

## JAGGED1 Expression Is Associated with Prostate Cancer Metastasis and Recurrence

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### Abstract

Recent studies suggest that NOTCH signaling can promote epithelial-mesenchymal transitions and augment signaling through AKT, an important growth and survival pathway in epithelial cells and prostate cancer in particular. Here we show that JAGGED1, a NOTCH receptor ligand, is significantly more highly expressed in metastatic prostate cancer as compared with localized prostate cancer or benign prostatic tissues, based on immunohistochemical analysis of JAGGED1 expression in human tumor samples from 154 men. Furthermore, high JAGGED1 expression in a subset of clinically localized tumors was significantly associated with recurrence, independent of other clinical parameters. These findings support a model in which dysregulation of JAGGED1 protein levels plays a role in prostate cancer progression and metastasis and suggest that JAGGED1 may be a useful marker in distinguishing indolent and aggressive prostate cancers.

### Introduction

The androgen receptor plays a critical role in prostate development (1). Withdrawal of androgens leads to a rapid decline in prostate cancer growth with significant clinical response, but this response is brief as tumor cells rapidly emerge, which are refractory to hormonal manipulation. Recent work from Chen *et al.* (2) suggests that increases in androgen receptor mRNA and protein are both necessary and sufficient to convert prostate cancer cells to a hormone-refractory state. Their model proposes that increased androgen receptor expression permits continued signaling even in the face of low levels of agonist ligands, and thereby limits the effectiveness of surgical or chemical castration. Thus, characterization of the androgen receptor ligand-response repertoire may identify downstream targets that contribute to the development of the hormone refractory state. Using a high throughput quantitative proteomic technique, Martin *et al.* (3) recently identified JAGGED1, a NOTCH receptor ligand, as a highly androgen-responsive gene product in the prostate cancer cell line LNCaP. Dysregulation of the NOTCH signaling pathway, which normally regulates many cell fate decisions, has been described in human T-cell leukemias and mucoepidermoid carcinoma (4) and has been recently proposed to play a role in epithelial-mesenchymal transitions in cancer (5). In the current study, we describe for the first time the increased expression of JAGGED1 protein in human prostate

tumors and its significant association with prostate cancer progression and metastasis.

### Materials and Methods

**Cell Culture.** LNCaP cells (American Type Culture Collection, Manassas, VA) were grown in RPMI 1640 without phenol red (Sigma, St. Louis, MO), supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD), 2 mmol/L L-glutamine, 50 units/ml penicillin, and 50  $\mu$ g/ml streptomycin. Cells were maintained in a 5% CO<sub>2</sub>, 95% air-humidified atmosphere at 37°C and cultured in phenol red-free medium with 5% stripped fetal bovine serum (BioWhittaker) 48 hours before the experiments. The cells were plated on 100-mm dishes with 50% density. Forty-eight hours later, the cells were treated with either vehicle control or 1 nmol/L synthetic androgen R1881 (New England Nuclear, Perkin Elmer, Boston, MA). All other cell lines were grown in DMEM containing 10% FCS, 2 mmol/L glutamine, 100 units/ml penicillin G, and 100  $\mu$ g/ml streptomycin at 37°C under 5% CO<sub>2</sub>.

**Immunoblot Analyses.** Washed cells were homogenized in lysis buffer containing 50 mmol/L Tris-HCl (pH 7.4), 1% NP40 (Sigma), and complete proteinase inhibitor mixture (Roche, Nutley, NJ). Protein concentrations were determined with the Bradford assay (Bio-Rad, Hercules, CA). Protein extracts were mixed with SDS sample buffer and electrophoresed in 10% SDS-polyacrylamide gels under reducing conditions. The separated proteins were transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ), which were incubated for 1 hour in blocking buffer (TBS with 0.1% Tween and 5% nonfat dry milk). The blots were incubated in blocking buffer with either affinity-purified JAGGED1 goat polyclonal antibody (AF1277, R&D Systems, Minneapolis, MN) at a 1  $\mu$ g/ml concentration in blocking buffer overnight at 4°C or rabbit anti-JAGGED1 (H-114, Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200 for 1 hour. After washing three times with TBS with 0.1% Tween buffer, the membrane was incubated with horseradish peroxidase-linked rabbit anti-goat IgG antibody (Zymed, South San Francisco, CA) or goat antirabbit antibody (DAKO USA, Carpinteria, CA) at a 1:5,000 dilution for 1 hour at 25°C. The signals were visualized with the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech) and autoradiography. Some blots were restained with rabbit antiprostate-specific antigen antibody (DAKO USA) for 2 hours or with rabbit anti- $\beta$ -tubulin antibody (1:1,000 dilution; Santa Cruz Biotechnology).

**Patient Population, Tissue Collection, and Tissue Microarray Construction.** Samples from 236 patients with benign, high-grade prostatic intraepithelial neoplasia and localized and metastatic prostate cancer were obtained from the rapid autopsy program and the radical prostatectomy series, respectively, of the University of Michigan Prostate Cancer SPORE Tissue Bank and assembled on tissue microarrays as described previously (6, 7). A subset of 95 men with clinically localized prostate cancer was evaluated for associations between JAGGED1 expression levels and tumor recurrence (as judged by a rise in prostate-specific antigen level). A total of 1,247 tissue microarray cores were evaluated.

**Immunohistochemical Analysis.** Immunohistochemistry was done on 5- $\mu$ m sections prepared from paraffin-embedded tissue microarrays. Slides were successively soaked in xylene; passed through graded alcohols; washed in distilled water; pretreated with 10 mmol/L citrate (pH 6.0; Zymed) in a steam pressure cooker (Decloaking Chamber, BioCare Medical, Walnut Creek,

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**Note:** M. Rubin and J. Aster share senior authorship. Supplementary data for this article may be found online at [http://rubinlab.tch.harvard.edu/supplemental\\_data/JAGGED1/index.jsp](http://rubinlab.tch.harvard.edu/supplemental_data/JAGGED1/index.jsp) and [www.ONCOMINE.org](http://www.ONCOMINE.org).

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CA); and washed again in distilled water. All additional steps were done at 25°C in a hydrated chamber. Slides were pretreated with Peroxidase Block (DAKO USA) for 5 minutes, blocked with 20% goat serum for 20 minutes, and treated with rabbit anti-JAGGED1 antibody (H-114, Santa Cruz Biotechnology) at 1:50 in DAKO diluent for 1 hour. After washing in 50 mmol/L Tris-Cl (pH 7.4), antirabbit horseradish peroxidase-conjugated antibody (Envision detection kit, DAKO) was applied for 30 minutes. After additional washing, immunoperoxidase staining was developed with a DAB chromogen kit (DAKO). Slides were counterstained with hematoxylin.

**Semiautomated Quantitative Image Analysis of JAGGED1.** Evaluation of JAGGED1 immunohistochemical staining was done with the Chromavision (Chromavision Medical Systems, Inc., San Juan Capistrano, CA) Automated Cellular Imaging System (ACIS II), an upgraded version of the previously described ACIS System (8). The system combines automated microscopy with computerized image analysis to generate a continuous immunohistochemical staining score between 0 to 255 intensity units. Each tissue microarray core was reviewed to ensure that intensity measurements were taken from diagnostic regions (benign, high-grade prostatic intraepithelial neoplasia, or cancer) and that staining scores obtained from nonrepresentative regions were excluded. Images of all tissue microarray cores used in this analysis, including those stained for JAGGED1, can be viewed at a supplementary web site.

**Statistical Analysis.** Patient information, including pretreatment prostate-specific antigen values, clinical and tumor stage, radical prostatectomy Gleason score, and surgical margin status, was prospectively collected and stored. After radical prostatectomy, patients were assessed annually for prostate-specific antigen recurrence-free survival with a cut point of >0.2 ng/ml to define biochemical evidence of micrometastatic recurrence or progression. Bivariate (univariate) analysis was done to examine the association of clinical and pathological parameters with JAGGED1 staining and recurrence-free survival. Cox proportional hazards regression models were used to analyze the relationship between recurrence-free survival and preoperative variables. A backward selection procedure was implemented to select the most parsimonious model. A 0.05 significance level was used for all decisions of significance. Analyses were run with SPSS 11.0.1 (SPSS, Inc., Chicago, IL). Kaplan-Meier analysis was used to establish prostate-specific antigen recurrence-free survival curves.

## Results and Discussion

**Characterization of JAGGED1 Antibodies.** We first tested the specificity of commercially available JAGGED1 antibodies on Western blots containing whole cell protein extracts from diverse cell types, including LNCaP cells. Immunostaining with a polyclonal

rabbit antibody raised against the cytoplasmic domain of JAGGED1 revealed a strong cross-reactive protein of the expected size of JAGGED1 only in LNCaP cells (Fig. 1A, lane 14), human umbilical vein endothelial cells (Fig. 1A, lane 10 and 11), and control 293 cells transfected with a pcDNA3 expression plasmid encoding JAGGED1 (Fig. 1A, lane 2). The levels of JAGGED1 in LNCaP cells increased substantially after incubation with the synthetic androgen analog R1881 for 1 to 3 days (Fig. 1B), as judged by stains done with a second polyclonal JAGGED1 antibody raised in goats, confirming that JAGGED1 increases in LNCaP cells stimulated with androgen (3). Based on pilot studies conducted with control cells embedded in paraffin (data not shown), we elected to use the polyclonal rabbit anti-JAGGED1 antibody for immunohistochemical staining of paraffin sections.

**JAGGED1 Protein Expression Is Associated with Prostate Cancer Progression.** To study the expression of JAGGED1 in human prostatic specimens, immunostaining was done on high-density tissue microarrays containing benign prostatic tissues, localized untreated prostatic adenocarcinomas, and hormone-naïve and -refractory metastatic tumors. Two samples from 18 different patients imprinted in duplicate were represented in these tissue microarrays (giving a total of 4 measurements per patient and 72 per category). The staining intensity of JAGGED1 was measured and quantified with the Chromavision Automated Cellular Imaging System II. For each patient a minimum, maximum, and mean staining intensity were measured for the 4 samples.

Representative examples of staining for JAGGED1 in benign prostatic tissue, localized cancer, and metastatic tumor are shown in Fig. 2A. Mean JAGGED1 staining intensity was increased significantly in clinically localized prostate cancer (score = 94.2; SE = 1.8) versus benign prostate tissue (score = 79.6; SE = 2.8;  $P < 0.001$ ) and again in metastatic tumor (score = 127.5; SE = 4.6) as compared with either clinically localized prostate cancer ( $P < 0.001$ ) or benign prostate tissue ( $P < 0.000$ ). There was no significant difference between the mean JAGGED1 staining between hormone naïve (score = 126.2; SE = 3.8) and hormone refractory metastatic prostate cancer (score = 129.1; SE = 9.7;  $P = 0.79$ ). The data for mean intensity measurements is summarized with error bars with 95% confidence intervals in Fig. 2B. The findings were unchanged when minimum or maximum staining intensities were evaluated. The tissue microarray

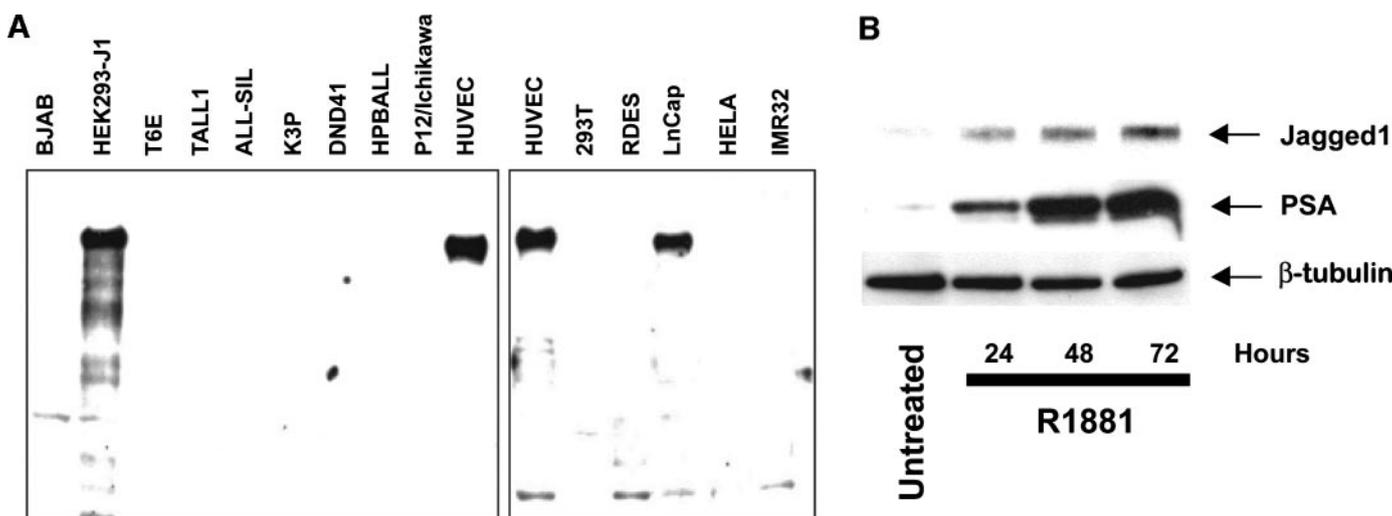


Fig. 1. Immunoblot analysis of JAGGED1 expression. A, rabbit anti-JAGGED1 antibody. Samples used to test the specificity of this antibody included whole cell extracts prepared from cell lines derived from human B cells (BJAB), murine (T6E), and human T-cell lines (TALL-1, ALL-SIL, K3P, DND-41, P12-Ichikawa, and HPB-ALL), human kidney (293T cells), Ewing's sarcoma (RDES), hormone-responsive human prostate cancer cells (LNCaP), cervical carcinoma (HELA), and neuroblastoma (IMR32). Extracts from primary human umbilical vein endothelial cells (HUVEC), and control human kidney 293T cells transfected with a JAGGED1 expression plasmid were also analyzed. Twenty-five micrograms of protein were loaded per lane. B, goat anti-JAGGED1 antibody. LNCaP cells were treated with synthetic androgen R1881 for 1 to 3 days before extracts were prepared. After JAGGED1 staining, the same blot was subsequently restained for prostate-specific antigen and  $\beta$ -tubulin.

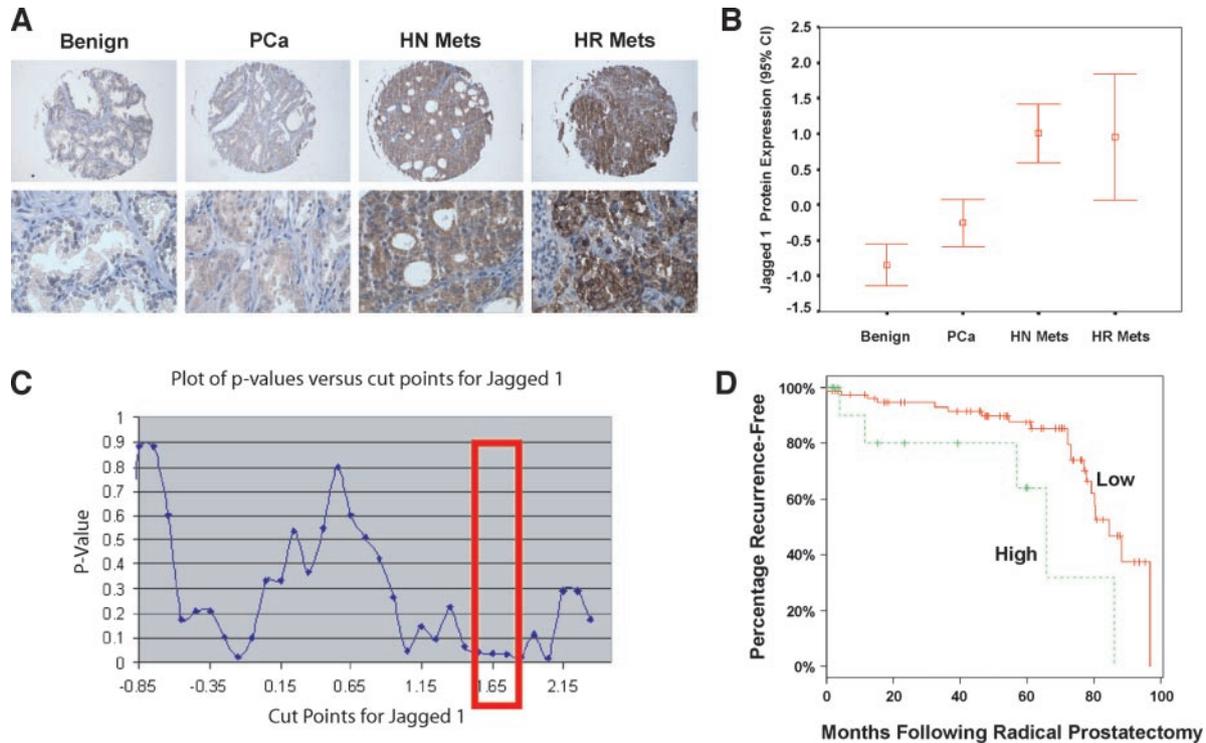


Fig. 2. A, representative JAGGED1 immunostaining in prostatic tissue samples. Images of all of the cores evaluated for this study are available online along with their associated clinical information and JAGGED1 intensity score. B, JAGGED1 immunostaining in benign prostatic tissues, localized cancer, and metastatic hormone naïve and refractory cancer (summary). Confidence intervals (95%) show normalized mean protein intensity units of JAGGED1 as determined by quantitative evaluation of immunohistochemistry; bars,  $\pm$ SE. C, association of JAGGED1 expression with risk of prostate-specific antigen recurrence. The significance of the association (log-rank test) is plotted across various cutoff points corresponding to increasing levels of the maximal JAGGED1 staining. D, Kaplan-Meier analysis of individuals with clinically localized prostate cancer expressing high (cutoff of 1.8) or low levels of JAGGED1 (log-rank test,  $P = 0.02$ ).

(HTMA3) images are available on a supplementary web site.<sup>8</sup> These data demonstrate an association between increased JAGGED1 expression and progression from localized to metastatic disease.

**High JAGGED1 Expression Is Associated with Prostate Cancer Recurrence after Radical Prostatectomy for Clinically Localized Disease.** The increased expression of JAGGED1 in localized prostate cancer and the additional up-regulation in metastatic tumor suggested that JAGGED1 might be a useful tissue biomarker to facilitate differentiating indolent from more aggressive prostate cancer. To examine this possibility, an association was sought between JAGGED1 levels and prostate cancer recurrence, defined as an increase in prostate-specific antigen of 0.2 ng/ml after radical prostatectomy or the development of overt metastatic disease. JAGGED1 immunostaining was done on 3 previously validated tissue microarrays. Analysis was restricted to a subset of patients ( $n = 95$ ) with clinically localized prostate cancer and 3 or more interpretable tissue cores. Patient demographics, which are representative of the total tissue microarray cohort, are presented in Table 1. Among these 95 patients, 26 (27.4%) experienced prostate-specific antigen failure as of January 2004.

The results of univariate analysis are shown in Table 2. Consistent with prior studies, Gleason score, preoperative prostate-specific antigen, extraprostatic extension, seminal vesicle invasion, positive surgical margins, and digital rectal examination were all significantly associated with prostate-specific antigen failure at the univariate level. Interestingly, in univariate analysis, a strong association between prostate-specific antigen recurrence and high levels of JAGGED1 staining was found (relative risks of 2.55 with 1.55 intensity cutoff, and 3.05 with 1.8 intensity cutoff, respectively; Fig. 2C). It should be noted that significant  $P$ s were found for JAGGED1 only when max-

imum intensity values for each patient were used. The variation in staining across cores is consistent with the heterogeneous nature of prostate cancer and suggests that broad tissue sampling may be needed to maximize the predictive power of JAGGED1 staining. A Kaplan-Meier analysis depicting the association between JAGGED1 (cutoff of 1.8) and the probability of prostate-specific antigen free survival is shown in Fig. 2D.

Table 1 Clinical demographics of 95 men with localized prostate cancer

	n %
Median age (years)	59 (range 43–80)
Preoperative PSA (ng/ml)	
< = 4	17 (17.9%)
and <10	56 (58.9%)
> = 10	22 (23.2%)
Digital rectal examination	
Negative	54 (56.8%)
Positive	41 (43.2%)
Gleason score	
≤6	35 (36.8%)
7	55 (57.9%)
≥8	5 (5.3%)
3 + 4	10 (10.5%)
4 + 3	45 (47.4%)
Surgical margin status	
Negative	64 (67.4%)
Positive	31 (32.6%)
Seminal vesicle invasion	
Negative	90 (94.7%)
Positive	5 (5.3%)
Extraprostatic extension	
Negative	72 (75.8%)
Positive	23 (24.2%)
Median tumor dimension (cm)	1.4 (range 0.2–3.6)
PSA failure (ng/ml)	
No	69 (72.6%)
Yes	26 (27.4%)

Abbreviation: PSA, prostate-specific antigen.

<sup>8</sup> [http://rubinlab.tch.harvard.edu/supplemental\\_data/JAGGED1/index.jsp](http://rubinlab.tch.harvard.edu/supplemental_data/JAGGED1/index.jsp)

Table 2 Parameters associated with PSA recurrence after radical prostatectomy for clinically localized prostate cancer

	P	Relative risk	95% CI	
			Lower bound	Upper bound
Univariate analysis				
Gleason score (2 cat, <7)	0.014	4.06	1.21	13.6
Preoperative PSA (3 cat)	0.025	2.43	1.31	4.51
Tumor dimension	0.082	2.08	0.89	4.83
Extraprostatic extension	0.0002	4.04	1.84	8.88
Seminal vesicle invasion	0.012	3.38	1.24	9.17
Positive surgical margins	0.0003	4.21	1.81	9.77
Digital rectal examination	0.038	2.29	1.02	5.15
JAGGED1 (cutoff = 1.55; max)	0.041	2.55	1	6.48
JAGGED1 (cutoff = 1.8; max)	0.021	3.05	1.12	8.31
Multivariable analysis				
Extraprostatic extension	0.0005	3.94	1.84	8.40
Preoperative PSA	0.042	1.97	1.03	3.79
JAGGED1 (cutoff = 1.8; max)	0.016	3.51	1.26	9.76

Abbreviations: PSA, prostate-specific antigen; CI, confidence interval; max, maximum.

The best multivariate model predictive of prostate cancer specific recurrence after radical prostatectomy for clinically localized prostate cancer included extraprostatic extension ( $P = 0.0005$ ; relative risk = 3.94), preoperative prostate-specific antigen ( $P = 0.042$ ; relative risk = 1.97), and JAGGED1 (maximum intensity values were used;  $P = 0.016$ ; relative risk = 3.51). Hence, high JAGGED1 protein level is a strong independent predictor of prostate cancer recurrence.

**JAGGED1 Expression in Other Cancers.** On the basis of these observations, we assessed JAGGED1 expression in other tumors with ONCOMINE II, a bioinformatics platform that provides a compendium of microarray data (9). We interrogated 90 cancer microarray data sets spanning 6,372 experiments for evidence of differential expression of JAGGED1 in benign and malignant tissues. In Supplemental Fig. 1, differential expression of JAGGED1 mRNA is seen between benign prostatic tissue and localized prostate cancer (study 1) and primary prostate cancer and metastatic prostate cancer (study 3; refs. 6, 10). Significant differential expression of JAGGED1 was also noted in squamous cell lung carcinoma (study 5,  $P = 0.43 \times 10^{-4}$ ) and carcinoid tumors (study 6,  $P = 9.9 \times 10^{-7}$ ; ref. 11). There was a trend toward differential JAGGED1 expression in fibroadenomas, pilocytic astrocytoma, and atypical teratoid/rhabdoid tumors, but it did not reach significance. Nevertheless, these microarray studies suggest a role of JAGGED1 in several human tumor types.

Our observations are significant in two ways. First, the correlation between high levels of JAGGED1 and an increased risk of prostate cancer recurrence after radical prostatectomy, independent of other pathological or clinical parameters, support the potential use of JAGGED1 as a prognostic marker in prostate cancer, a possibility that needs to be validated in a larger clinical cohort. Secondly, this study adds to other recent diverse data supporting a role for JAGGED1-NOTCH signaling in prostatic development and malignant transformation. NOTCH1 mRNA is highly expressed in human prostate cancer cell lines (12) and is markedly increased in metastatic disease in a SV40 large T antigen mouse model of prostate cancer (13). Levels of JAGGED1 transcript are also increased in metastatic human tumors (6, 14) and are modulated by androgen in the ventral prostate of orchietomized rats (15) and in LNCaP cells (16). Of additional interest, JAGGED1 and NOTCH2 extracellular domains accumulate in conditioned LNCaP media after androgen stimulation (3). Shedding of these domains may occur during receptor activation, raising the interesting possibility that soluble JAGGED1 might be a useful biomarker in patients with prostate cancer. Consistent with this idea, JAGGED1, along with prostate-specific antigen, is among the top 15 most abundant proteins present in LNCaP-conditioned media (3).

An evaluation of JAGGED1 gene expression using the ONCOMINE, a compendium of microarray data, suggests that JAGGED1 expression is also high in a number of other cancers. Independent studies have shown high levels of JAGGED1 expression in cervical carcinoma (17) and in mesotheliomas versus primary mesothelial cultures (18), suggesting potential diagnostic and prognostic utility in a broad range of tumors. JAGGED1/NOTCH1 signaling in some contexts promotes epithelial-mesenchymal transitions (5), an activity which could explain the strong correlation between prostate cancer progression and JAGGED1 expression, which we report here. It will be of interest to investigate whether increased levels of JAGGED1 correlate with NOTCH receptor activation in prostate cancer and other tumor types and to determine whether such tumors are sensitive to inhibitors of NOTCH signaling. Conversely, other studies have suggested the existence of a "reverse" JAGGED1 signaling pathway that activates the transcription factor AP-1 (19) and contributes to transformation of RKE cells in culture (20). Our findings lay the groundwork for exploration of the importance of canonical JAGGED1/NOTCH signaling and possible NOTCH-independent JAGGED1 signaling in prostate cancer.

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#### References

- Yong EL, Lim J, Qi W, Ong V, Mifsud A. Molecular basis of androgen receptor diseases. *Ann Med* 2000;32:15-22.
- Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;10:33-9.
- Martin DB, Gifford DR, Wright ME, et al. Quantitative proteomic analysis of proteins released by neoplastic prostate epithelium. *Cancer Res* 2004;64:347-55.
- Weng AP, Aster JC. Multiple niches for Notch in cancer: context is everything. *Curr Opin Genet Dev* 2004;14:48-54.
- Zavadil J, Cermak L, Soto-Nieves N, Bottlinger EP. Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. *EMBO J* 2004; 23:1155-65.
- Dhanasekaran SM, Barrette TR, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. *Nature (Lond)* 2001;412:822-6.
- Perrone EE, Theoharis C, Mucci NR, et al. Tissue microarray assessment of prostate cancer tumor proliferation in African-American and white men. *J Natl Cancer Inst (Bethesda)* 2000;92:937-9.
- Bauer KD, de la Torre-Bueno J, Diel IJ, et al. Reliable and sensitive analysis of occult bone marrow metastases using automated cellular imaging. *Clin Cancer Res* 2000; 6:3552-9.
- Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 2004;6:1-6.
- Singh D, Febbo PG, Ross K, et al. Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 2002;1:203-9.
- Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc Natl Acad Sci USA* 2001;98:13790-5.
- Zayzafoon M, Abdulkadir SA, McDonald JM. Notch signaling and ERK activation are important for the osteomimetic properties of prostate cancer bone metastatic cell lines. *J Biol Chem* 2004;279:3662-70.
- Shou J, Ross S, Koeppen H, de Sauvage FJ, Gao WQ. Dynamics of notch expression during murine prostate development and tumorigenesis. *Cancer Res* 2001;61:7291-7.
- LaTulippe E, Satagopan J, Smith A, et al. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res* 2002;62:4499-506.
- Nantermet PV, Xu J, Yu Y, et al. Identification of genetic pathways activated by the androgen receptor during the induction of proliferation in the ventral prostate gland. *J Biol Chem* 2004;279:1310-22.
- Velasco AM, Gillis KA, Li Y, et al. Identification and validation of novel androgen-regulated genes in prostate cancer. *Endocrinology* 2004;145:3913-24.
- Gray GE, Mann RS, Mitsiadis E, et al. Human ligands of the Notch receptor. *Am J Surg Pathol* 1999;154:785-94.
- Kettunen E, Nissen AM, Ollikainen T, et al. Gene expression profiling of malignant mesothelioma cell lines: cDNA array study. *Int J Cancer* 2001;91:492-6.
- LaVoie MJ, Selkoe DJ. The Notch ligands, Jagged and Delta, are sequentially processed by alpha-secretase and presenilin/gammasecretase and release signaling fragments. *J Biol Chem* 2003;278:34427-37.
- Ascano JM, Beverly LJ, Capobianco AJ. The C-terminal PDZ- ligand of JAGGED1 is essential for cellular transformation. *J Biol Chem* 2003;278:8771-9.