Expression of Notch1 to -4 and their Ligands in Renal Cell Carcinoma: A Tissue Microarray Study

L.M. ANTÓN APARICIO[1,2,3,10], V. MEDINA VILLAAMIL[2], G. APARICIO GALLEGO[2], I. SANTAMARINA CAÍNZOS[2], R. GARCÍA CAMPELO[3], L. VALBUENA RUBIRA[4], S. VÁZQUEZ ESTÉVEZ[5], 10, L. LEÓN MATEOS[6,10], J. L. FIRVIDA PEREZ[7,10], M. RAMOS VÁZQUEZ[8,10], O. FERNÁNDEZ CALVO[7,10] and M. VICTORIA BOLÓS[9]

[1]UDC, Medical Department, A Coruña, 15006, Spain;
[2]INIBIC, CHU A Coruña, Oncology Group, A Coruña, 15006, Spain;
[3]CHUAC, Medical Oncology Department, A Coruña, 15006, Spain;
[4]Modelo Hospital, Pathology Department, A Coruña, 15011, Spain;
[5]CH Xeral Calde, Medical Oncology Department, Lugo, 27004, Spain;
[6]CHUS, Medical Oncology Department, Santiago de Compostela, 15705, Spain;
[7]CHOU, Medical Oncology Department, Ourense, 32005, Spain;
[8]COG, Medical Oncology Department, A Coruña, 15009, Spain;
[9]Pfizer Oncology, Medical Scientific Regional, Madrid, 28054, Spain;
[10]GGIO-GU, A Coruña, 15006, Spain

Abstract. *Background: Mutations in signalling pathways essential for embryonic development often lead to tumourigenesis, as is also true for Notch. The aim of this study was to assess the relationship between Notch1 to -4 and their ligands with anatomopathological features of the patients with renal cell carcinoma (RCC). Materials and Methods: This study investigated the pattern of protein expression in RCC specimens using tissue microarray technology. A total of 80 paraffin-embedded RCC samples were retrospectively analysed together with ACHN and A.704 cell lines. Results: Notch1 showed significant positive correlation with chromophobe RCC, no broken capsule, Furhman grade I and when the number of nodes involved was small [(N=1); p=0.039, 0.016, 0.037 and 0.001, respectively)]. Notch3 showed higher expression when the tumour was located in the right kidney (p=0.048). Conclusion: Notch1 may be useful in the future as a biomarker for the differential diagnosis of different RCC histological subtypes. Notch1 to -3 may also have potential use as a strong prognostic factor.*

Correspondence to: Vanessa Medina Villaamil, BS, INIBIC, CHU A Coruña, Oncology Group, Xubias de Abaixo s/n, 15006 A Coruña, Spain. Tel: +34 981178272, Fax: +34 981178273, e-mail: vanesa.medina.villaamil@sergas.es

Key Words: Notch, notch ligands, immunohistochemistry, renal cancer biomarkers, tissue array.

The Notch pathway is an evolutionary conserved intercellular signalling pathway that is involved in numerous biological processes including cell-fate determination, cellular differentiation, proliferation, survival and apoptosis (1-3).

It is known that Notch receptors also play a role in tumour angiogenesis and there are at least two distinct mechanisms for activating signalling in tumour endothelium, namely: (i) increased Jagged1, delta-like ligand (DLL) 1, or DLL4 expression activates Notch signalling in neighbouring endothelial cells (ECs), stimulating tumour angiogenesis and tumour growth (4), and (ii) Notch receptor activation is required for vascular endothelial growth factor-induced up-regulation of target genes (5).

Notch has emerged as a critical element for kidney development. There is accumulating evidence that Notch signalling affects survival, proliferation and cell fate at various stages of kidney development, including the decision of the kidney side cell population to self-renew or differentiate (6, 7). There are several studies showing that the Notch signalling pathway has an important role during the development of the mammalian kidney and key members of the Notch pathway are expressed during nephrogenesis.

In the adult kidney, localisation of the Notch pathway members has been performed. Most members of this pathway showed expression in proximal tubules, including Notch1, Notch2, Notch3, Jagged2 (also cortical collecting ducts), Jagged1 (only weakly expressed in glomerular and vascular bundles), Deltex2 (Dtx2) and Deltex3 (Dtx3) (6).

Notch signalling is associated with both oncogenic and tumour-suppressive roles and has been documented in various cancers. Specifically, the Notch signalling cascade is constitutively active in human clear-cell renal cell carcinoma (cRCC) cell lines and primary tumours. It has been demonstrated that the Notch signalling pathway components are expressed in cRCC cells and that Notch pathway elements are overexpressed in primary cRCCs with significantly higher levels of Notch1 and Jagged1 compared with normal kidney (8). Previous experimental studies reported that Notch3 and Jagged1 mRNAs are elevated in cRCC (9).

The purpose of the present study was to investigate, for the first time, whether the expression of Notch1 to -4 receptors and their ligands (Jagged1, DLL1, 3 and 4) may be useful as biological markers in renal cell carcinoma (RCC). The study examined whether their expression is associated with a specific histological subtype. In addition the relationship with pathologic features was also tested.

Materials and Methods

Cell lines. The tumour cell lines ACHN and A.704 were used as a surrogate model of renal cancer. Both cell lines were maintained in Dulbecco's Modified Eagle's Medium-High Glucose (Sigma-Aldrich Co, Ltd Irvine, Ayrshire, UK) supplemented with 10% foetal calf serum inactivated, 1% non-essential amino acids (NEAA) and 1% penicillin, 1% streptomycin and 1% amphotericin at 37˚C in 5% CO_2. In the case of A.704, it was necessary to include 1 mM sodium pyruvate (NaP) to the culture medium. Cells from adherent cultures were recovered with 1% trypsin/EDTA cell-dissociating reagent.

To obtain the cytospin of the cell lines, an aliquot of cultured cells was separated and centrifuged at 1,500rpm for 10 min in the Universal 32 Hettich (Sigma Laborzentrifugen GmbH, Osterode, Germany). The pellet was diluted in phosphate buffered saline (PBS) supplemented with 5% foetal bovine serum. After preparing the crystals with filters and funnels, 500 μl of the cell suspension were added into each well and centrifuged at 1,500 rpm for 10 min in the Universal 32 Hettich. The crystals were dried at room temperature and fixed by immersion in cold acetone for 10 min.

Patients and tissue samples. The patient cohort included 80 patients treated with a partial or radical nephrectomy for RCC, including chromophobe RCC (chRCC), papillary RCC (pRCC) and cRCC variants, recruited between 1996 and 2006. Immunohistochemistry (IHC) studies were performed and clinical data from an established kidney cancer database were reviewed. Patients were staged using radiographic studies and postoperative pathological data, according to the 1997 tumour-node-metastasis (TNM) criteria proposed by the American Joint Committee on Cancer. Tumour grading was performed using the Fuhrman grading scale (10). The patient group consisted of 53 men and 27 women, aged 34 to 87 years, with a mean age of 64 years. Among the tissue samples there were 57 cRCC, 15 chRCC, 6 pRCC, 2 samples of undetermined histological type and 11 renal healthy controls. Tumour laterality was almost equally distributed between the left and the right kidney (49.4 and 50.6%, respectively). Renal pelvic invasion was presented in 11% of tumours. Only 2.5% of the cases studied showed invasion of lymphatic vessels. Renal capsule rupture was found in 15% of tumours. Only one case (1.2%) showed invasion of the veins, while 7.3% had renal hilar invasion. Finally, the tumour size ranged from 2-140 cm in length.

Construction of tissue microarray blocks and immunohistoanalysis. Archival tumour specimens from the patient cohort were obtained from the histopathological archives of the Pathology Department of the Modelo Hospital, A Coruña, Spain, with the approval of the Institutional Review Board.

For IHC evaluation, tissue microarrays (TMA) were constructed using a manual tissue arrayer with accessory (Durviz, Valencia, Spain). Representative haematoxylin-eosin-stained samples of all tumours were reviewed to assess the histological type and grade. To identify viable, morphologically-interesting areas of the specimen, three cylindrical core biopsies (2 mm in diameter) were taken from different sites of each tumour sample and precisely arrayed in a recipient paraffin tissue microarray block to construct a TMA containing 48 samples (a 6×8 matrix was designed). Eleven specimens from healthy kidneys were also analysed for comparison as TMA control.

For immunofluorescence on cell lines of the antibodies studied, the signals were generated by a secondary antibody mouse anti-rabbit (Santa Cruz, CA, USA), IgG conjugated to phycoerythrin diluted 1:400. Nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Stained sections were mounted in glycogen (Sigma-Aldrich) and visualised using a BX61 fluorescence microscope (Olympus, Postfach, Hamburg, Germany).

The primary antibodies used are listed in Table I. The working dilution was determined using positive controls: for Notch1, breast carcinoma was used; for Notch2 to -4, DLL1, DLL3-4 and normal kidney was used and for Jagged1, astrocytoma was used.

Two pathologists, blinded to the pathological data, independently evaluated immunoreactivity of the TMA. Discrepancies were resolved by simultaneous re-examination of the slides by both investigators.

Assessment of antibody staining score. The immunoreactivity score was evaluated by multiplying the percentage of positive cells (PP%) and the staining intensity (SI). First, the PP% was scored as 0 for <1%, 1 for 1-24%, 2 for 25-49%, 3 for 50-74%, and 4 for ≥75%. Secondly, the sections were counterstained with Mayer's haematoxylin and permanently mounted. Staining was evaluated in three high-power fields and the samples were scored as cells strongly positive (3+), moderately positive (2+), weakly positive (1+) or negative (0). The results from three cores in the same patient were averaged to obtain a mean value for subsequent statistical analysis.

Each slide was carefully examined in the area of the tumour that contained the biggest proportion of positively stained cancer cells. Staining pattern (membranous, cytoplasmic, or nuclear) was also recorded.

Statistical analysis. Statistical data analyses were performed using the statistical software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). First, the associations among different antibodies and pathological factors were explored at the 95% confidence level using non-parametric statistics, Mann-Whitney *U* and Kruskal-Wallis tests, *p*-values <0.05 being considered the cut-off point for significance. Correlations between variables were tested according to the Spearman and Pearson correlation tests.

Table I. *List of antibodies*.

	Notch1	Notch2	Notch3	Notch4	Jagged1	DLL1	DLL3	DLL4
Clone	A6	NA	M-134	H-225	H-114	NA	P8145	NA
Isotype	IgG2b	Synthetic peptide	IgG	IgG	IgG	IgG	IgG	IgG
Titer	3 μg/ml	1:250	1:50	1:100	1:50	10 μg/ml	15 μg/ml	10 μg/ml
Antigen retrieval	Citrate	Citrate	Citrate	Citrate	EDTA	EDTA	Citrate	Citrate
Company	Abcam	Lifespan	Santa Cruz Biotechnology	Santa Cruz Biotechnology	Santa Cruz Biotechnology	AbD Serotec	Aviva Systems Biology	AbD Serotec
Clonality	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal

NA, Not available.

Results

Frequency and pattern of expression of Notch1-4 and their ligands. A recombinant anti-Notch1 antibody was used encoding the ligand binding region of human Notch1 endothelial growth factor repeats 11 and 12. A total of 41.3% of the cases studied showed a weak positive membranous staining in a small percentage of cells (20%). A total of 55.1% of the cases had a high score because of a high intensity of staining and a medium-to-high percentage of positive cells (40-50%). In cRCC and healthy controls, immunoreaction was detected in the membrane and cytoplasm of tubules.

A rabbit polyclonal antibody was used for Notch2 against amino acid residues 2396-2409 of human Notch2 (the entire protein had 2471 amino acids). A total of 38.8% of samples had positive and markedly active cell reactivity for anti-Notch2. A total of 7.5% of the samples were negative for this antibody. Finally, positivity existed but only weakly and in a low number of cells in 30.4% of samples. Notch2 showed only membrane reaction in cRCC samples, while it showed cytoplasm and membrane staining in the other groups. Notch3 is a rabbit polyclonal antibody raised against amino acids 2107-2240 of Notch3 of mouse origin. Only 12.5% of samples were positive with strong (+++) reactivity and a high percentage of cells (75%) for Notch3, while 12% of the cases were negative. A total of 68.9% showed moderate-to-weak positivity (++/+) in a low number of cells. Notch3 was detected in the membrane of chRCC and cRCC being detected in membrane and cytoplasm in pRCC and healthy controls. Finally, Notch4 is a rabbit polyclonal antibody raised against amino acids 1779-2003 mapping at the *C*-terminus of Notch4 of mouse origin; 22.5% of the cases studied were negative for this anti-Notch4. Only 6.3% of the cases had a severe reaction, reflecting the presence of this protein in a large number of cells. A total of 68.9% of the cases showed a weak positivity in a low percentage of cells. Notch4 was seen in the membrane of cRCC and pRCC. In cRCC samples it was detected in the membrane and cytoplasm and in normal kidney immunoreaction was observed in the cytoplasm of tubules. Nuclear reaction not was observed in any case.

Jagged1 is a polyclonal antibody raised against amino acids 1110-1223 of human origin. Only little expression of this protein was observed in the samples tested: 4.3% of them were negative and the remaining 95.7% had a score between1-2. Positive cases showed immunoreactions on the cell surface.

Antiserum to human DLL1 was raised by immunisation of rabbits with highly purified antigens. The synthetic peptide sequence amino acids 155-173 of mouse DLL1, also known as Drosophila Delta homologue 1, corresponding to 722 amino acid single-pass type 1 membrane protein. Only 5% of the samples were negative for this antibody, while 58.8% showed a weak expression for anti-DLL1.The remaining 22.6 was found reactive to this antibody in a medium-to-high way. Visualisation of the protein was mainly membrane.

DLL3 is a polyclonal antibody produced in rabbits immunised with a synthetic peptide corresponding to a region of human DLL3 with an internal ID of P28145. About 70% of the samples tested showed a high expression of this protein with membrane localisation. No sample was negative.

Finally, DLL4 is a polyclonal antibody raised against the internal region of human DLL4. A total 65% of the samples studied had a high score. No sample was negative for this protein. The location of staining was cell membrane and cytoplasm.

The immunofluoresence analysis on A.704 and ACHN cell lines showed expression of Notch1 and Notch3 in the case of A.704 and, for the ligands, Jagged1 showed remarkable expression. In the case of ACHN, the immunoreactivity observed was remarkable for Jagged1 and DLL4 (Figure 1A-D).

IHC expression and its correlation with pathological variables. Representative examples of reactivity for Notch1, Notch3 are shown in Figures 2A-D and 3A-D.

The statistical analysis revealed that the expression of Notch1 is related significantly with chRCC histological type (p=0.039). The highest score occurred in the samples from tumours with right location (p=0.487). In addition, a significant relationship was found between Notch1 and degree of tissue differentiation, Fuhrman grade, in the case of well-differentiated tumours (p=0.023). These samples were from

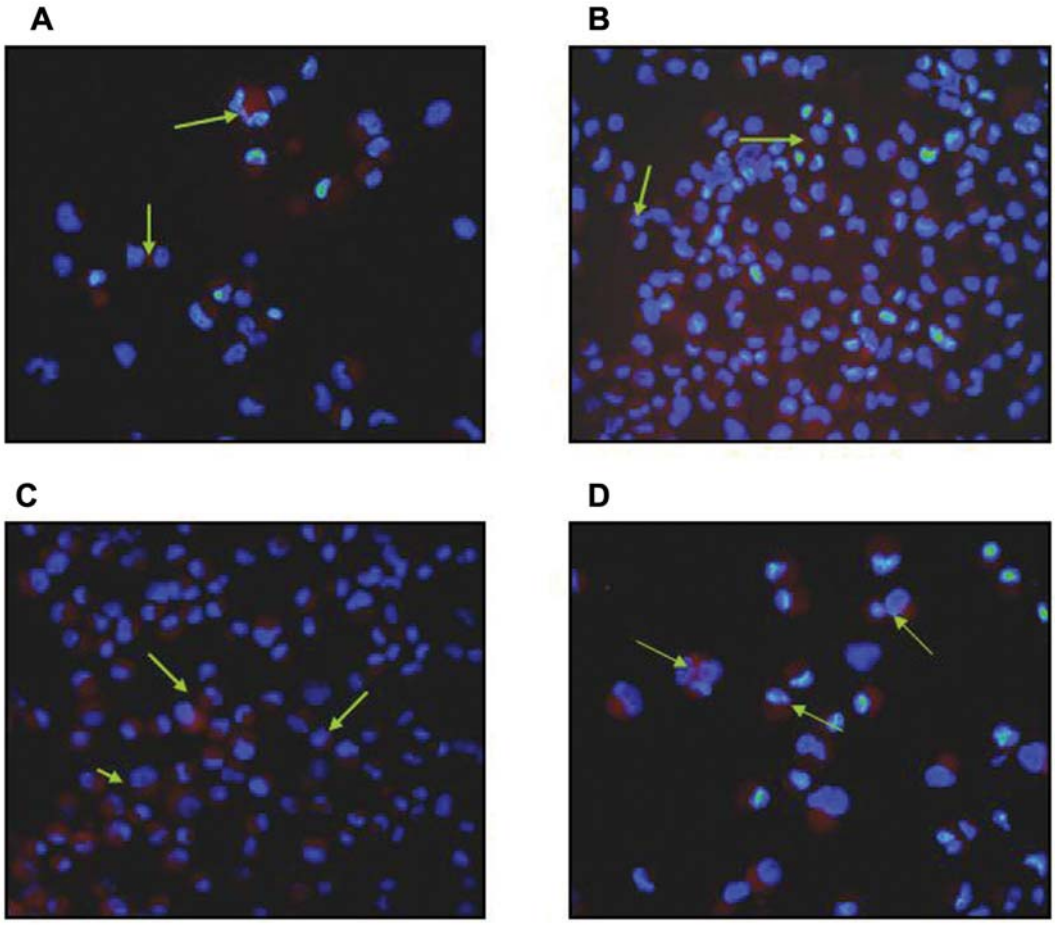

Figure 1. *Arrows indicate representative examples of immunofluorescence in renal cancer cell lines showing membrane and cytoplasm reaction in A.704 cell line for Jagged1 (A) and Notch1 (B) and in ACHN membrane and cytoplasm localisation for Jagged1 (C) and DLL4 (D)(magnification, ×20).*

tumours that showed no invasion of the renal pelvis or the hilium and neither the veins nor a broken renal capsule showed higher reactivity for this anti-Notch1 antibody. In no case except for broken capsule (p=0.016) was a statistically significant correlation observed. In the case of the invasion of the renal lymphatics, the score was higher in the samples from tumours with lymphatic invasion (p=0.850). Finally, tumours diagnosed as N1 showed more Notch1 expression at the protein level (p<0.001).

The expression of Notch2 protein was highest in the cases of chRCC (p=0.533) in comparison to the other two histological subtypes studied (cRCC and pRCC). When the samples came from tumours located in the right kidney, the Notch2 expression was increased (p=0.449). Regarding the degree of tissue differentiation, it was found that poorly differentiated tumours expressed more Notch2 (p=0.627). When the samples came from tumours with invasion of the renal pelvis, lymph vessels hilum of the kidney and broken renal capsule, expression of this protein was increased but there was no statistical significance found indicating that the expression of Notch2 can be affected by any of these pathological parameters. In regard to tumour invasion of the veins, the expression of Notch2 was increased in cases where there was no such invasion (p=0.948). Regarding T tumours, Notch2 expression was highest in cases diagnosed as T1-2 (p=0.239). With regard to the number of involved nodes, an intense expression of Notch2 was observed in a large number of cells for N0-1 tumours (p=0.885).

For Notch3 the expression was higher in samples from women (p=0.050). The expression was very similar in the three histological types studied (cRCC, chRCC and pRCC). However, there was no statistical increase in cRCC (p=1.00). The expression of Notch3 was higher in cases of right tumour location (p=0.048). The tumour samples that showed a Fuhrman grade IV showed increased reactivity to this anti-Notch3 (p=0.436), showing little or no positivity for this antibody in cases of Furhamn grade I. The positivity increased in the tumours invading the renal pelvis, lymph

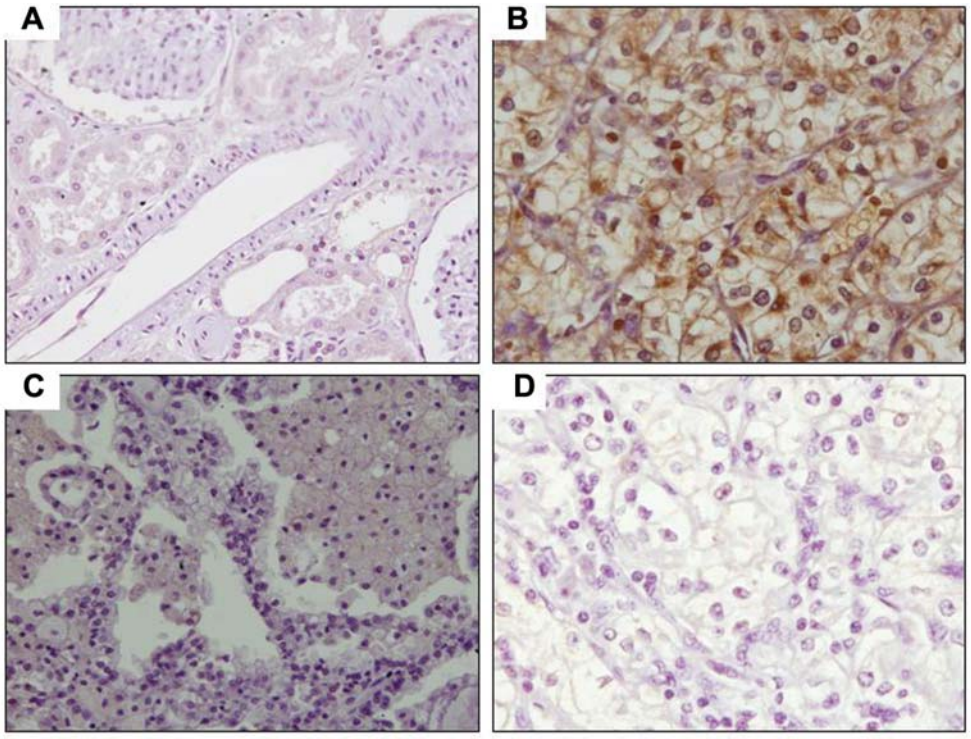

Figure 2. *Representative examples of IHC for Notch1 in: (A) normal kidney mucosa with reaction in membrane and cytoplasm of tubules (magnification, ×20), (B) cRCC with 75% positivity cells (+++) (magnification, ×20), (C) pRCC with 20% positivity cells (+) (magnification, ×40) and (D) chRCC with 10% positivity cells (+) (magnification, ×40).*

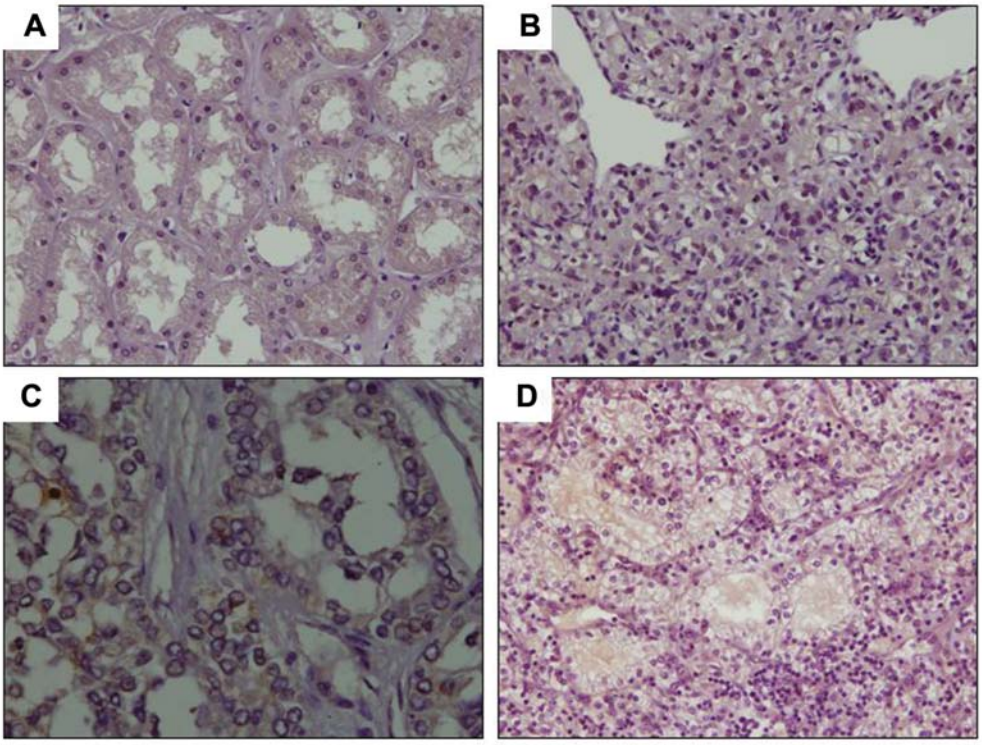

Figure 3. *Representative examples of IHC for Notch3 in: (A) normal kidney mucosa with membrane and cytoplasm reaction (magnification factor, ×20), (B) cRCC with 5% positivity cells (magnification factor, ×20), (C) pRCC with 60% positivity cells (++) (magnification factor, ×40) and (D) chRCC with 70% positivity cells (++) (magnification factor, ×20).*

Table II. *Statistical significance of associations between molecular variables.*

Variable		Notch1	Notch2	Notch3	Notch4	Jagged1	DLL1	DLL3	DLL4
Notch1	ϱ	-------	0.106	–0.272	–0.060	0.332	–0.312	–0.203	–0.199
	p	-------	0.350	**0.015**	0.594	**0.024**	**0.005**	0.072	0.076
Notch2	ϱ	-------	-------	0.437	0.285	0.246	0.172	0.142	0.348
	p	-------	-------	**0.01**	**0.010**	0.099	0.126	0.209	**0.002**
Notch3	ϱ	-------	-------	-------	0.529	–0.012	0.402	0.246	0.462
	p	-------	-------	-------	**0.01**	0.935	**0.01**	**0.028**	**0.01**
Notch4	ϱ	-------	-------	-------	-------	–0.034	0.193	0.057	0.334
	p	-------	-------	-------	-------	0.825	0.086	0.614	0.002
Jagged1	ϱ	-------	-------	-------	-------	-------	0.011	0.049	0.146
	p	-------	-------	-------	-------	-------	0.940	0.745	0.334
DLL1	ϱ	-------	-------	-------	-------	-------	-------	0.402	0.370
	p	-------	-------	-------	-------	-------	-------	**0.01**	**0.001**
DLL3	ϱ	-------	-------	-------	-------	-------	-------	-------	0.620
	p	-------	-------	-------	-------	-------	-------	-------	**0.01**

Significant *p*-values ($p<0.05$) are indicated in bold. ϱ, Spearman's rho.

vessels, hilum and renal veins and broken renal capsule but in no case was it able to explain the increased expression of Notch3 in a statistical way. The tumours with less depth of invasion, T1-2, were those that showed greater expression of Notch3 ($p=0.940$). Regarding the number of nodes involved by tumour, the larger number of nodes invaded showed greater expression of Notch3 ($p=0.003$).

With regard to the receptors of these proteins, the expression of DLL1 showed statistical significance in tumours with ruptured renal capsule ($p=0.042$). Finally, both this protein, DLL1, and another member of the family of ligands, DLL4, showed greater expression in cases with N0-1 tumours ($p=0.001$ and $p=0.020$, respectively).

Associations among Notch1 to -4, Jagged1, Delta1, Delta2 and Delta3 protein expression. The statistical significance of associations between the various molecular variables is listed in Table II. A negative correlation was found between Notch1 and Notch3 or DLL1 (Spearman's rho=–0.312, $p=0.005$ and Spearman's rho=–0.272, $p=0.015$, respectively). Notch1 showed a strong positive correlation with Jagged1 (Spearman's rho=0.332, $p=0.029$). In the case of Notch2, the positive correlation with Notch3 presented considerable statistical significance (Spearman's rho=0.437, $p=0.01$). Notch2 also presented a statistical value close to 0.4 with DLL4 (Spearman's rho=0.348, $p=0.002$). Finally, Notch2 was positively correlated with Notch4 (Spearman's rho=0.285; $p=0.010$). In the case of Notch3, a strong linear association between this protein and Notch4 was found (Spearman's rho=0.529, $p=0.01$), as well as DLL1 and DLL3 (Spearman's rho=0.402, $p<0.001$ and Spearman's rho=0.246, $p=0.028$, respectively). Notch4 also showed positive correlation with DLL4 (Spearman's rho=0.334, $p=0.002$).

Discussion

Notch is involved in cell-fate decisions in embryonic and adult stem cells and progenitors. Notch is also implicated in terminal differentiation events in specific tissues. Additionally, Notch promotes the development of new cell types at the boundaries of two distinct cell populations. Emerging evidence has shown that deregulated expression of Notch receptors, ligands and targets is observed in many types of tumours (11, 12).

Depending on the tumour type, Notch can promote or limit tumour growth through either cell autonomous or cell non-autonomous effects on differentiation, cellular metabolism, cell-cycle progression, angiogenesis and self-renewal. Deregulated expression of wild-type Notch receptors, ligands and target genes is observed in a growing number of haematological tumours. Mutations in Notch receptors have also shown tumourigenic properties in preclinical models (13).

However, evidence shows that the Notch signalling pathway has opposing functions in tumour development, where it may act either as an oncogene or as a tumour suppressor, depending on the cell context. In fact, it seems that the result of alteration in Notch signalling is dependent on its normal function in a given tissue. In this setting, Notch may act as an oncogene in those tissues where it is involved in stem-cell self-renewal or in cell-fate decision. In contrast, Notch signalling may have a tumour suppressor role in those tissues in which Notch promotes terminal differentiation events (13).

Notch signalling is involved in mammary gland stem-cell self-renewal (14, 15). The self-renewal of the intestine is regulated by highly evolutionary conserved signalling pathways, which include Notch (16, 17). Notch signalling has an important role in haematopoiesis as a mediator of cell-fate determination and in the self-renewal of haematopoietic stem cells (18, 19).

Notch signalling is associated with both oncogenic and tumour suppressive roles and has been documented in various cancer types. Aberrant Notch signalling has been linked to a wide variety of tumours. Notch can either promote or limit tumour growth depending on the tumour type (20). Notch may play a role in tumourigenesis by inhibiting differentiation, promoting survival, or accelerating proliferation.

The association between deregulation, an oncogenic fashion, of the Notch pathway and human cancer is firmly established in T-cell lymphoblastic leukaemia, lymphoma and medulloblastoma (21-25). In other neoplasms, such as non-small cell lung cancer and skin cancer, Notch has a tumour suppressor function (26, 27). Notch2 has been suggested to drive embryonic brain tumour growth, whereas Notch3 has been implicated in choroid plexus tumours (27). Finally, Notch1 and its ligands, Delta-like-1 and Jagged-1, have been implicated in glioma cell survival and proliferation (28).

Nevertheless, the patterns of expression of Notch receptors and their ligands in RCC, as well as their relation with pathological findings, remain poorly understood. Accordingly, the present study was designed to evaluate the expression and pathological significance of these molecules in human RCC. Based on IHC staining, an up-regulation was found in the case of Notch1 and DLL3 and DLL4 ligands. In contrast, the expression of the other markers was barely noted, presenting a down-regulation in the cohort examined. All four Notch receptors and ligands were expressed in non-neoplasic tissues. Previous studies (29) found no or weak expression of Notch1 in RCC tissues as well as in cell lines.

The correlation between tissue expressions of different markers showed an inverse relationship between Notch1 and the ligands DLL1, DLL3 and DLL4. The other receptors studied showed a positive correlation with these ligands in RCC tissues. These results suggest that Notch1 pathway may be initiated by the Jagged-1 or -2 ligands. Previous studies (30) in human papillary bladder cancer highlighted the relationship between Notch1 and Jagged-1 expression.

A study by TMA in hepatocellular carcinoma found that the positive expression of Notch1 was parallel with Jagged1 expression (31).

Another explanation for this finding of inverse correlation between receptor and ligand may be due to the fact that ligands can also affect Notch signalling through interactions with Notch within the same cell (*cis*-interactions) (32, 33). Compared with the *trans*-activating interactions, *cis*-interactions between Notch ligands and Notch inhibit Notch signalling. The mechanism underlying *cis*-inhibition of Notch signalling is unknown, but may involve sequestration of cell-surface Notch that precludes its availability for interactions with ligands on neighbouring cells (34). The Notch signalling pathway also interacts with a number of different signalling systems and many of these also affect ligand expression (35). In particular, fibroblast growth factor, platelet-derived growth factor, transforming growth factor beta, vascular endothelial growth factor, hedgehog and Wnt have been found to modulate ligand expression and produce specific cellular responses. The majority of these signalling pathways increase ligand expression.

The importance of Notch ligands in cancer and other pathological states involving aberrant angiogenesis have identified Notch ligands as potential and promising therapeutic targets (36-39).

Statistical analysis showed that Nocth1 has an inverse correlation with Fuhrman grade, consistent with data by Sun *et al*. 2009; and maximum tumour size, with increasing tumour grade decreases the expression of this receptor, suggesting that the Notch pathway may be related to tumourigenesis of RCC. It will be interesting to use an antibody that recognises the active nuclear form in order to study whether this pathway is active in this tumour type, thus confirming its involvement in tumourigenesis in the RCC. Moreover, Jagged-1 showed a positive correlation with Fuhrman grade, while Notch3 and 4 receptors were positively correlated with tumour size as so as ligands DLL1, 3 and 4. These associations suggest that the ligands expression is involved in tumours with most rapid growth and spread.

In other types of solid tumours such as ovarian carcinoma the expression of Notch1 increases gradually with the poor differentiating tissues of cancer, indicating that Notch1 may be involved and play an oncogenic role in the development of ovarian cancer (40).

The results of the present study have increased the understanding of the significance of Notch1 and Notch3 receptors in the tumourigenesis of RCC since it was found that Notch1 has its highest expression in the samples diagnosed with chRCC histological type and in tumours that have not broken renal capsule and in a number of metastatic regional lymph low, suggesting a possible diagnostic role of this marker. Notch3 expression was characteristic of tumours with right-hand location.

In the case of ligands, the statistical analysis showed that DLL1 is characteristic of tumours with broken renal capsule and, in cases of low metastasis to lymph nodes, DLL1-4 showed the highest expression.

These findings suggest the usefulness of these markers as diagnostic factors, in the case of Notch1, and prognosis, in the case of Notch1-3 and DLL1-4. Since Notch signalling is such a complicated and comprehensive pathway, the mechanisms underlying the roles of Notch signalling in tumourigenesis need further research by techniques more accurate and reproducible than IHC. In summary, the present study linked Notch signalling with RCC, suggesting its role in the tumourigenesis of these tumours.

Acknowledgements

We thank María Fraga Mariño for helpful advice about immunofluorescence technique. The research in our Hospital was supported by the Fundación Del Complejo Hospitalario Universitario A Coruña (CHUAC), Spain.

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Received December 27, 2010
Revised February 16, 2011
Accepted February 17, 2011