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Notch1 activation is a poor prognostic factor in patients with gastric cancer

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Background: Aberrant Notch1 activation has been studied in many malignant tumours, but its role in gastric cancer remains unknown. This study is aimed to investigate the prognostic significance of Notch1 activation in patients with gastric cancer.

Methods: In this study, we prospectively enrolled two independent sets of patients with gastric cancer from China and defined the activation of Notch1 by immunohistochemical staining of its active form, intracellular domain of Notch1 (ICN1). The prognostic value of Notch1 activation and clinical outcomes in gastric cancer were evaluated.

Results: Expression of ICN1 is elevated in gastric cancer tissues and is predominately localised in the cell cytoplasm and/or membrane. High ICN1 expression positively correlates with tumour invasion depth ($P=0.032$), lymph node metastasis ($P<0.001$), advanced TNM stage ($P=0.003$) and reduced overall survival ($P=0.0004$) in the training set. Multivariate Cox regression analysis identified ICN1 as an independent prognostic factor ($P=0.031$), which could be incorporated into the TNM system to generate a better predictive model for patient outcomes. The *c*-index was 0.7375 when assessed with the TNM stage and improved to 0.8037 when ICN1 expression was added in the training set. These results were validated in the validation set.

Conclusions: Notch1 activation was correlated with gastric cancer progression and defined as a novel independent prognostic factor. Combining ICN1 expression with TNM stage may provide a better predictive model for outcomes of gastric cancer patients.

Although the incidence of gastric cancer has declined in recent years for improvement on prevention strategy, it remains the fourth common malignant tumour and the second most common cause of tumour-associated death in the world (Jemal *et al*, 2011). New cases occurred in China, accounting for nearly half of the world total (Yang, 2006). A larger number of patients are diagnosed as unresectable owing to atypical symptoms at early stage. Traditional prognostic model for gastric cancer was based on tumour cell-centred phenotype such as the TNM stage. However, increasing evidences have shown that patients in the same stage may have quite different outcomes, partly owing to the heterogeneity of tumour (Lim *et al*, 2005; Stock and Otto, 2005). Thus, elucidation of the molecular mechanisms underlying the tumour heterogeneity may add some prognostic information for the current TNM prognostic model and may provide novel therapeutic targets for patients with gastric cancer.

Notch1 is a type I transmembrane heterodimeric receptor that directly activates transcription of gene targets upon productive interactions with Notch ligands (Fortini, 2009; Kopan and Ilagan, 2009). Sequential cleavage of Notch1 is required for activation of the full-length receptors. Intracellular domain of Notch 1 (ICN1), the active form of Notch1, is released by proteolysis from the full-length Notch1 and transferred to the cell nucleus to associate with DNA-binding protein to assemble a transcription complex that activates downstream target genes. Previous studies have identified a multifaceted functional role of Notch signal in diverse cellular processes such as cell differentiation, proliferation, apoptosis, adhesion, migration, epithelial-to-mesenchymal transition (EMT), angiogenesis and oncogenesis (Artavanis-Tsakonas *et al*, 1999; Bolos *et al*, 2007). Although aberrant expression of Notch1 has been reported in a growing number of solid tumours, whether Notch1 signalling will be oncogenic or tumour suppressive

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was decided by the specific cell type and the presence of specific cytokines/growth factors in the cellular microenvironment and the dosage of signalling (Leong and Karsan, 2006).

Previous studies revealed that high expression of Notch1 in breast cancer (Reedijk *et al*, 2005), colorectal tumour (Reedijk *et al*, 2008), glioblastoma (Kanamori *et al*, 2007), oral squamous cell carcinoma (Joo *et al*, 2009) and melanoma (Balint *et al*, 2005; Liu *et al*, 2006) is correlated with tumour progression, whereas Notch 1 acts as a tumour suppressor gene in prostate cancer (Whelan *et al*, 2009), liver cancer (Wang *et al*, 2009), pancreatic cancer (Mullendore *et al*, 2009) and skin tumour (Panelos *et al*, 2008). Moreover, evidence obtained from studies of cervix cancer suggests that Notch signalling during the initial stages of tumourigenesis can prevent tumour formation, in contrast to later stages of tumour development, in which Notch activation is required (Talora *et al*, 2002). On the basis of these results, the activation of Notch1 in different tumours may have diametrical functions. Previous studies have revealed that Notch receptors (Notch1–3) and Notch ligand Jagged1 are expressed in human gastric cancer (Sander and Powell, 2004). Activation of Notch1 signal could promote progression of gastric cancer, partly via cyclooxygenase-2 (Yeh *et al*, 2009). However, the clinical significance of Notch1 activation and its correlation with gastric cancer progression remains largely unknown and needs to be further established.

In this study, we discovered that Notch1 activation, as indicated by elevated ICN1 levels, associates with gastric cancer progression. Intratumoral levels of ICN1 were positively correlated with tumour invasion depth, TNM stage and poor overall survival (OS) in gastric cancer patients. Notch1 activation can be used as an independent prognostic factor and should be incorporated with TNM stage to generate a better predictive model for outcomes of gastric cancer patients. These data identified the potential prognostic significance of Notch1 activation and may open a new avenue for therapeutic intervention with Notch1 for advanced-stage gastric cancer patients.

MATERIALS AND METHODS

Patients and specimens. Two independent sets comprising 191 gastric cancer patients who received standard gastrectomy with lymph node resection from two institutional clinical centres were enrolled in our study. The training set that comprised 101 consecutive patients with total or partial gastrectomy was obtained from Zhongshan Hospital (Shanghai, China) between 2000 and 2005. The validation set that comprised 90 consecutive patients was obtained from Nantong Tumor Hospital (Jiangsu, China) between May 2007 and February 2008 with the same enrolment criteria. All specimens were obtained from patients with informed consent approved by the Clinical Research Ethics Committee of each hospital. Clinicopathological variables including age, sex, location, tumour size, tumour differentiation, Lauren classification and TNM stage were collected for each patient. Tumour size was defined as the longest diameter according to the pathology report. None of these patients received any preoperative anticancer treatment. All specimens were pathologically reassessed independently by two gastroenterology pathologists according to the 2010 International Union against Cancer TNM classification system.

Tissue microarray, immunohistochemistry and evaluation of immunohistochemical findings. Formalin-fixed, paraffin embedded surgical specimens were used for tissue microarray construction and for subsequent immunohistochemistry (IHC) study. Two cores were taken from each surgical sample by using punch cores that measured 1.5 mm in the greatest dimension from the centre of the tumour foci. Tissue blocks were mounted on glass slides by sequencing. The IHC protocols were as described previously (Liu *et al*, 2011). The primary

antibody against ICN1 (Abcam, Cambridge, MA, USA) was used. The specificity of the antibody was demonstrated by IHC with peptide competition. The intensity of immunohistochemical staining of ICN1 was scored by two gastroenterology pathologists using the semi-quantitative immunoreactivity scoring (IRS) system as described previously (Weichert *et al*, 2008). Immunoreactivity score was derived by multiplying the intensity of immunohistochemical staining and the percentage of immunoreactive cells ranged from 0 to 12, and we defined 8 as the cutoff value for high and low expression according to the 'minimum *P*-value method' on the basis of its relation with OS. The negative control staining was treated equally with the primary antibody omitted.

Statistical analysis. The analysis was performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Pearson's χ^2 test or Fisher's exact test was used to compare qualitative variables. Kaplan–Meier analysis was used to determine the survival. Log-rank test was used to compare patient survival between subgroups. Numbers at risk were calculated at the beginning of each time period. The Cox proportional hazards regression model was used to perform univariate and multivariate analyses, and $P > 0.10$ was the criterion used for variable deletions when performing stepwise regression. Receiver operating characteristic (ROC) curve analysis was used to determine the predictive value of the parameters. All significance tests were two-sided and $P < 0.05$ was considered statistically significant. Results are reported according to the REMARK (Reporting Recommendations for Tumour Marker Prognostic Studies) guidelines (McShane *et al*, 2005).

RESULTS

Patient characteristics. The detailed characteristics of patients were shown in Table 1. There were more patients diagnosed at an advanced stage (TNM III + IV) in the validation set compared with the training set (67.7% vs 46.5%) and more distal gastric cancer in the validation set compared with the training set (64.5% vs 41.6%). Overall survival was defined as the interval between surgery and last visit or death. The follow-up median time was 77 and 35 months for the training and validation sets, respectively. Such heterogeneity may help confirming that the predictor has universal applicability across heterogeneous population of patients from different districts.

Inmunohistochemical findings. To ascertain the expression of activated Notch1 in gastric tumour tissue, we examined the expression of ICN1 in the training and validation sets of specimens and matched the non-tumour specimens by IHC staining. The expression of ICN1 was mainly localised in the cell cytoplasm and/or membrane in the tumour area, and showed variable staining intensity, whereas stroma cells showed negative staining (Figure 1A–D). The expression level was elevated in tumour tissues compared with non-tumour tissues. High ICN1 expression was more easily seen in advanced tumour stage and the percentage of positive IHC staining increased with tumour progression according to the TNM stage (Figure 1E). Conclusively, the expression of ICN1 was elevated in gastric tumour tissues compared with non-tumour tissues, especially in advanced tumour stage, indicating the potential oncogenic role of Notch1 activation in gastric cancer.

Relation between ICN1 expression and clinicopathological features in gastric cancer patients. The correlation between ICN1 expression and clinicopathological features in gastric cancer patients were shown in Table 1. According to the cutoff value derived from IRS score, 48.5% (49–101) and 50% (45–90) were ICN1 low expression in the training and validation sets, respectively. Intracellular domain of Notch1 expression was correlated with tumour invasion depth ($P = 0.032$ and 0.013 ,

Table 1. Relation between intratumoral ICN1 expression and clinical characteristics in the training and validation sets of patients with gastric cancer

Factor	Training set					Validation set				
	Patients		ICN1 expression			Patients		ICN1 expression		
	No.	%	Low	High	P-value	No.	%	Low	High	P-value
All patients	101	100	49	52		90	100	45	45	
Age (years) ^a					0.059					0.822
≤60	50	49.5	29	21		29	32.2	15	14	
>60	51	50.5	20	31		61	67.8	30	31	
Gender					0.317					0.227
Female	38	37.6	16	22		23	25.6	14	9	
Male	63	62.4	33	30		67	74.4	31	36	
Localisation					0.408					0.558
Proximal	10	9.9	3	7		19	21.1	11	8	
Middle	49	48.5	26	23		13	14.4	5	8	
Distal	42	41.6	20	22		58	64.5	29	29	
Tumour size ^a (cm)					0.869					0.270
<3.5	61	60.4	30	31		16	17.8	10	6	
≥3.5	40	39.6	19	21		74	82.2	35	39	
Differentiation					0.450					0.048
Well	6	6.0	4	2		1	1.1	1	0	
Moderately	36	35.6	15	21		36	40.0	23	13	
Poorly	59	58.4	30	29		53	58.9	21	32	
Lauren classification					0.123					0.822
Intestinal type	75	74.3	33	42		61	67.8	30	31	
Diffuse type	26	25.7	16	10		29	32.2	15	14	
T classification					0.032					0.013
T1	31	30.7	21	10		4	4.4	3	1	
T2	10	9.9	6	4		8	8.9	7	1	
T3	4	4.0	2	2		9	10.0	7	2	
T4	56	55.4	20	36		69	76.7	28	41	
N classification					<0.001					0.076
N0	43	42.6	29	14		20	22.2	14	6	
N1	19	18.8	9	10		16	17.8	10	6	
N2	14	13.9	8	6		26	28.9	10	16	
N3	25	24.7	3	22		28	31.1	11	17	
Distant metastasis					0.243					0.242
No	98	97.0	49	49		87	96.7	45	42	
Yes	3	3.0	0	3		3	3.3	0	3	
TNM stage					0.003					0.031
I	35	34.7	22	13		6	6.7	5	1	
II	19	18.8	13	6		23	25.6	15	8	
III	44	43.6	14	30		58	64.4	25	33	
IV	3	2.9	0	3		3	3.3	0	3	

Abbreviations: ICN1 = intracellular domain of Notch1; TNM = tumour node metastasis. P-value <0.05 marked in bold font shows statistical significant.

^aSplit at median.

respectively) and TNM stage ($P=0.003$ and 0.031 , respectively) in the two independent sets. In addition, in the training set, ICN1 expression was associated with lymph node metastasis ($P<0.001$), and in the validation set, ICN1 expression was correlated with tumour differentiation ($P=0.048$). High ICN1 expression was more easily seen in patients with increased tumour invasion depth and advanced TNM stage. In conclusion, intratumoral ICN1 expression may correlate with gastric cancer progression.

Association between ICN1 expression and OS in patients with gastric cancer. To further investigate the relation between ICN1

expression and OS, we compared the OS between ICN1 high expression and ICN1 low expression subgroups by using Kaplan–Meier analysis and log-rank test. Intracellular domain of Notch1 low expression has a survival benefit compared with high expression (Figure 2A, $P=0.0004$ and 0.0001 , respectively) in two independent sets, indicating that intratumoral ICN1 expression may be an important molecular mechanism in identifying outcomes for gastric cancer patients. For further investigation of the effect of ICN1 expression in stratifying patients with different TNM stratum, we combined TNM I + II and TNM III + IV as an early-stage tumour and as an advanced-stage tumour, respectively.

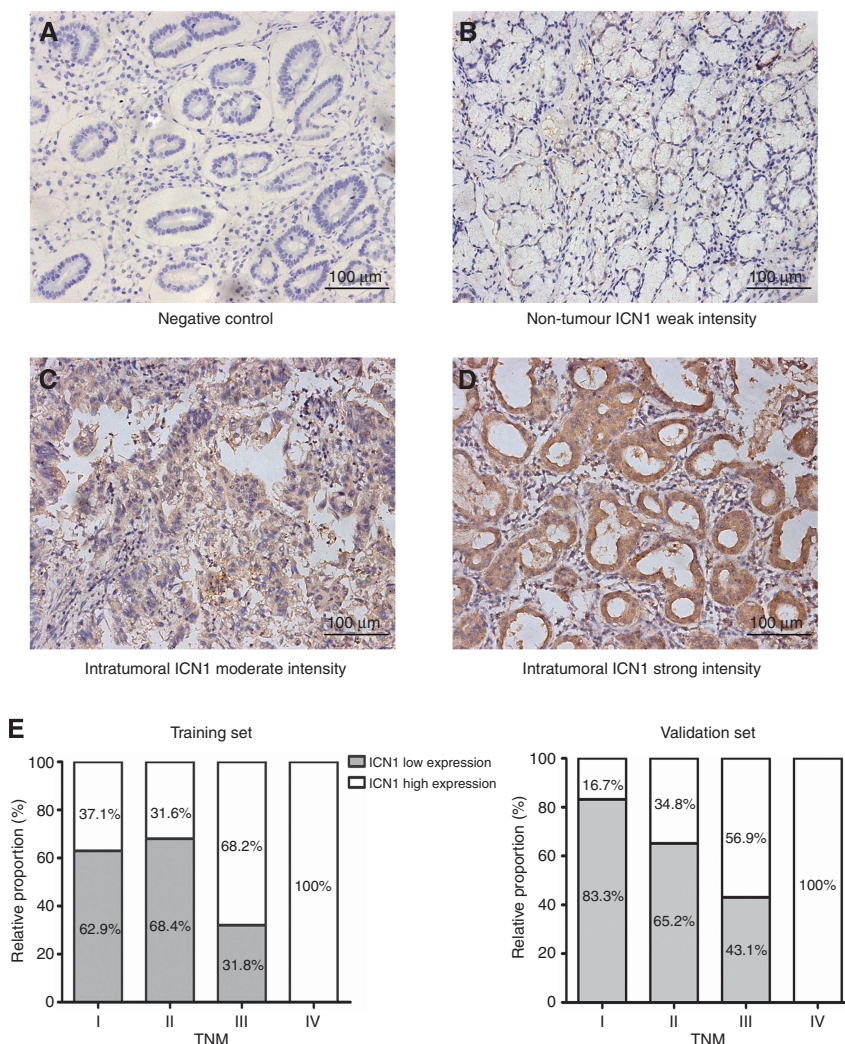


Figure 1. Expression of ICN1 in sections of gastric cancer. Representative microphotographs of ICN1 expression (A–D). Negative control (A); non-tumour weak intensity (B); intratumoral moderate intensity (C); intratumoral strong intensity (D). (E) The percentage of patients with ICN1 high or low expression according to the TNM stage in the training (left panel) and validation (right panel) sets. Original magnification: $\times 200$.

In the early stage, ICN1 expression did not show prognostic significance with OS (Figure 2B, $P=0.6817$ and 0.1702 , respectively), whereas for patients in advanced stage, ICN1 low expression had a great survival benefit compared with high expression (Figure 2C, $P=0.0094$ and 0.0026 , respectively) in the training and validation sets. All these results here showed that ICN1 expression correlates with OS for patients with gastric cancer, especially for the advanced stage.

Prognostic factor. To identify the prognostic significance of clinicopathological factors for OS, univariate Cox analysis was conducted. T classification (HR, 7.996; 95% CI, 1.905–35.559, $P=0.005$), N classification (HR, 14.251; 95% CI, 3.394–59.843, $P<0.001$), distant metastasis (HR, 13.121; 95% CI, 3.538–48.665, $P<0.001$), TNM stage (HR, 10.648; 95% CI, 3.713–30.538, $P<0.001$) and ICN1 expression (HR, 4.072; 95% CI, 1.752–9.466, $P=0.001$) were identified as risk factors that may affect the OS of gastric cancer patients in the training set, whereas distant metastasis (HR, 3.346; 95% CI, 1.012–11.069, $P=0.048$), TNM stage (HR, 2.879; 95% CI, 1.521–5.450, $P=0.001$) and ICN1 expression (HR, 2.797; 95% CI, 1.645–4.755, $P<0.001$) were identified as risk factors for OS in the validation set (Table 2). Further adjustment of covariate factors by using multivariate Cox analysis identified TNM stage (HR, 8.547; 95% CI, 2.931–24.925, $P<0.001$ and HR, 2.427; 95% CI, 1.267–4.646, $P=0.007$) and ICN1 expression

(HR, 2.579; 95% CI, 1.093–6.086, $P=0.031$ and HR, 2.414; 95% CI, 1.407–4.143, $P=0.001$) as independent prognostic factors for the training and validation sets, respectively (Figure 3A). Patients with ICN1 high expression in the advanced stage showed more risk for a reduced OS (HR, 3.688; 95% CI, 1.219–10.715, $P=0.016$ and HR, 2.475; 95% CI, 1.338–4.577, $P=0.004$), whereas patients in early stage did not have such a risk (HR, 0.625; 95% CI, 0.065–6.014, $P=0.625$ and HR, 2.187; 95% CI, 0.689–6.924, $P=0.184$) in the two independent sets (Figure 3A). In conclusion, intratumoral ICN1 expression may be a novel defined independent prognostic factor for gastric cancer patients, especially for advanced tumour.

Combination of ICN1 expression and TNM stage and ROC analysis. To generate a more sensitive predictive model for outcomes of patients with gastric cancer, we combined ICN1 expression and TNM stage to create a prognostic score system. Receiver operating characteristic analysis was used to compare the prognostic validity of the combination between ICN1 or TNM stage alone. The combination of ICN1 and TNM stage showed better prognostic value (AUC (95%), 0.854 (0.770–0.916)) compared with TNM stage (AUC (95%), 0.793 (0.701–0.867), $P=0.0263$) or ICN1 expression (AUC (95%), 0.687 (0.587–0.776), $P<0.0001$) alone in the training set and (AUC (95%), 0.756 (0.654–0.840)) compared with TNM stage (AUC (95%), 0.683 (0.577–0.777), $P=0.0149$) or ICN1 expression (AUC (95%), 0.675 (0.568–0.770), $P<0.01$) alone

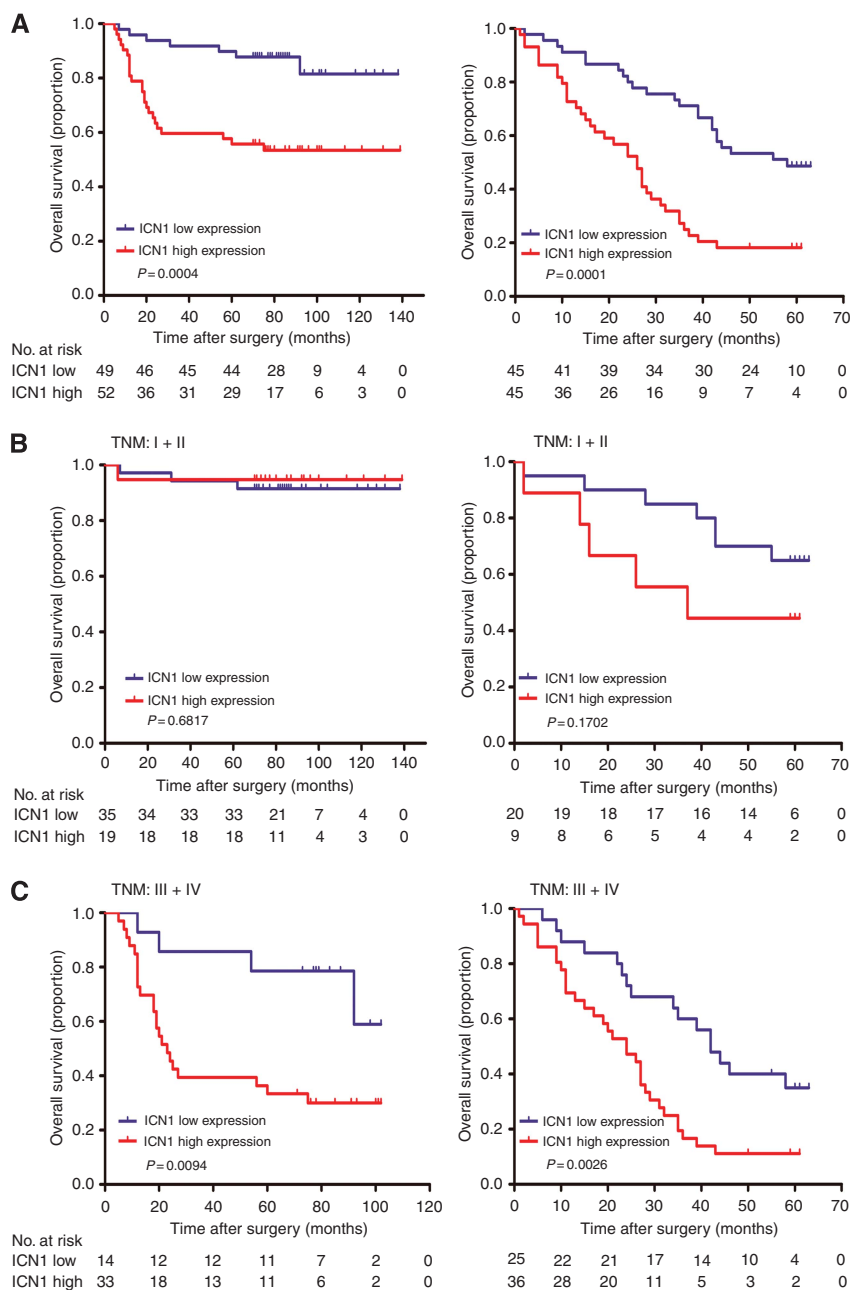


Figure 2. Kaplan–Meier analysis for OS of patients with gastric cancer according to the ICN1 expression. (A–C) Kaplan–Meier analysis for OS of patients with gastric cancer according to ICN1 expression in (A) all patients (left panel, training set, $n=101$, $P=0.0004$; right panel, validation set, $n=90$, $P=0.0001$), (B) TNM I + II (left panel, training set, $n=54$, $P=0.6817$; right panel, validation set, $n=29$, $P=0.1702$) and (C) TNM III + IV (left panel, training set, $n=47$, $P=0.0094$; right panel, validation set, $n=61$, $P=0.0026$). P -value was calculated by log-rank test.

in the validation set (Figure 3B). The c -index was 0.7375 when assessed with TNM stage and improved to 0.8037 when ICN1 expression was added in the training set (Table 3). The AIC was 244.32 when assessed with TNM stage and reduced to 231.31 when ICN1 expression was added in the training set (Table 3). These results were validated in the validation set (Table 3). All these results proved that combination of ICN1 and TNM stage can generate a better predictive model for OS of gastric cancer patients.

DISCUSSION

Traditional predictive model for outcomes of patients with gastric cancer relies on TNM stage and is limited in its ability to

discriminate a subset of patients for the heterogeneity of tumour. In fact, tumour cells acquired different biological phenotype by various mechanisms in disease initiation and progression. Thus, elucidation of the molecular mechanisms underlying the tumour heterogeneity may shed light on better prediction for patient outcomes. In our study, we have demonstrated the prognostic power of Notch1 activation for gastric cancer and revealed that Notch1 activation may be a potential mechanism under tumour heterogeneity. These results have both prognostic and therapeutic implications.

Notch signalling defines an evolutionarily conserved local cell interaction mechanism that participates in a variety of cellular processes (Bolos *et al*, 2007). Aberrant expression of Notch has been reported in various solid malignancies and function as an oncogene or as a tumour suppressor gene (Balint *et al*, 2005;

Table 2. Univariate Cox regression analyses for overall survival in the training and validation sets of patients with gastric cancer

Factor	Overall survival					
	Training set			Validation set		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (years) ^a : >60 vs ≤60	1.632	0.792–3.364	0.184	1.358	0.774–2.382	0.287
Gender: male vs female	1.010	0.490–2.085	0.978	0.937	0.522–1.681	0.826
Localisation: distal vs middle + proximal	1.818	0.896–3.689	0.098	0.886	0.528–1.487	0.646
Differentiation: poorly vs moderately + well	1.376	0.659–2.876	0.395	1.404	0.836–2.356	0.199
Lauren classification: diffuse vs intestinal	1.434	0.675–3.046	0.349	1.668	0.985–2.824	0.057
Tumour size (cm) ^a : ≥3.5 vs <3.5	1.297	0.639–2.633	0.471	2.388	0.953–5.984	0.063
T classification: T2 + T3 + T4 vs T1	7.996	1.905–35.559	0.005	22.806	0.299–1737.244	0.157
N classification: N1 + N2 + N3 vs N0	14.251	3.394–59.843	<0.001	1.844	0.933–3.645	0.078
Distant metastasis: yes vs no	13.121	3.538–48.665	<0.001	3.346	1.012–11.069	0.048
TNM stage: III + IV vs I + II	10.648	3.713–30.538	<0.001	2.879	1.521–5.450	0.001
ICN1 expression: high vs low	4.072	1.752–9.466	0.001	2.797	1.645–4.755	<0.001

Abbreviation: 95% CI = 95% confidence interval; HR = hazard ratio; ICN1 = intracellular domain of Notch1; TNM = tumour node metastasis. P-value <0.05 marked in bold font shows statistical significant. ^aSplit at median.

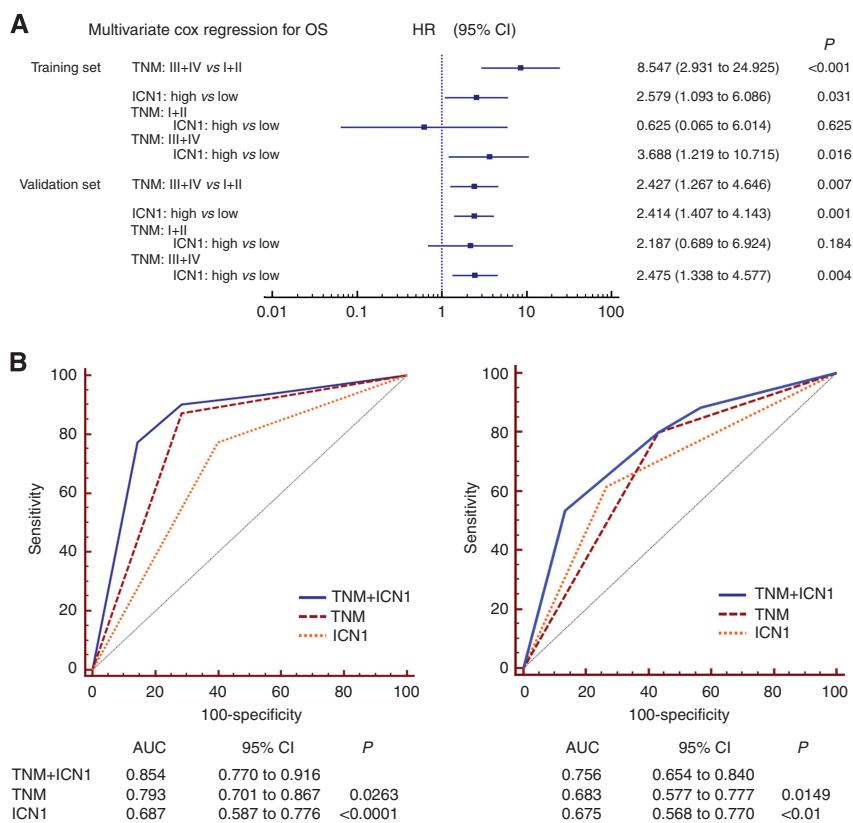


Figure 3. Multivariate Cox analysis and ROC analysis for the predictive value of ICN1 expression in gastric cancer patients. (A) Multivariate Cox analysis identified independent prognostic factors for the training and validation sets. (B) Receiver operating characteristic analysis of the sensitivity and specificity for the predictive value of combined TNM and ICN1 stratification model, TNM model, ICN1 model in the training (left panel) and validation (right panel). P-value <0.05 was considered statistically significant.

Reedijk *et al*, 2005; Liu *et al*, 2006; Kanamori *et al*, 2007; Panelos *et al*, 2008; Reedijk *et al*, 2008; Joo *et al*, 2009; Mullendore *et al*, 2009; Wang *et al*, 2009; Whelan *et al*, 2009). In this study, we focused mainly on Notch1 and found that Notch1 activation, represented by ICN1 expression, correlates with outcomes of

gastric cancer patients and can be used as a novel independent prognostic factor. Early-stage gastric cancer exhibited relative lower portion of ICN1 staining, whereas advanced-stage gastric cancer showed elevated ICN1 staining (Figure 1). Intracellular domain of Notch1 high expression in advanced-stage gastric

Table 3. Comparison of the prognostic accuracies of TNM staging and ICN1 expression

Model	C-index	AIC
Training set		
ICN1	0.6715	262.37
TNM	0.7375	244.32
TNM + ICN1	0.8037	231.31
Validation set		
ICN1	0.6360	473.45
TNM	0.6046	475.92
TNM + ICN1	0.6696	465.64

Abbreviations: AIC = Akaike information criterion; C-index = Harrell's concordance index; ICN1 = intracellular domain of Notch1.

cancer indicated its possible role in the maintenance of stem cell-like property. All these results further confirmed the functional role of ICN1 expression in tumour progression.

Further analysis of the relation between ICN1 expression and OS of gastric cancer patients revealed a negative correlation. Survival of advanced-stage gastric cancer patients can be stratified by ICN1 expression, whereas patients in early stage cannot (Figure 2). Incorporating ICN1 expression into current prognostic TNM staging system improved the prognostic value for OS (Figure 3B). These results showed that ICN1 expression might add some prognostic information for patients with gastric cancer and lead to a more precise classification integrated with TNM stage. However, the results of combination of intratumoral ICN1 expression into the current prognostic model need an independent and larger data set to validate.

Numerous mechanisms have suggested to have a role in Notch-induced solid tumorigenesis, but the exact mechanisms were diverse in different tumours. Activated Notch signalling can suppress p53 activity in T-acute lymphoblastic leukemia and breast cancer to promote oncogenesis through increased cell survival and genomic instability (Beverly *et al*, 2005; Mungamuri *et al*, 2006). Increased Notch signalling has also been associated with the maintenance of a stem-like cell population in medulloblastoma by interacting with hedgehog signalling (Hallahan *et al*, 2004), and by activation of PI3K-AKT pathways in melanoma (Liu *et al*, 2006). Activation of the Notch1 signalling pathway could promote gastric cancer progression at least in part through COX-2 (Yeh *et al*, 2009). All these results revealed a multifaceted pathway of Notch1 activation underlies tumour progression. However, the exact molecular function roles of Notch1 activation in gastric cancer progression remain far from being fully elucidated and is awaiting further investigation.

As Notch1 activation indicate tumour progression and poor outcome in patients with gastric cancer, targeting Notch1 signalling steps, including ligand/receptor binding, release of ICN1, interaction of ICN1 and downstream target genes, as well as ICN1 protein stability, may have antitumour effects. Currently, blocking Notch1 signalling by suppressing the proteolytic step, which is mediated by γ -secretase, leads to the generation of ICN1 becoming plausible (Shih and Wang, 2007). Inhibitors of γ -secretase have been studied worldwide in the past decades for their potential to block the generation of A β peptide that is associated with Alzheimer's disease (Selkoe and Kopan, 2003). Thus, γ -secretase inhibitors tested clinically for Alzheimer's disease may be applicable to treat tumours that have constitutive Notch1 activation. Previous studies have also revealed that Notch signalling pathway activation may have a critical role in the regulation of chemotherapy resistance via promoting EMT and stem-cell like

property (Wang *et al*, 2010). Blocking Notch1 activation may restore the sensitivity of tumour to chemotherapy treatment. These data suggest that targeting Notch1 signal with γ -secretase inhibitors may be a novel and efficient strategy for the treatment in advanced gastric cancer patients alone or combined with traditional cytotoxic drugs.

Although our study proved the prognostic significance of Notch1 activation in patients with gastric cancer, there are some limitations. The study is retrospective in nature and the number of patients enrolled is relatively small. A larger, multicentred, prospective data are needed to validate these results.

In conclusion, we have identified that Notch1 activation correlates with gastric cancer progression and can be used as a novel prognostic factor for patient outcomes. Incorporating ICN1 expression into TNM stage can provide a better prognostic model for patients with gastric cancer. Targeting Notch1 activation may open a new avenue for treatment of advanced-stage gastric cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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