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Expression of the RNA-binding protein HuR and its clinical significance in human stage I and II lung adenocarcinoma

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Summary. The ubiquitously expressed RNA-binding protein Hu antigen (HuR) participates in the post-transcriptional regulation of mRNAs bearing U- and AU-rich sequences. Expression of HuR is increased in cancers of the breast, colon, ovary and lung and cytoplasmic immunoreactivity for HuR was found to be closely related to poor outcomes in patients with these tumors.

Since the regulation of HuR function is closely linked to its subcellular localization, we evaluated, by quantitative immunohistochemistry, the impact on clinical outcome of both nuclear and cytoplasmic levels (integrated density: ID) of HuR and of nuclear/cytoplasmic ratio (N/C) in 54 lung adenocarcinomas from stage I and II patients.

Nuclear and cytoplasmic Hur IDs and N/C were not associated with age, smoking or tumor diameter. Low N/C was significantly associated with lymph-node involvement at presentation. Cox's regression analysis showed that high cytoplasmic, but not nuclear, HuR ID and low N/C were directly associated with the risk of death and metastasis. In the multivariate analysis, low HuR N/C retained an independent negative prognostic significance relative to the risk of metastasis and death. Moreover, the levels of N/C allowed us to discriminate subjects with the highest risk of metastasis and death among patients with lung adenocarcinomas expressing high levels of cytoplasmic HuR.

In conclusion, the measure of the ratio between nuclear and cytoplasmic HuR levels allows a sensitive prognostic evaluation of the clinical outcome in early stage lung adenocarcinoma patients.

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Introduction

The family of Hu/elav RNA-binding proteins participate in the post-transcriptional regulation of mRNAs bearing U- and AU-rich sequences, and comprises the neuronal proteins HuB, HuC and HuD, and the ubiquitously expressed RNA-binding protein Hu antigen (HuR) (Antic and Keene, 1997).

The regulation of HuR functions is closely linked to its subcellular localization. In the nucleus, HuR seems to participate in splicing and export of pre-mRNAs, while in the cytoplasm HuR may render target mRNAs stable, induces their translation, or performs both functions (Keene, 1999). HuR may initially bind to ARE-containing mRNAs in the nucleus, accompanies them to the cytoplasm and then returns rapidly to the nucleus after release from the mRNA. HuR localization varies during the cell cycle and it is predominantly localized in the nucleus of resting cells (Wang et al., 2000a,b) where it binds proteins SET α/ß, pp32 and APRIL. Several stress-activated signaling pathways modulate the cytoplasmic localization of HuR and its RNA-binding functions (Wang et al., 2000a,b; Brennan and Steitz, 2001; Gallouzi et al., 2001). Moreover, activation of the mitogen-activated protein (MAP) kinases p38 and ERK enhances the cytoplasmic presence of HuR and its ability to bind to and stabilize target mRNAs (Tran et al., 2003; Yang et al., 2004).

The expression of HuR is increased in cancers of the breast, colon, ovary and lung and cytoplasmic immunoreactivity for HuR was found to be closely related to poor outcomes in patients with these tumors (Blaxall et al., 2000; López de Silanes et al., 2003;

Denkert et al., 2004, 2006; Heinonen et al., 2005; Wang et al., 2011). The identification of HuR as a shuttling protein rationalizes the possibility that HuR may have different subcellular localizations during different functional and/or developmental stages (Fan and Steitz, 1998a). Thus, the quantitative evaluation of HuR distribution between nucleus and cytoplasm of cancer cells may best reflect the functional relevance of HuR expression in tumor biology.

Lung cancer represents the leading cause of cancer death, at least in Western countries. Clinically, it is classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC, by far the most frequent, is histopathologically subclassified as squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (Herbst et al., 2008).

There are important differences between the molecular changes present in adenocarcinomas and other lung cancers, indicating different pathogenetic mechanisms (Sato et al., 1994; Gazdar and Minna, 1997). These findings suggest the need to study adenocarcinoma as a separate entity, both from a clinical and molecular point of view.

In the present paper, we analyzed, by quantitative immunohistochemistry, the expression of HuR in 54 lung adenocarcinomas from stage I and II patients. Moreover, given the importance of cellular localization on the functions of HuR, we assayed the level of HuR expressed both in the nucleus and cytoplasm of tumor cells and evaluated the impact of the ratio between nuclear and cytoplasmic HuR expression on clinical outcome.

Materials and methods

Patients

Patients with completely resected pathological stage I and II lung adenocarcinomas, with age range 18 to 75 years, no previous malignancy, performance status (ECOG) 0-1 and weight loss in the three months prior to diagnosis lesser or equal to 5%, were enrolled in this study, after approval by the Bioethic Committee of the Università Cattolica del S. Cuore (Prot. Cm.(A.231)/C.E./2011). Cyto/histologic confirmation of the diagnosis was obtained by CT guided trans-thoracic fine needle aspiration biopsy. Patients judged to be resectable were operated by the same surgical team. Only anatomical resections were performed, no less than lobectomy. In all cases hilar-mediastinal radical homolateral lymphadenectomy was performed. Two pathologists (L.L and F.O.R.) independently reviewed all the histologic slides from the 54 patients in a blind fashion and according to the most recent classification criteria based on the predominant growth pattern of adenocarcinoma (Travis et al., 2011), instead of mixed growth pattern as considered in WHO classification (Travis et al., 2004). The tumors were classified as lung adenocarcinomas of mixed histologic type (20 acinar predominant, 14 papillary predominant and 20 solid predominant with mucin production). Based on definitive pathological staging, patients with pStage Ia and Ib (T1N0 and T2N0) were randomized to receive adjuvant radiotherapy or not, as previously described (Trodella et al., 2002). Patients with stage II disease were treated with adjuvant radio-chemotherapy. Chemotherapy agents used were: cisplatin (75 mg/m^2, day 1) and etoposide (100 mg/m^2, day 1-3). In the present study none of the patients treated with postoperative radiotherapy experienced a G3-5 toxicity. Non symptomatic radiologic findings were collected in 44% of patients and only one patient had an acute pneumonitis, completely recovered with medical intervention. Patients included in the study entered a follow-up program, carried out simultaneously by the oncologist radiotherapist, the surgeon and the pneumologist.

Immunohistochemistry

Tumor tissues were fixed in formalin and paraffin-embedded according to standard procedures. Consecutive tissue sections of representative blocks from each case, were deparaffinized in xylene, rehydrated, treated with 0.3% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity, and subjected to heat-induced epitope retrieval in a microwave oven using the Dako ChemMate detection kit (Dako, Milano, Italy). All slides were simultaneously processed for immunohistochemistry on the DAKO autostainer (Dako), using the Vectastain ABC peroxidase kit (Vector Laboratories, Burlingame, CA). Sections were incubated with rabbit polyclonal antibody against HuR (1:100; Santa Cruz Biotechnology, Inc., CA). Endogenous biotin was saturated by a biotin blocking kit (Vector Laboratories). As positive internal control the human lung adenocarcinoma cell line HCC44 was used. Negative controls were performed using non-immunized rabbit serum or omitting the primary antibody. After overall evaluation of the HuR tumor immunostaining (nuclear and/or cytoplasmic HuR expression), three 20 x fields were chosen so as to reflect the overall immunostaining of the entire tumor section. The intensity of HuR expression was evaluated as nuclear and cytoplasmic immunostainings by the following procedure: nuclei were lightly counterstained with hematoxylin and, in digitalized images, selected by slice thresholding of color images utilizing the Select -> Color Range command in Adobe Photoshop CS3 software (Adobe Systems Incorporated, San Josè, CA). Once the intensity of the nuclear immunostainings were measured, nuclei were removed from digitalized images and the intensity of the cytoplasmic immunostainings were evaluated. The intensity of immunohistochemical staining (integrated density, ID) was evaluated as previously reported (Ranelletti et al., 2001). Data were also expressed as the ratio between nuclear and cytoplasmic (N/C) HuR expression. Three pathologists

without prior knowledge of the clinic-pathological parameters did the computerized image analysis of all samples.

Statistical analysis

Cox-Mantel method was used to evaluate the prognostic role of HuR IDs as continuous covariates. All medians and life tables were computed using the product-limit estimate by Kaplan and Meier, and the curves were examined by the log-rank test. Univariate and multivariate analyses were performed by Cox's proportional hazards model. Metastasis-free and overall survival was calculated from the date of first surgery to that of metastasis occurrence or of death from disease. All p values were two-sided. Statistical analyses were done by JMP 7 software (SAS Institute Inc., Cary, NC).

Results

HuR expression

HuR immunostaining was localized in both the nucleus and cytoplasm of tumor cells. Shown in Fig. 1 are tumors with prevalent nuclear (1A), mixed (nuclear and cytoplasmic) (1B) or prevalent cytoplasmic (1C) HuR expression, as well as negative tumor (1D). In tumor stroma heterogeneous nuclear immunostaining was present in tumor-associated macrophages and fibroblasts. In peri-tumoral normal lung tissue, both type I and II pneumocytes, as well as alveolar macrophages, showed prevalent nuclear HuR expression (not shown). In the overall tumor population, nuclear HuR IDs ranged from 0.8 to 18.3 (median=9.3; mean ± SE=9.1±0.6); cytoplasmic HuR IDs ranged from 0.45 to 57.5

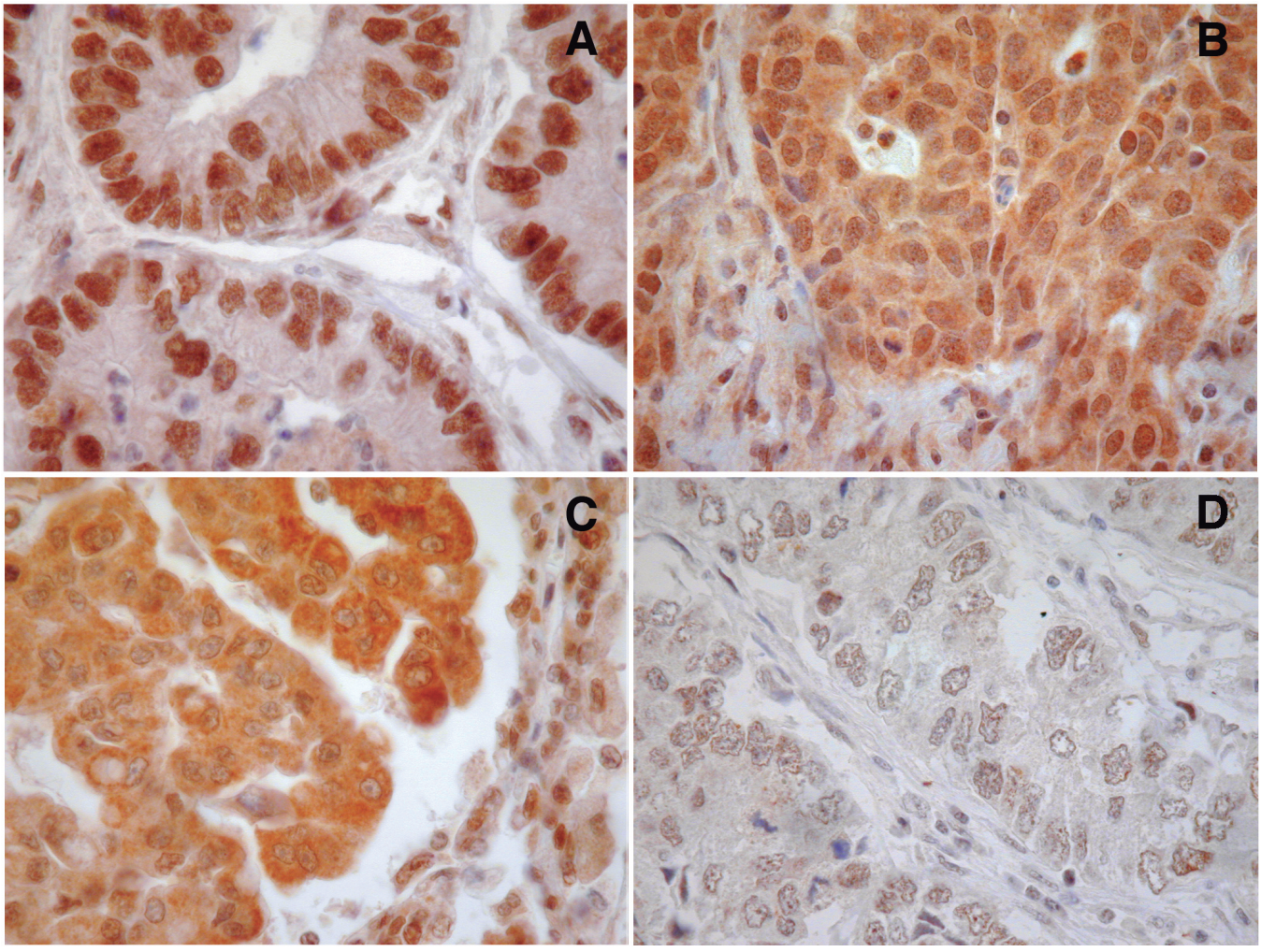

Fig. 1. Immunohistochemical analysis of HuR expression in adenocarcinomas. Shown are tumors with prevalent nuclear **(A),** nuclear and cytoplasmic **(B)** or prevalent cytoplasmic **(C)** HuR immunolocalizations, as well as negative tumors **(D).** x 400

(median=13.7; mean ± SE=19.0±1.9). Considering that HuR function is closely linked to its subcellular localization, we measured the ratio between nuclear and cytoplasmic HuR expression as reported in the Materials and Methods and shown in Fig. 2. In the overall tumor population, N/C ranged from 0.19 to 1.97 (median=0.56; mean ± SE=0.7±0.06). In the example reported in figure 1, the tumors with prevalent nuclear (A), mixed (B) and cytoplasmic (C) patterns showed N/C of 1.4, 0.49 and 0.19, respectively. In the overall tumor population, nuclear and cytoplasmic HuR IDs were directely associated (Spearman's rank test: $p<0.0001$).

HuR expression according to clinico-pathological characteristics

Nuclear and cytoplasmic Hur IDs and N/C were not associated with age, gender, smoking, or tumor diameter, while both cytoplasmic ID and N/C differed significantly in relation to tumor histology. In particular, as revealed by Tukey-Kramer test, adenocarcinomas with acinar and papillary predominant pattern showed higher cytoplasmic HuR IDs and lower N/C than adenocarcinomas with a predominant solid pattern. Moreover, cytoplasmic HuR IDs and N/C did not differ significantly between acinar and papillary predominant tumors. Only low N/C was significantly associated with lymph-node involvement at presentation (Table 1).

Survival analyses

With a median follow-up of 36 months (range 3-194), metastases occurred in 19 out of 54 (32.2%) cases. At the end of the study, 12 out of 54 (22.2%) patients had died of cancer. Cox's regression analysis, using nuclear and cytoplasmic HuR IDs and N/C as continous covariates, showed that high cytoplasmic, but not nuclear, HuR IDs and low N/C were directly associated with the risk of death and metastasis. The risk ratios of metastasis, per unit change of regressors were 1.04 (95% C.I.=1.01-1.07; p=0.013) and 0.19 (95% C.I.=0.04-0.70; p=0.009) for cytoplasmic HuR ID and N/C, respectively. The risk ratios of death, per unit change of regressors were 1.06 (95% C.I.=1.02-1.10; p=0.0011) and 0.02 (95% C.I.=0.0008-0.27; p=0.0007) for cytoplasmic HuR ID and N/C, respectively. The plots of the estimtes of metastasis-free and overall surviving as a function of nuclear and cytoplasmic HuR IDs and N/C levels showed that the increase of cytoplasmic, but not nuclear, HuR IDs and the decrease of N/C were associated with a reduction of the surviving fraction of patients at 5-year follow-up (Fig. 3).

For survival analysis, nuclear and cytoplasmic Hur IDs and N/C continous variables were converted to binomial variables of high versus low expression around the median values. This choice was made based on an initial analysis of the distribution of variable values and according to the results of ROC curve analyses. Tumors with nuclear HuR IDs≥9.3 (27 out of 54) or cytoplasmic

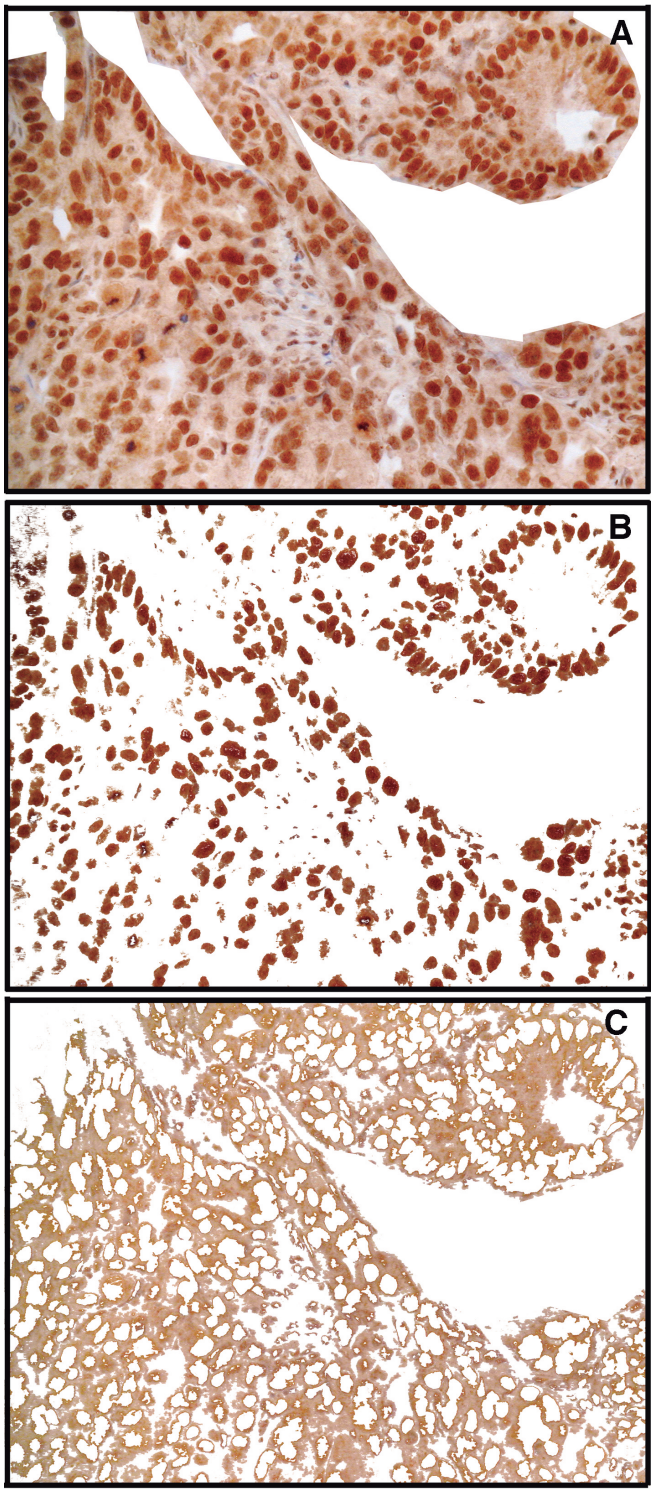

Fig. 2. Cell nuclei **(B)** and cytoplasms **(C)** of the tumor section **(A)** were separated in digitalized image by slice thresholding of color image as outlined in Materials and Methods. x 200

HuR IDs≥13.5 (29 out of 54) were classified as positive. Relative to N/C, tumors with ratios ≥0.56 were considered of nuclear pattern type (28 out of 54). The survival curves according to cytoplasmic, but not nuclear, HuR IDs or to N/C status showed a significant relationship between positive cytoplasmic HuR IDs or low N/C and short metastasis-free and overall survival (Fig. 4). Kaplan-Meier analyses of survival curves revealed that the 5-year metastasis-free survival was 54% (95% C.I.=33. 6-75.3) and 82% (95% C.I.=66.7-98.1) (likelihood ratio: p=0.04) for patients with cytoplasmic HuR positive and negative tumors, respectively. For patients with low and high N/C, the 5-year metastasis-free survival was 49% (95% C.I.=26.5-70.7) and 83% (95% C.I.=56.3-95.3) (likelihood ratio: p=0.0007), respectively. The 5-year overall survival was 52% (95% C.I.=28.5-75.7) and 94% (95% C.I.=83.9-105.0) (likelihood ratio: p=0.01) for patients with cytoplasmic HuR positive and negative tumors, respectively. For patients with low and high N/C, the 5-year overall survival was 39% (95% C.I.=14.0-63.6) and 100% (likelihood ratio: p<0.0001), respectively.

Then, we further examined whether adjusting for N/C adds more information to the relationship between high cytoplasmic HuR ID levels and the metastases-free and overall survival rates. Weibull model estimates of the overall and metastasis-free survival as a function of cytoplasmic HuR IDs, for patients stratified according to

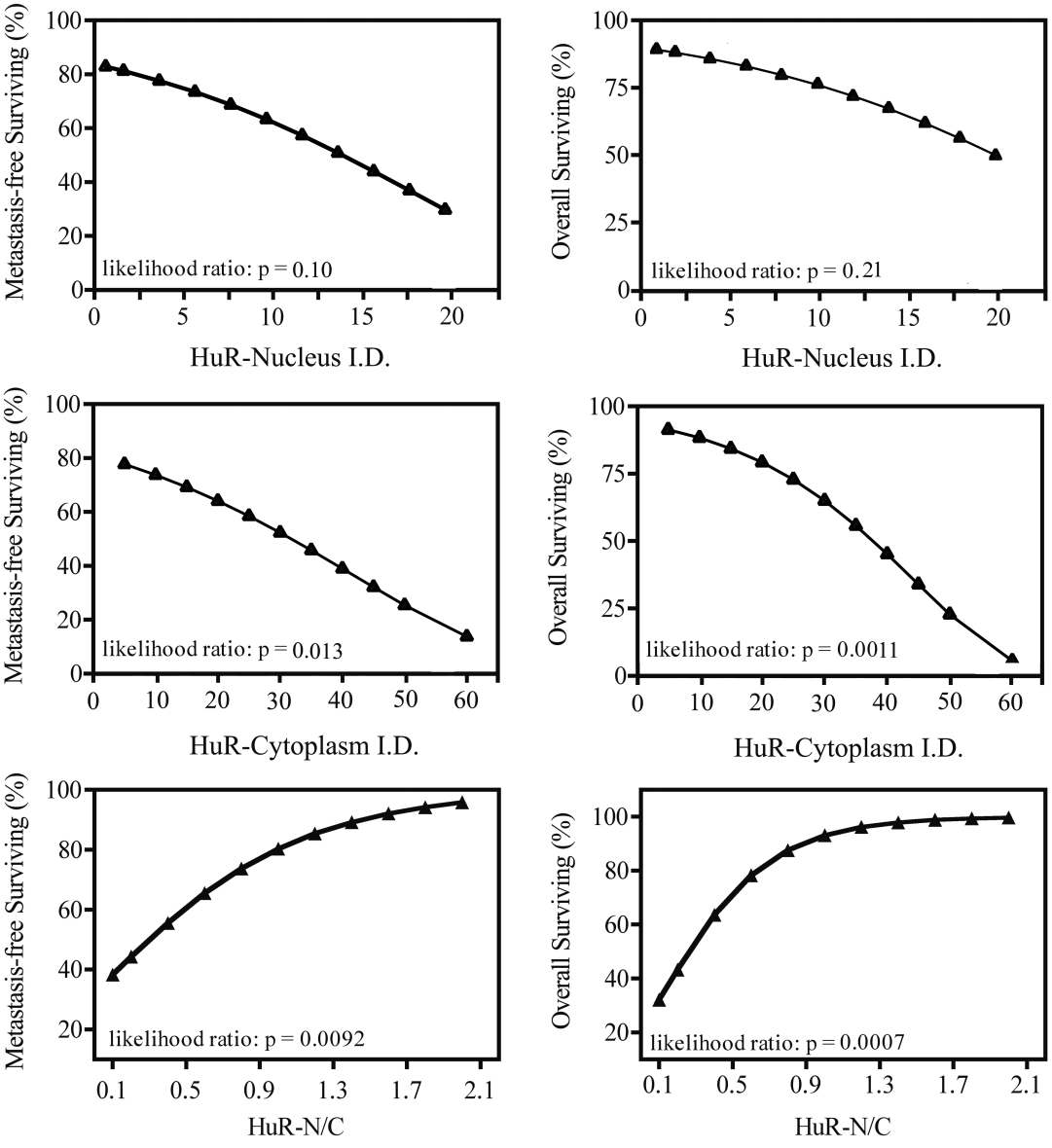

Fig. 3. Plots of the estimtes of metastasis-free and overall survival as a function of nuclear and cytoplasmic HuR integrated density (I.D.) values and of the ratio between nuclear and cytoplasmic HuR levels (N/C). The proportional hazards model was evaluated at each covariate value and the proportion of patients without event at 5-year follow-up was estimated from the computed survival functions.

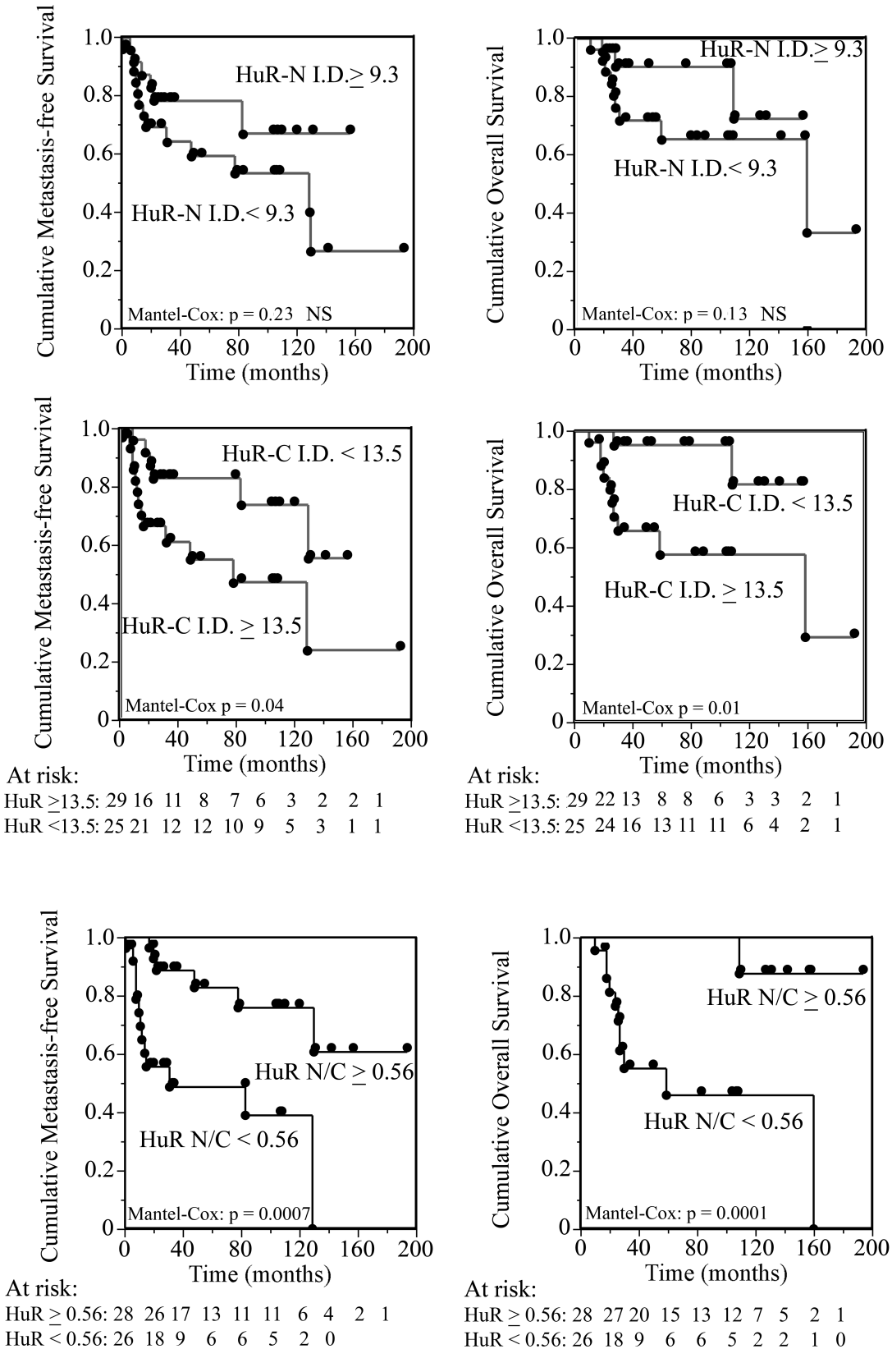

Fig. 4. Kaplan-Meier analysis of survival curves as a function of HuR nuclear (N), cytoplasmic (C) and nuclear to cytoplasmic ratio (N/C) stratus in lung adenocarcinoma patients. The reported cut-off values correspond to the median values of variables.

the N/C status (≥0.56 or <0.56) of their tumors, showed (Fig. 5) that, at 5-year follow-up, the estimated proportions of patients with metastasis were highest in the sub-group of subjects with tumors displaying low N/C status. Relative to overall survival, the estimated proportions of dead patients were highest in the sub-group of subjects with tumors displaying low N/C status.

Having observed that acinar and papillary predominant tumors showed a lower N/C than solid ones, we evaluated the prognostic role of N/C separately in the sub-group of papillary and acinar tumors and in that of solid tumors. Kaplan-Meier analyses of survival curves revealed that, independently from the adenocarcinoma pattern, at 5-year follow-up, the proportion of overall and metastasis-free surviving patients was significantly higher in the sub-group of patients with tumors displaying high (≥0.56) N/C status (data not shown).

As revealed by univariate analysis, cases with lymph-node involvement, cytoplasmic HuR positive and low N/C tumor expression status were associated with an increased risk of metastasis, while those with a tumor diameter ≥3 cm, lymph-node involvement, cytoplasmic HuR positive and low N/C tumors were associated with an increased risk of death (Table 2).

In multivariate analysis, low HuR N/C retained an independent negative prognostic significance relative to the risk of metastasis, while tumor diameter ≥3 cm, lymph-node involvement, low HuR N/C retained an independent negative prognostic significance relative to the risk of death (Table 3).

Discussion

Several studies focusing on cancers of breast, colon, ovary and lung (Blaxall et al., 2000; López de Silanes et al., 2003; Heinonen et al., 2005; Denkert et al., 2004,

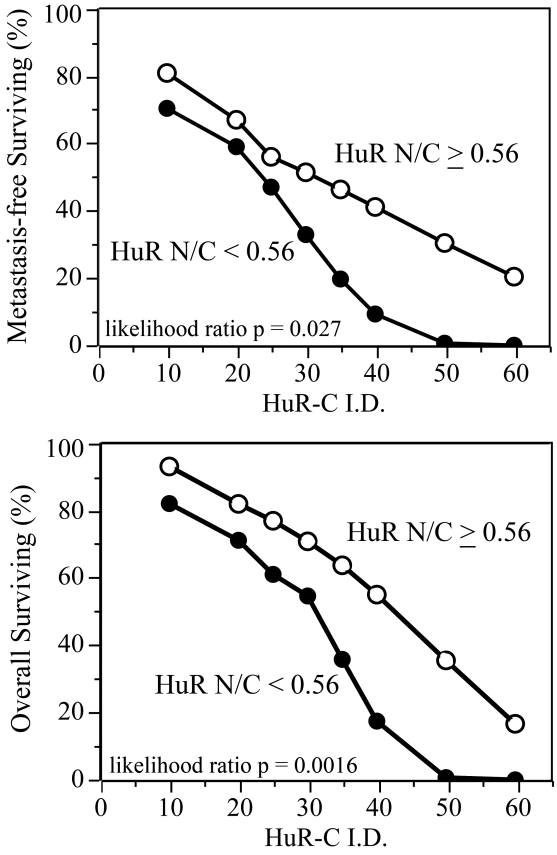

Fig. 5. Plots of the Weibull model estimates, at 5-year follow-up, of the proportion of metastasis-free and overall surviving patients, as a function of cytoplasmic HuR integrated density levels (HuR-C I.D.) of their tumors. Patients were stratified according to the high or low N/C ratio of HuR expression in their tumors.

Table 1. HuR immunostaining, expressed as nuclear (HuR-N), cytoplasmic (HuR-C) and nuclear/cytoplasmic ratio (HuR-N/C), according to clinico-pathological characteristics in 54 stage I-II lung adenocarcinoma patients.

		n	HuR-N (I.D)	p	HuR-C (I.D)	p	HuR-N/C	p
Age	<66	32	9.59±0.761		19.55±2.53		0.76±0.08	
	≥66	22	8.46±0.92	0.38[2]	18.26±3.05	0.88	0.64±0.10	0.49
Sex	M	39	9.28±0.69		17.81±2.27		0.79±0.07	
	F	15	8.75±1.12	0.52	22.18±3.66	0.30	0.50±0.11	0.042
Smoking	No	14	9.07±0.69		18.32±2.25		0.77±0.07	
	Yes	40	9.29±1.16	0.86	21.05±3.81	0.30	0.54±0.12	0.12
Tumor diameter (cm)	<3	25	9.11±0.87		17.72±2.85		0.71±0.08	
	≥3	29	9.15±0.81	0.98	20.16±2.65	0.53	0.71±0.09	0.97
Lymph-node involvement	No	43	9.06±0.66		17.62±2.14		0.77±0.07	
	Yes	11	9.10±1.30	0.94	24.54±4.23	0.07	0.44±0.14	0.03
Tumor histology	Acinar	20	10.58±0.94		26.25±3.52		0.52±0.07	
	papillary	14	9.58±1.17		20.32±13.91		0.57±0.06	
	solid	20	7.29±0.95	0.07[3]	8.15±1.81	0.002	0.98±0.13	0.01

[1]: The results are expressed as mean±S.E. of HuR integrated densities (I.D.). [2]: Mann-Whitney test. [3]: Kruskal-Wallis test.

2006; Wang et al., 2011) showed a significant correlation between HuR expression and advancing stages of malignancy, supporting a role for HuR in cancer development in these tissues. Much evidence indicated that HuR expression plays crucial roles in mRNA stabilization/translation of numerous growth, motility and angiogenic factors, including epidermal growth factor, vascular endothelial growth factor, platelet-derived growth factor, transforming growth factor beta, hypoxia inducible factor-1α and parathyroid hormone-related protein, all shown to be important in lung tumorigenesis (Hastings, 2004; López de Silanes et al., 2005; Wang et al., 2009; Monego et al., 2010).

Given the importance of HuR localization in cellular compartments on its functions (Fan and Steitz, 1998a), we assayed the level of HuR expressed both in the nucleus and cytoplasm of tumor cells, differently from a previous study in lung carcinoma (Wang et al., 2011), and evaluated the impact of the ratio between nuclear and cytoplasmic HuR expression on clinical outcome.

In this study, we found that the ratio between nuclear and cytoplasmic HuR levels is an independent prognostic marker of a poor clinical outcome in early stage lung adenocarcinomas. Our data are in agreement

Table 2. Univariate analysis of prognostic variables in 54 stage I-II lung adenocarcinoma patients.

		Metastasis-free Survival			Overall Survival		
Covariates		RR[1]	(CI 95%)[2]	p	RR	(CI 95%)	p
Age (years):	<66	1			1		
	≥66	0.70	(0.3-1.8)	0.46	0.79	(0.4-1.4)	0.43
Sex:	Male	1			1		
	Female	2.85	(1.1-7.4)	0.06	2.32	(0.7-7.7)	0.18
Smoking:	No	1			1		
	Yes	0.60	(0.2-1.5)	0.30	1.29	(0.3-4.8)	0.70
Tumor diameter (cm)	<3	1			1		
	≥3	0.72	(0.3-1.8)	0.49	9.81	(1.3-76.4)	0.003
Lymph-node involvement	No	1			1		
	Yes	4.35	(1.6-11.7)	0.007	8.12	(2.2-29.3)	0.002
HuR-N (I.D.)	>9.3	1			1		
	≥9.3	1.90	(0.7-5.1)	0.18	2.40	(0.6-9.2)	0.19
HuR-Cyt (I.D.)	<13.5	1			1		
	≥13.5	2.7	(1.0-7.2)	0.048	5.9	(1.2-27.8)	0.025
HuR-N/C	>0.56	1			1		
	≥0.56	5.1	(1.8-14.5)	0.001	21.7	(2.7-176.4)	0.0001

[1]: Unadjusted relative risk. [2]: 95% confidente intervals.

Table 3. Multivariate analysis of prognostic variables in 54 stage I-II lung adenocarcinoma patients.

		Metastasis-free Survival			Overall Survival		
Covariates		RR[1]	(CI 95%)[2]	p	RR	(CI 95%)	p
Age (years)	<66	1			1		
	≥66	0.63	(0.2-1.8)	0.39	0.86	(0.2-4.6)	0.86
Sex:	Male	1			1		
	Female	1.62	(0.4-6.0)	0.47	4.07	(0.9-18.7)	0.07
Smoking	No	1			1		
	Yes	1.10	(0.3-4.3)	0.89	2.98	(0.3-27.7)	0.34
Tumor diameter (cm)	<3	1			1		
	≥3	0.80	(0.3-2.2)	0.66	14.18	(0.8-245.7)	0.05
Lymph-node involvement	No	1			1		
	Yes	2.47	(0.8-7.2)	0.09	7.96	(1.6-39.4)	0.011
HuR-N/C	>0.56	1			1		
	≥0.56	3.68	(1.1-12.2)	0.038	24.77	(1.9-318.0)	0.014

[1]: Unadjusted relative risk. [2]: 95% confidente intervals.

with the recent observation on the association in NSCLC between HuR expression and a poor clinical outcome (Wang et al., 2011).

In our series of patients, radiotherapy is not a confounding factor in negative matter because no treatment related death was recorded, and no beneficial effect on systemic disease can be advocated. The survival rate of pStage Ia and Ib patients treated with radiotherapy did not differ significantly from that of untreated ones (data not shown). Moreover, radiotherapy ideally affects local recurrence, while the ratio between nuclear and cytoplasmic HuR levels was inversely associated with metastatic spread.

HuR is predominantly present in the nucleus of normal unstimulated cells. It has to be transported to the cytoplasm in order to stabilize mRNAs encoding proteins involved in malignant transformation (Keene, 1999). The export of HuR from the nucleus is mediated by its association with transportin 1 and 2 (Rebane et al., 2004) by the shuttling sequence termed 'HNS' in the hinge region (Fan and Steitz, 1998b), and by its association with pp32 and APRIL, which includes the nuclear export signal recognised by the export receptor chromosome maintenance region 1 (CRM1) (Brennan et al., 2000; Gallouzi et al., 2001).

Heat stress enhances HuR-mediated export of hsp70 mRNA to the cytoplasm, probably through HuR's association with protein ligands pp32 and APRIL (Brennan et al., 2000). Moreover, signaling through mitogen-activated protein kinases p38 and ERK enhances the cytoplasmic presence of HuR and its ability to bind to and stabilize target mRNAs (Tran et al., 2003; Yang et al., 2004).

Expression of HuR is increased in virtually all cancer tissues compared to the normal-tissue counterparts (López de Silanes et al., 2003). Given that HuR has never been reported to be mutated in cancer, but influences the expression of target mRNAs by post-translational alterations, the ensuing influence on the patterns of expressed oncogenes represents an epigenetic event which depends, at least in part, on the regulation of HuR shuttling, and then on its subcellular localization (Keene et al., 1999; López de Silanes et al., 2005). In fact, it has been documented that Hur proteins have both nuclear and cytoplasmic functions, since these proteins shuttle back and forth between the two cellular compartments (Hinmana and Loua, 2008). Interestingly, it has been reported that HuR is exported to the cytoplasm in oral carcinoma cells in a different manner than in normal cells, and this is likely to occur through the perturbation of a normal export pathway (Hasegawa et al., 2009).

Consistently with the above observations, we found that the measure of the ratio between nuclear and cytoplasmic HuR levels is a more sensitive assessment of lung tumor biology than a separate evaluation of nuclear and/or cytoplasmic HuR expression. Indeed, the levels of N/C allowed us to discriminate subjects with the highest risk of metastasis and death, particularly among patients with lung adenocarcinomas expressing high levels of cytoplasmic HuR.

Gemcitabine is a pyrimidine nucleoside anti-metabolite agent which is active in several human malignancies, including NSCLC (Toschi et al., 2009). It has been reported that HuR both targets and regulates the protein expression of deoxycytidine kinase, the key enzyme involved in metabolizing the prodrug gemcitabine into its active di- and tri-phosphate metabolites. Modulation of the expression of deoxycytidine kinase through HuR overexpression dramatically sensitized pancreatic cancer cells to gemcitabine in vitro (Costantino et al., 2009). Accordingly, the HuR cytoplasmic status in patient tumor cells from a training set strongly correlated with gemcitabine response (Costantino et al., 2009). These observations, which should be extended to NSCLC patients, suggests that HuR may serve as both prognostic and predictive biomarker. Additional studies are needed to elucidate whether HuR might be a promising molecular target for lung adenocarcinoma therapy.

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