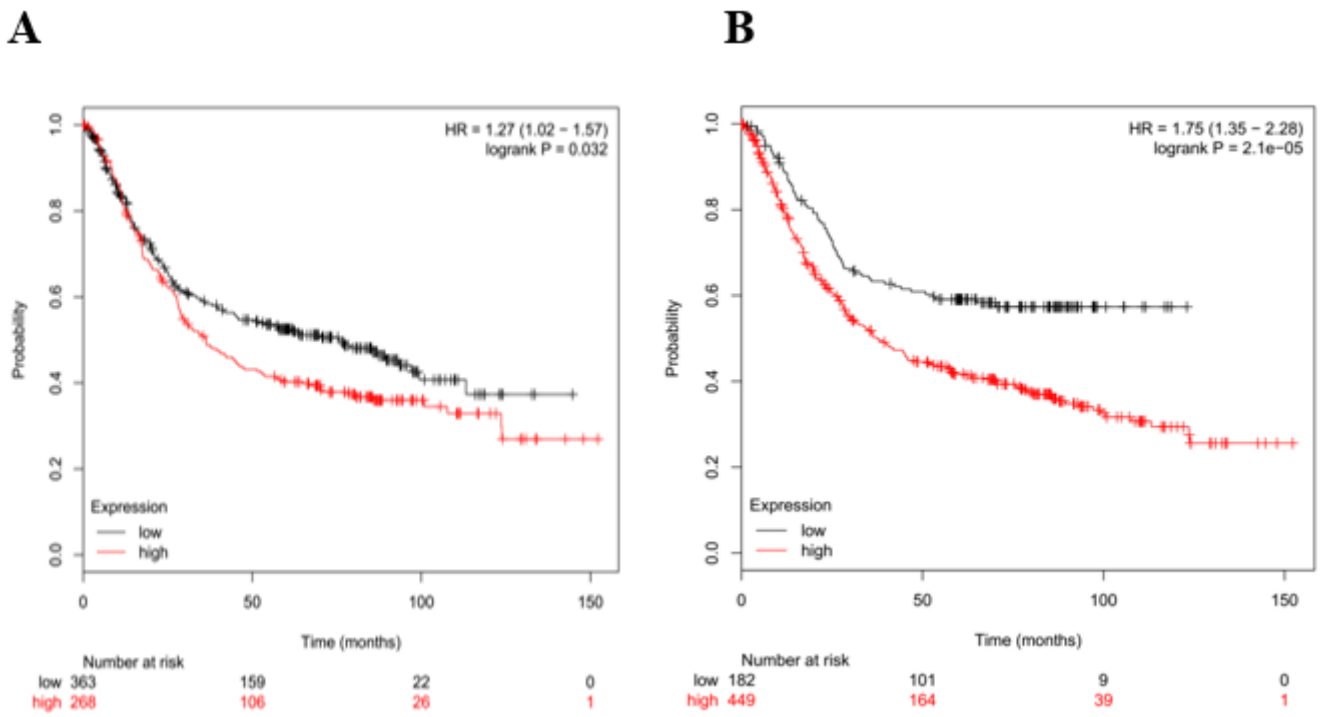

Figure 1

For DLL1, The Affymetrix ID is: 224215_s_at. (A) OS curves are plotted for GC patients (n = 631). The Affymetrix ID is: 227938_s_at. (B) OS curves are plotted for GC patients (n = 631).

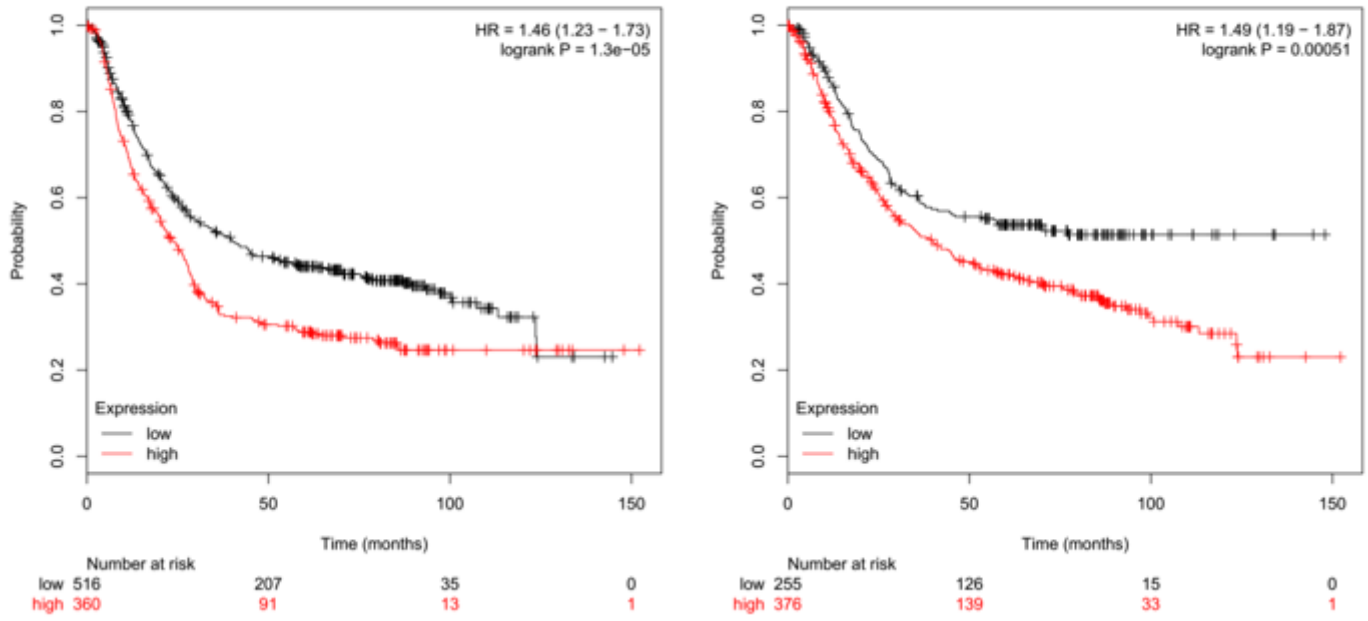

Figure 2

For DLL3, The Affymetrix ID is: 219537_x_at. (A) OS curves are plotted for GC patients (n = 876). The Affymetrix ID is: 222898_s_at. (B) OS curves are plotted for GC patients (n = 631).

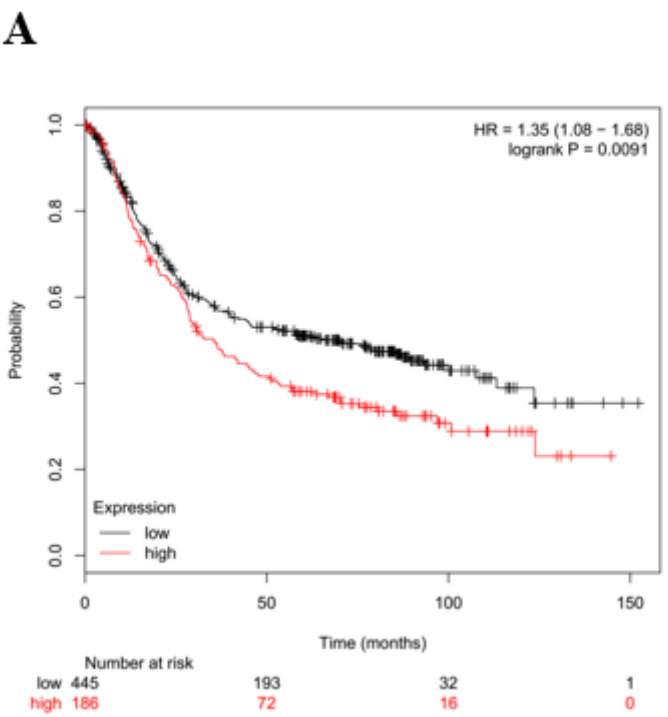

Figure 3

For DLL4, The Affymetrix ID is: 223525_at. (A) OS curves are plotted for GC patients (n = 631).

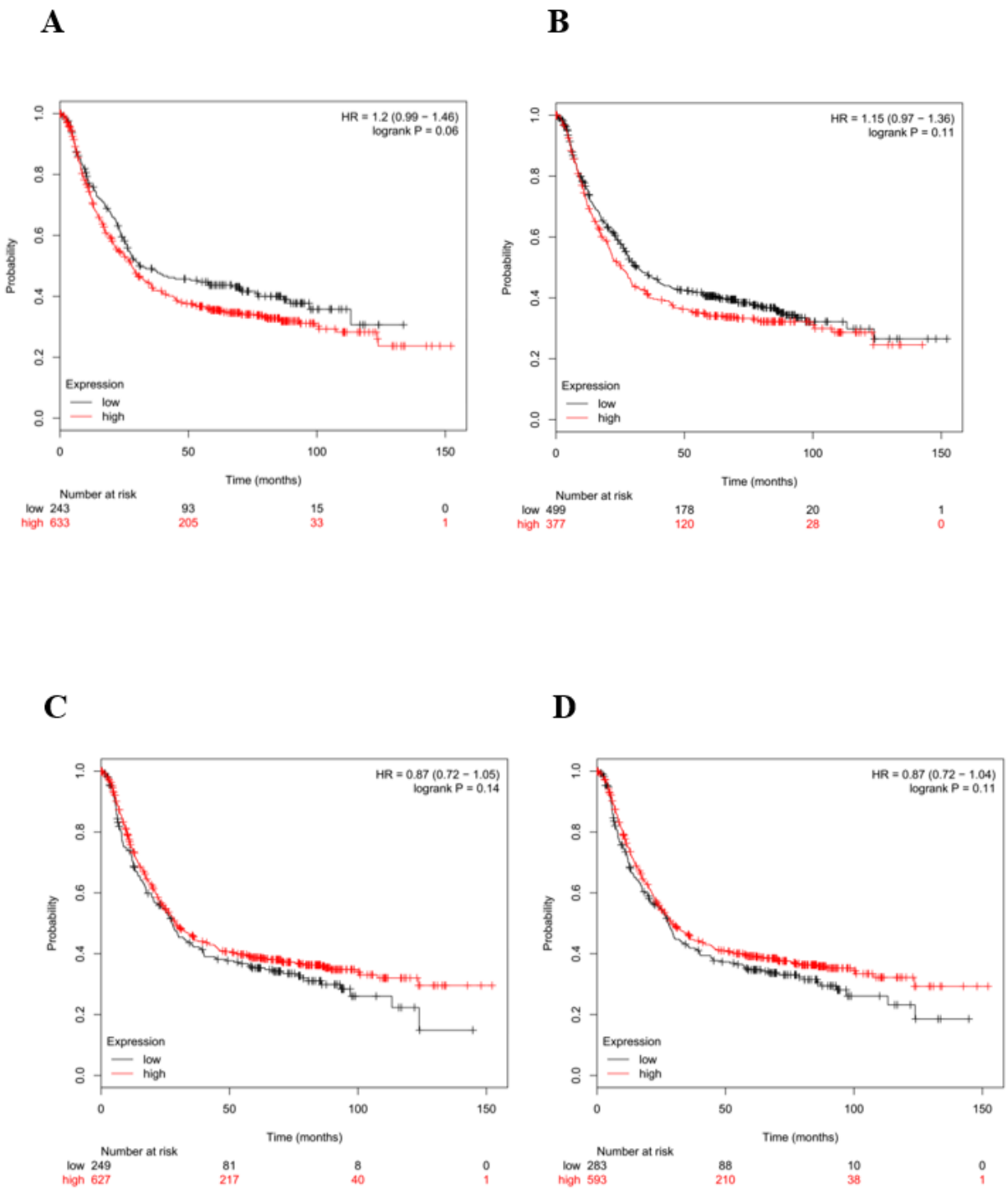

Figure 4

For JAG1, The Affymetrix ID is: 216268_s_at. (A) OS curves are plotted for GC patients (n = 876). The Affymetrix ID is: 209098_s_at. (B) OS curves are plotted for GC patients (n = 876). The Affymetrix ID is: 209099_x_at. (C) OS curves are plotted for GC patients (n = 876). The Affymetrix ID is: 216268_s_at. (D) OS curves are plotted for GC patients (n = 876).

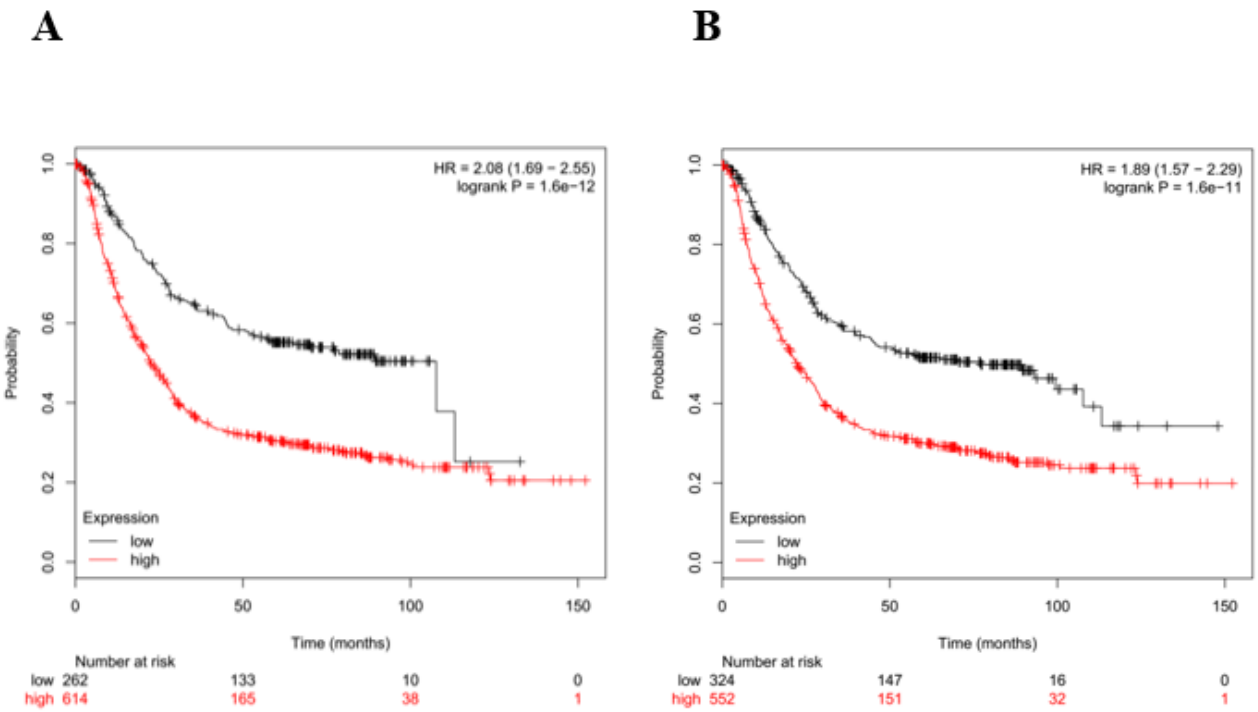

Figure 5

For JAG2, The Affymetrix ID is: 209784_s_at. (A) OS curves are plotted for GC patients (n = 876). The Affymetrix ID is: 32137_at. (B) OS curves are plotted for GC patients (n = 876).