RON is not a prognostic marker for resectable pancreatic cancer

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ABSTRACT

Background: The receptor tyrosine kinase RON exhibits increased expression during pancreatic cancer progression and promotes migration, invasion and gemcitabine resistance of pancreatic cancer cells in experimental models. However, the prognostic value of RON expression in pancreatic cancer is unknown.

Methods: RON expression was characterized in several large cohorts, including a prospective study, totaling 492 pancreatic cancer patients and relationships with patient outcome and clinico-pathologic variables were assessed.

Results: RON expression was associated with outcome in a training set, but this was not recapitulated in the validation set, nor was there any association with therapeutic responsiveness in the validation set or the prospective study.

Conclusion: Although RON is implicated in pancreatic cancer progression in experimental models, and may constitute a therapeutic target, RON expression is not associated with prognosis or therapeutic responsiveness in resected PC.

INTRODUCTION

The c-MET-related receptor tyrosine kinase RON (MST1R) is overexpressed in pancreatic cancer (PC) relative to non-malignant pancreas (1). RON signaling enhances migration, invasion and survival of PC cells and promotes resistance to gemcitabine (2, 3), making it a potential therapeutic target. In gastroesophageal cancer, RON overexpression is associated with poor survival (4). The goal of this study was to use a comprehensive cohort of PC patients to assess RON as a biomarker of prognosis or therapeutic responsiveness.

METHODS

Patients, Tissue Microarrays and Immunohistochemistry

Clinico-pathologic and outcome data for 492 consecutive patients with a diagnosis of pancreatic ductal adenocarcinoma who underwent pancreatic resection were obtained from teaching hospitals associated with the Australian Pancreatic Cancer Network (www.pancreaticcancer.net.au; Table 1). This cohort consisted of a training set of 76 patients, a validation set of 316 patients and a further cohort of 100 patients accrued prospectively for the International Cancer Genome Consortium (www.icgc.org). Detailed methods for tissue microarray construction, the assessment of immunostaining and statistical analysis were described previously (5). Immunostaining was performed using anti-RON β (C-20) antibody (Santa Cruz Biotechnology) at a dilution of 1:100 for 60 min. Positive RON expression was defined as a modified H-score (intensity x %) >210 as it was the most discriminant cut-off point in the training set (Figure 1A and B).

For the ICGC cohort, we extracted RNA from tumors using Qiagen Allprep® (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions, assayed for quality on

an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA), and hybridized to Illumina Human HT-12 V4 microarrays. mRNA expression data were available for 80 of 100 patients. Raw idat files were processed using *IlluminaGeneExpressionIdatReader* (Cowley *et al.* manuscript in preparation). Following array quality control, data were vst transformed and robust spline normalized, using the *lumi* R/Bioconductor package (6). Expression levels of RON were discretized using the mean and the 25th and 75th percentiles.

RESULTS

RON expression (H-score >210) was a biomarker of poor prognosis in the training set (Figure 1C). However, in the larger validation set RON expression was not prognostic (Figure 1D). RON expression did not co-segregate with chemotherapy responsiveness, however a trend towards better qualitative response was seen for the RON low or absent group (Figure 1E and F). There was no association between RON expression and tumor stage (Chi-squared *P*=0.1226), tumor size (*P*=0.6289) lymph node metastases (*P*=0.9424), grade (*P*=0.3324), perineural (*P*=0.3351) or vascular invasion (*P*=0.2095). Expression of RON was not associated with prognosis in the prospective ICGC cohort (Figure 1G). In addition, a 3-gene expression signature of RON+MSP+MT-SP1, and combinations of 2 of these genes, were also investigated since MT-SP1 processes MSP, the RON ligand, to an active form, and high expression of these 3 genes in breast cancer is associated with poor prognosis (7). However, none of these signatures was associated with differential survival (data not shown).

DISCUSSION

PC remains one of the most lethal of human cancers. There is a lack of effective therapies or biomarkers with clinical utility in this disease. Pre-clinical studies identified RON as a potential predictive biomarker for gemcitabine response (2, 3). The current study examined RON as a prognostic and predictive biomarker in three large well-annotated cohorts of patients (total of 492) with resectable PC using immunohistochemistry and gene expression arrays. Apart from the training cohort, RON expression was not associated with survival. The validation cohort consisted of 316 patients and has a 99% power to detect a hazard ratio of 1.90 (assuming HR and an expression pattern similar to the training cohort with a Type I error of 0.05). However, RON overexpression was only observed in 5.4% of patients in the validation set as compared to 22.1% in the training set. A post hoc analysis of the validation set using different cut-points for RON expression could not demonstrate any differential survival. It is likely that due to the smaller number of patients, the training cohort returned a false-positive result. This highlights the importance of independent validation in biomarker discovery and development. The current study did demonstrate that RON is expressed in a large proportion of PC (training set: 61 of 64, 95.3%; validation set: 253 of 281, 90.0%), which is consistent with previous published data (1). Although these data do not support RON as a prognostic or predictive biomarker in resectable PC, RON may still prove to be an effective therapeutic target due to its potential role in PC progression.

Figure Legend:

Figure 1. Assessment of RON as a prognostic marker in pancreatic cancer. (A) and (B), Pancreatic cancers with high and low immunostaining for RON, respectively. Kaplan-Meier survival curves for patients stratified based upon RON expression: (C), the training cohort; (D), the validation cohort; (E), patients with high RON expression, with and without adjuvant chemotherapy; (F), patients with low or absent RON-expression, with and without adjuvant chemotherapy; (G), the ICGC validation cohort.

Figure 1

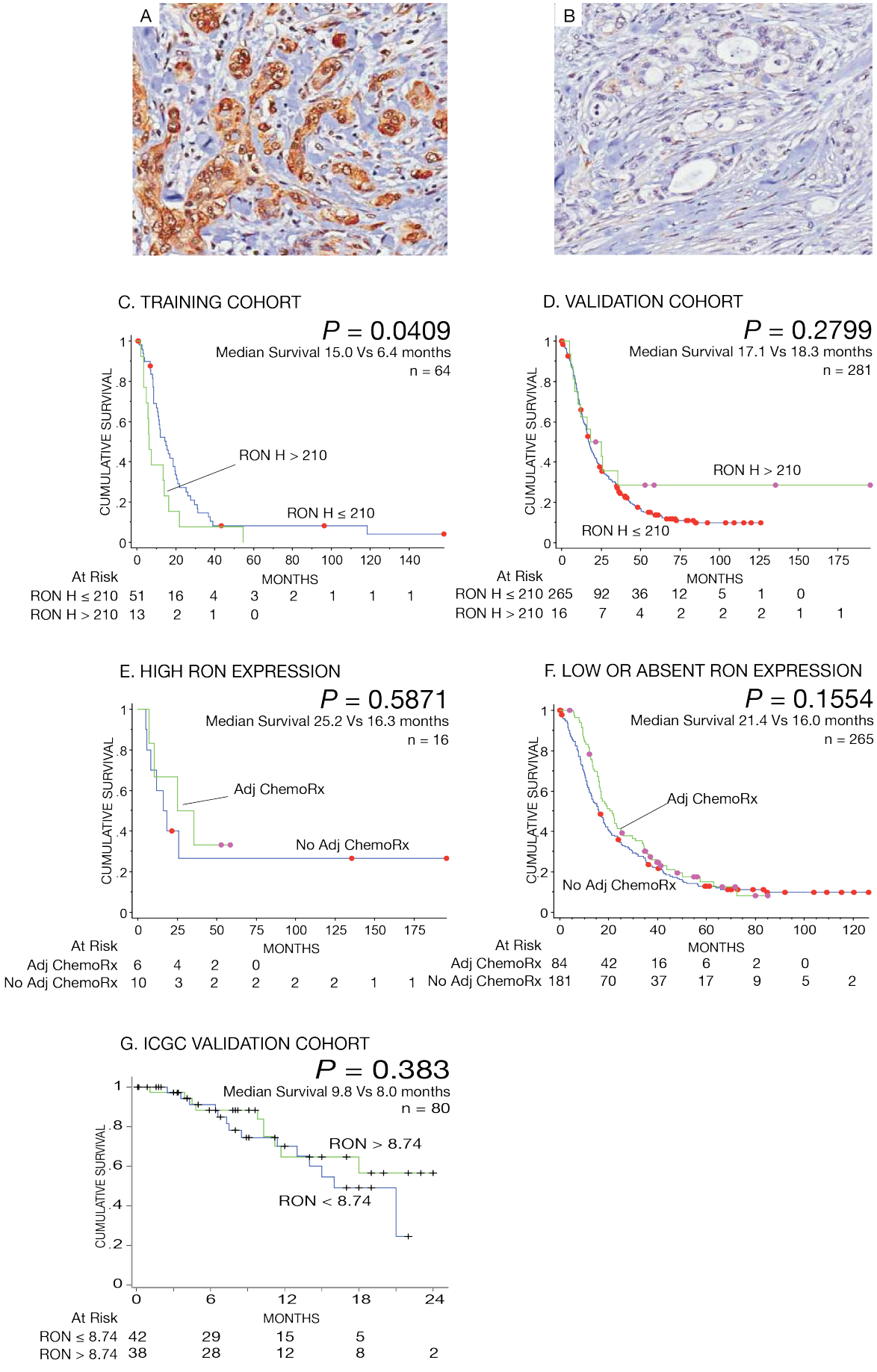
A
B
C. TRAINING COHORT
P = 0.0409
Median Survival 15.0 Vs 6.4 months
n = 64
CUMULATIVE SURVIVAL
RON H > 210
RON H ≤ 210
MONTHS
At Risk
RON H ≤ 210 51 16 4 3 2 1 1 1
RON H > 210 13 2 1 0
D. VALIDATION COHORT
P = 0.2799
Median Survival 17.1 Vs 18.3 months
n = 281
CUMULATIVE SURVIVAL
RON H > 210
RON H ≤ 210
MONTHS
At Risk
RON H ≤ 210 265 92 36 12 5 1 0
RON H > 210 16 7 4 2 2 2 1 1
E. HIGH RON EXPRESSION
P = 0.5871
Median Survival 25.2 Vs 16.3 months
n = 16
CUMULATIVE SURVIVAL
Adj ChemoRx
No Adj ChemoRx
MONTHS
At Risk
Adj ChemoRx 6 4 2 0
No Adj ChemoRx 10 3 2 2 2 2 1 1
F. LOW OR ABSENT RON EXPRESSION
P = 0.1554
Median Survival 21.4 Vs 16.0 months
n = 265
CUMULATIVE SURVIVAL
Adj ChemoRx
No Adj ChemoRx
MONTHS
At Risk
Adj ChemoRx 84 42 16 6 2 0
No Adj ChemoRx 181 70 37 17 9 5 2
G. ICGC VALIDATION COHORT
P = 0.383
Median Survival 9.8 Vs 8.0 months
n = 80
CUMULATIVE SURVIVAL
RON > 8.74
RON < 8.74
MONTHS
At Risk
RON ≤ 8.74 42 29 15 5
RON > 8.74 38 28 12 8 2

Table 1: Patient Cohorts Characteristics

	Training Cohort			Validation Cohort			ICGC Cohort		
Variables	**n = 76 No. (%)**	**Median DSS (months)**	***P* value (Logrank)**	**n = 316 No. (%)**	**Median DSS (months)**	***P* value (Logrank)**	**n = 100 No. (%)**	**Median DSS (months)**	***P* value (Logrank)**
Sex									
Male	45 (59.2)	16.3		157 (49.4)	18.3		61 (61.0)	18.4	
Female	31 (40.8)	8.5	0.0340	159 (50.6)	16.9	0.5792	39 (39.0)	18.3	0.5467
Age (years)									
Mean	62.1			66.7			66.9		
Median	64.5			69.0			68.0		
Range	35.0 – 83.0			28.0 – 87.0			34.0 – 90.0		
Outcome									
Follow-up (months)	0.3 – 158.0			0.1 – 195.8			0.1 – 29.8		
Median follow-up	158.0			68.7			14.1		
Death PC	68 (89.5)			259 (82.2)			33 (33.0)		
Death other	5 (6.6)			15 (4.8)			9 (9.0)		
Death Unknown	0 (0.0)			3 (1.0)			3 (3.0)		
Alive	1 (1.3)			38 (11.8)			55 (55.0)		
Lost to FU	2 (2.6)			1 (0.3)			0 (0.0)		
Stage[a]									
I	16 (21.1)	19.6		23 (7.3)	41.0		8 (8.0)	17.4	
II	59 (77.6)	11.5		282 (89.2)	17.8		87 (87.0)	18.8	
III	0 (0.0)			0 (0.0)			1 (1.0)	----[b]	
IV	1 (1.3)	22.0	0.2828	11 (3.5)	7.6	<0.0001	4 (4.0)	12.0	****[c]
T Stage									
T1	12 (15.8)			16 (5.1)			3 (3.0)		
T2	29 (38.2)	13.6		33 (10.4)	26.6		12 (12.0)	17.4	
T3	35 (46.1)	14.7	0.4857	267 (84.5)	16.8	0.0084	84 (84.0)		
T4	0 (0.0)			0 (0.0)			1 (1.0)	18.4	0.4297
N Stage									
N0	37 (48.7)	19.8		119 (37.9)	21.2		24 (24.2)	17.4	
N1	39 (51.3)	9.7	<0.0001	197 (62.1)	16.7	0.0267	75 (75.8)	18.4	0.4714
Grade									
I	7 (9.2)			26 (8.3)			4 (4.0)		
II	43 (56.6)	15.0		209 (65.9)	17.7		61 (61.0)	----	
III	26 (34.2)	11.2	0.0283	81 (25.8)	18.3	0.5971	33 (33.0)		
IV							2 (2.0)	15.1	0.0011
Tumor size									
≤ 20mm	15 (19.7)	17.1		77 (24.8)	32.0		14 (14.0)	18.3	
> 20mm	61 (80.3)	11.9	0.1232	236 (75.2)	16.0	<0.0001	86 (86.0)	18.4	0.8056
Margins									
Clear	40 (52.6)	18.6		195 (61.7)	22.4		66 (66.0)	----	
Involved	36 (47.4)	9.7	0.0004	121 (38.3)	13.3	<0.0001	34 (34.0)	13.9	0.0335
Tumor Location									
Head	62 (81.6)	15.6		258 (81.5)	18.8		85 (85.0)	18.4	
Others	14 (18.4)	7.4	0.0004	58 (18.5)	13.0	0.0312	15 (15.0)	13.6	0.0488
Perineural Invasion									
Negative	24 (32.0)	15.6		82 (26.1)	25.6		20 (20.6)	----	
Positive	51 (68.0)	13.6	0.1909	226 (73.9)	17.4	0.1180	77 (79.4)	17.4	0.0211
Vascular Invasion									
Negative	45 (60.0)	15.0		161 (53.5)	21.2		39 (40.6)	----	
Positive	30 (40.0)	10.1	0.0141	140 (46.5)	16.2	0.0070	57 (59.4)	15.9	0.0348
Adj Chemotherapy									
Yes	13 (17.1)	13.6		98 (31.2)	22.4		65 (68.4)	21.4	
No	63 (82.9)	14.1	0.7737	218 (68.8)	16.5	0.0451	30 (31.6)	12.0	0.0007
RON Expression[d]									
Low or absent	51 (27.9)	15.0		265 (71.9)	17.1		42 (52.5)	9.8	
High	13 (72.1)	6.4	0.0409	16 (28.1)	18.3	0.2799	38 (47.5)	8.0	0.3830

[a] AJCC 7th Edition

[b] Median survival has not been reached yet

[c] Rank test cannot be tested as one or more groups contained no censored observations

[d] High RON expression was defined as H>210 for immunohistochemistry or greater than 8.74 on normalized Log2 score for gene expression array

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