

Cytoplasmic Expression of HuR is Related to Cyclooxygenase-2 Expression in Colon Cancer

Sung-Jig Lim, M.D.¹
 Suk-Hwan Lee, M.D.²
 Sun Hyung Joo, M.D.²
 Jeong Yoon Song, M.D.²
 Sung Il Choi, M.D.²

Departments of¹Pathology and²Surgery,
 East-West Neo Medical Center,
 KyungHee University
 College of Medicine, Seoul, Korea

Correspondence: Sung Il Choi, M.D.
 Department of Surgery, East-West Neo Medical
 Center, KyungHee University School of Medicine,
 #149, Sangil-dong, Gangdong-gu,
 Seoul 134-727, Korea
 Tel: 82-2-440-6136
 Fax: 82-2-440-6295
 E-mail: drchoi@khu.ac.kr
 Received October 2, 2008
 Accepted November 11, 2008
 This work was supported by the KyungHee
 University Research Fund in 2006 (KHU-
 20061221).

Purpose

HuR, human family embryonic-lethal abnormal vision-like protein, can bind to mRNA and stabilizes the nucleic acid in the cytoplasm, resulting in more efficient translation. HuR is predominantly present in the nucleus and shuttles between the nucleus and cytoplasm. HuR stabilizes cyclooxygenase-2 (Cox-2) mRNA in several cancers, including breast, stomach, lung and brain cancer.

Materials and Methods

We investigated the expression and cellular location of HuR, as well as evaluated Cox-2 expression in 79 colorectal cancer patients with the use of immunohistochemical methods. The biological implications of HuR localization and Cox-2 expression in colorectal carcinoma were evaluated.

Results

Nuclear HuR expression was observed in 59 (74.7%) tumors and cytoplasmic HuR expression was seen in 25 (31.6%) tumors. Cox-2 immunoreactivity was noted in 42 (53%) tumors. The expression of cytoplasmic HuR was significantly associated with Cox-2 expression ($p=0.004$). Cytoplasmic expression of HuR showed a correlation with lymphatic invasion ($p=0.025$) and the presence of a lymph node metastasis ($p=0.027$). The presence of nuclear HuR showed no correlation with Cox-2 expression or any other of the clinicopathological parameters that were examined.

Conclusion

These results suggest that cytoplasmic translocation of HuR is associated with Cox-2 expression for some colorectal carcinomas.

Key words

Colorectal neoplasms, HuR, Cyclooxygenase-2

Introduction

The regulation of gene expression in eukaryotes is controlled at both the transcriptional and posttranscriptional level (1). Post-transcriptional gene regulatory events, especially regulation of mRNA turnover, have emerged as fundamental and effective means to alter the expression of functionally related genes (2). The investigation of post-transcriptional regulatory mechanisms centers on the association of a given RNA-binding protein with its target mRNA (3). The proteins make mRNA stable by binding to AU-rich elements (AREs) in the 3'-untranslated region of some mRNAs. Thus, the binding effectively lengthens the presence of a given mRNA and consequently, the amount of protein expressed is

increased. Among the ARE-binding proteins identified, the human family embryonic lethal abnormal vision (ELAV)-like proteins that consist of four members (Hel-N1/HuB, HuC, HuD and HuR) have been well characterized (4). HuR is expressed in many cell types, mainly in the cellular nucleus, while the other three proteins are usually expressed in terminally differentiated tissue (4). Several studies have shown that HuR can shuttle between the nucleus and cytoplasm and localization of HuR in the cytoplasm is required for its mRNA stabilizing function (5). HuR binds to labile transcripts containing AREs, such as mRNAs for proto-oncogenes, cytokines and cytokine-response genes in the nucleus. The HuR-mRNA complex is then transported to the cytoplasm (6), where the stabilized mRNA can be translated into protein more efficiently.

Increased expression of cyclooxygenase-2 (Cox-2) has been

implicated in carcinogenesis. There is evidence that mRNA stability and translational efficiency are major steps for the control of Cox-2 expression, although an increase of Cox-2 gene transcription partly accounts for the increase of Cox-2 protein expression (7,8). There is an ARE in the 3'-untranslated region of Cox-2 mRNA that provides the binding site for HuR, resulting in increased mRNA half-life (9). Cox-2 is overexpressed in several malignant tumors and the increase in expression may be due to dysregulation of mRNA stability (10-12).

Recently, a correlation between the protein expression level of HuR and Cox-2 was found in colon, breast, stomach, ovary and uterine cervical cancer cells (9,13-16). There have been no reports on the relationship between HuR and Cox-2 expression in colorectal cancer tumors from patients in South Korea.

Colorectal cancer is one of the leading causes of cancer-associated death and the third most common malignant tumor found in patients in Western countries (17). The incidence and the mortality rate of colorectal cancer have also been on the increase in South Korea (18). We investigated the expression and cellular location of HuR and measured Cox-2 expression with the use of immunohistochemical methods. The biological implications of HuR localization and Cox-2 expression in colorectal carcinoma were also evaluated.

Materials and Methods

We collected 79 cases of primary colorectal cancer patients from May 2006 to December 2007 at the East-West Neo Medical Center. The clinical characteristics of patients were evaluated from hospital records. Resected tissue specimens were fixed in 10% buffered formalin and were embedded in paraffin. Histological sections cut from paraffin blocks were stained with hematoxylin and eosin, and all of the stained slides were reviewed. For effective and fast detection, tissue array slides were made. Two cores of representative cancer tissue (2.0 mm in diameter) were obtained from the original paraffin block and the cores were inserted into a new paraffin block with 40 holes. The tissue microarray block also contained three normal colorectal cores. Serial sectioned slides were produced and hematoxylin and eosin staining was performed.

Immunohistochemical procedures were carried out on 4- μ m tissue sections from each tissue microarray block using the Bond Polymer Intense Detection system (Leica Microsystems, North Ryde, NSW Australia) according to the manufacturer's instruction with minor modifications. In brief, 4- μ m sections of formalin-fixed and paraffin-embedded tissues were deparaffinized by the use of Bond Dewax Solution (Leica Microsystems), and an antigen retrieval procedure was performed using Bond ER solution (Leica Microsystems) for 30 minutes at 100°C. Endogenous peroxidase was quenched by incubation with hydrogen peroxide for five minutes. Sections were incubated for 15 minutes at ambient temperature with a monoclonal COX-2 antibody (1 : 400 dilution; Dako, Glostrup, Denmark) and a monoclonal HuR antibody (3A2,

1 : 300 dilution; Zymed, South San Francisco, CA), respectively, using biotin-free polymeric horseradish peroxidase (HRP)-linker antibody conjugate system in a Bond-max automatic slide stainer (Leica Microsystems). As a positive control for Cox-2 expression, we used a human colon carcinoma cell line known to overexpress Cox-2. When more than 10% of the cancer cells showed cytoplasmic staining for Cox-2, the tumor was judged as positive for Cox-2 expression. We observed normal breast glands as a positive control for HuR staining. We evaluated the percentage of positive cells and intensity of staining for the evaluation of the HuR staining status. The percentage of positive cells was scored as follows: 0 (0% positive cells), 1 (< 10% positive cells), 2 (10 to < 50% positive cells), 3 (50 to < 80% positive cells), 4 (> 80% positive cells). The intensity of cytoplasmic and nuclear staining was scored as follows: 0 (negative staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining). For the immunoreactive score, values from 0 to 12 were multiplied by the percentage of the positive cell score and intensity of the staining score. We classified the cases as having negative or weak expression when the immunoreactive score was 0 to 5; a score from 6 to 12 was regarded as strong expression.

All statistical analyses were performed using MedCalc 9.6.0.1 statistical software. The relationships between variables were studied using the chi-squared test. A probability value below 0.05 was considered as statistically significant.

Table 1. Characteristics of patients

	Characteristics	No. of patients (%)
Age	≤ 63.5	40 (50.6)
	> 63.5	39 (19.4)
Sex	Male	48 (60.8)
	Female	31 (39.2)
Site	Rectal	35 (44.3)
	Non-rectal	44 (55.7)
Differentiation	Well	4 (5.1)
	Moderate	72 (91.1)
	Poorly	3 (3.8)
Lymphatic invasion	Present	21 (26.6)
	Negative	58 (73.4)
Vascular invasion	Present	12 (15.2)
	Negative	67 (84.8)
pT	1	2 (2.5)
	2	7 (8.9)
	3	53 (67.1)
	4	17 (21.5)
pN	0	37 (46.8)
	1	21 (26.6)
	2	21 (26.6)
pM	0	72 (91.1)
	1	7 (8.9)

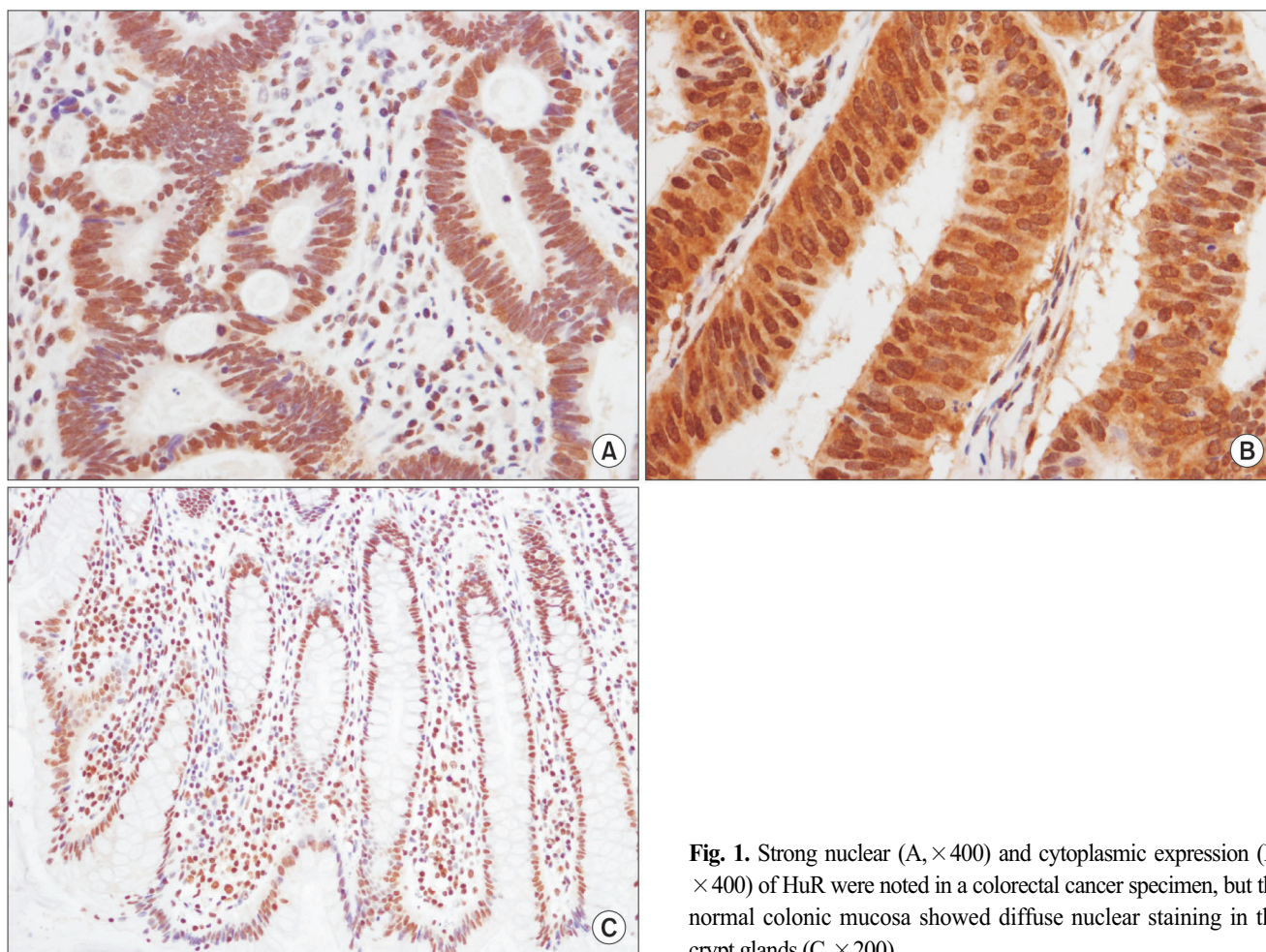


Fig. 1. Strong nuclear (A, $\times 400$) and cytoplasmic expression (B, $\times 400$) of HuR were noted in a colorectal cancer specimen, but the normal colonic mucosa showed diffuse nuclear staining in the crypt glands (C, $\times 200$).

Results

The mean and median age of the patients at the time of the initial diagnosis was 62.4 years (age range, 39~85 years) and 63.5 years, respectively. Of all of the patients, 48 (60.8%) were male. In the study population, 67.1% of patients had moderately differentiated tumors, 5.1% of the patients had well differentiated tumors and 3.8% of the patients had poorly differentiated tumors. Of the 79 patients, 35 patients (44.3%) had a rectal cancer while 44 (55.7%) patients had a non-rectal colon cancer. A lymph node metastasis was present in 42 patients (53.2%). Baseline patient characteristics are shown in Table 1. HuR expression in the nucleus and cytoplasm of cancer cells was present in 59 (74.7%) and 25 (31.6%) cases, respectively (Fig. 1A, B). As shown in Table 2, cytoplasmic expression of HuR significantly correlated with Cox-2 expression ($p=0.004$) and lymphatic invasion ($p=0.027$). Other clinicopathological factors such as age, sex, site, differentiation, vascular invasion and TNM stage showed no significant correlation with HuR cytoplasmic expression. For nuclear expression of HuR, we could not observe any association between nuclear expression of HuR and the

clinicopathological factors (Table 2). Cox-2 expression was seen in 42 (53%) colorectal cancer cases (Fig. 2). The expression of Cox-2 in the adjacent normal colorectal mucosal epithelium was very weak or negative. Cox-2 expression showed a significant correlation with differentiation ($p=0.027$). Cox-2 expression increased with a higher T stage, but the finding was not statistically significant ($p=0.051$). Other clinicopathological factors examined showed no significant correlations (Table 3).

Discussion

In this study, we demonstrated that colorectal cancer showed predominantly nuclear expression (74.7%) of HuR, and increased cytoplasmic expression of HuR was significantly associated with increased lymph node metastasis ($p=0.027$) and lymphatic invasion ($p=0.025$), whereas nuclear expression of HuR showed no association with the examined clinicopathological parameters. Furthermore, we found that cytoplasmic expression of HuR was

Table 2. Cytoplasmic and nuclear expression of HuR and association with clinicopathological factors

	HuR cytoplasmic expression		p	HuR nuclear expression		p
	Positive	Negative		Positive	Negative	
Age			0.217			0.953
≤ 63.5	16	24		32	8	
> 63.5	22	17		30	9	
Sex			0.515			0.642
Male	25	23		39	9	
Female	13	18		23	8	
Site			0.544			0.986
Rectal	15	20		27	8	
Non-rectal	23	21		35	9	
Differentiation			0.127			0.505
Well	1	3		4	0	
Moderate	34	38		56	16	
Poorly	3	0		2	1	
Lymphatic invasion			0.025			0.064
Positive	15	6		13	8	
Negative	23	35		49	9	
Vascular invasion			0.278			0.409
Positive	8	4		11	1	
Negative	30	37		51	16	
pT			0.091			0.114
1	0	2		2	0	
2	4	3		5	2	
3	22	31		45	8	
4	12	5		10	7	
pN			0.027			0.656
0	12	25		30	7	
1	14	7		17	4	
2	12	9		15	6	
pM			0.916			0.995
Positive	3	4		6	1	
Negative	35	37		56	16	
Cox-2			0.004			0.176
Positive	27	15		30	12	
Negative	11	26		32	5	

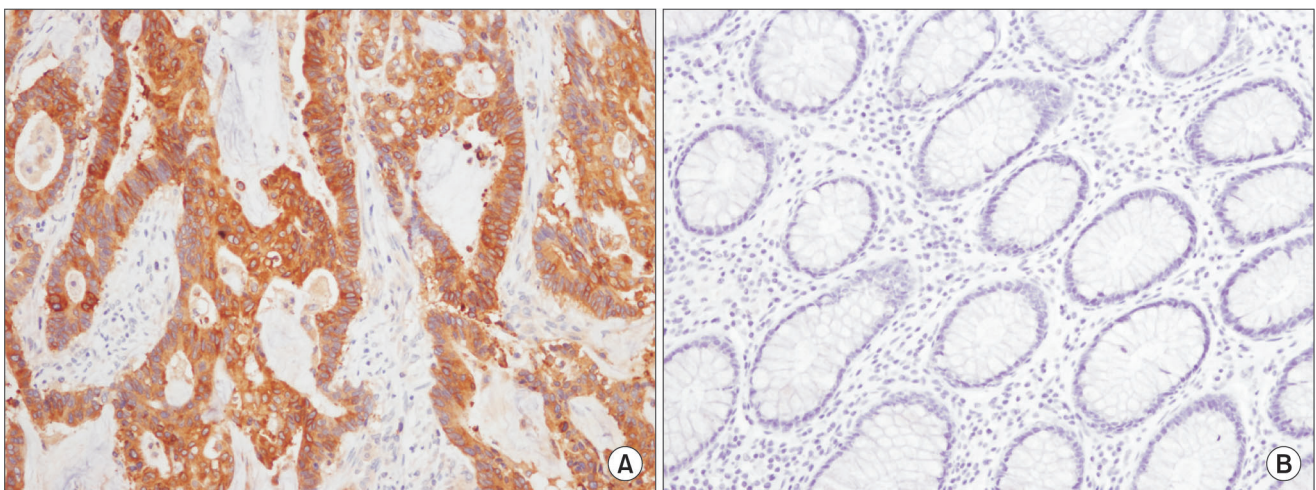
**Fig. 2.** Diffuse strong cytoplasmic expression of Cox-2 for an adenocarcinoma in the colon (A, $\times 200$) was seen, and no staining was seen in the normal mucosa (B, $\times 200$).

Table 3. Cox-2 expression and association with clinicopathological factors

	Cox-2 expression		p
	Positive	Negative	
Age			0.729
≤ 63.5	20	20	
> 63.5	22	17	
Sex			0.650
Male	27	21	
Female	15	16	
Site			0.685
Rectal	20	15	
Non-rectal	22	22	
Differentiation			0.027
Well	0	4	
Moderate	39	33	
Poorly	3	0	
Lymphatic invasion			0.864
Positive	12	9	
Negative	30	28	
Vascular invasion			0.580
Positive	5	7	
Negative	37	30	
pT			0.051
1	0	2	
2	2	5	
3	27	26	
4	13	4	
pN			0.314
0	17	20	
1	14	7	
2	11	10	
pM			0.158
Positive	6	1	
Negative	36	36	
Cytoplasmic HuR expression			0.004
Positive	27	11	
Negative	15	26	
Nuclear HuR expression			0.176
Positive	30	32	
Negative	12	5	

significantly associated with Cox-2 expression. Our results are in accord with the previous studies that reported that the HuR protein translocates from the nucleus to the cytoplasm during tumorigenesis (13-16). Eleven cases of negative Cox-2 expression showed positive cytoplasmic HuR expression in our study. This finding suggests that the possibility of other additional regulatory mechanisms for Cox-2 expression exist. Even though the regulation of mRNA stability of Cox-2 is thought to be an important regulatory step, other mechanisms such as transcriptional control can also exist. Further large-scale studies are needed for the determination of the mechanism of regulation of Cox-2 expression and stabilization of Cox-2 mRNA in cancer cells.

Although the ability of HuR to shuttle from the nucleus to cytoplasm is important for mRNA stabilization, the exact mechanism is not known. Several mechanisms for controlling the cellular location have been studied. Recent studies have shown that mitogenic-activated protein kinase-2 increased cytoplasmic translocation of HuR and the stability of ARE-containing mRNAs (19). The translocation of HuR to the cytoplasm can be induced by stress caused by agents such as UV light, DNA damaging agents or T cell activation, while AMP-activated kinase can inhibit the translocation of HuR to the cytoplasm (20-22).

There are some other mRNAs, such as the mRNAs for tumor necrosis factor α , cyclin A, cyclin B1, MARCKS, uPA and uPA receptor that are stabilized by HuR (23-25). Based on the known functions of HuR, we believe that HuR might play an important role in cell cycle regulation, apoptosis, angiogenesis, inflammation, and tumor growth. Moreover, HuR can be a potential target for molecular tumor therapy with consideration of these multiple effects.

Conclusion

The cytoplasmic expression of HuR may be a part of a regulatory pathway (s) that controls the expression of Cox-2 in colorectal cancer. Additional studies with a larger number of specimens are required to determine if HuR might be a potential target for tumor therapy.

References

- Chen CY, Shyu AB. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem Sci.* 1995;20:465-70.
- Fan J, Yang X, Wang W, Wood WH 3rd, Becker KG, Gorospe M. Global analysis of stress-regulated mRNA turnover by using cDNA arrays. *Proc Natl Acad Sci USA.* 2002;99:10611-6.
- Frederickson RM. Fluidigm. Biochips get indoor plumbing. *Chem Biol.* 2002;9:1161-2.
- Ma WJ, Cheng S, Campbell C, Wright A, Furneaux H. Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *J Biol Chem.* 1996;271:8144-51.
- Fan XC, Steitz JA. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J.* 1998;17:3448-60.
- Gallouzi IE, Steitz JA. Delineation of mRNA export pathways by the use of cell-permeable peptides. *Science.* 2001;294:1895-901.
- Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3'-untranslated region. *J Biol Chem.* 2000;275:11750-7.
- Ristimäki A, Narko K, Hla T. Down-regulation of cytokine-induced cyclo-oxygenase-2 transcript isoforms by dexamethasone: evidence for post-transcriptional regulation. *Biochem J.* 1996;318:325-31.
- Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, et al. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J Clin Invest.* 2001;108:1657-65.
- Tatsuguchi A, Matsui K, Shinji Y, Gudis K, Tsukui T, Kishida T, et al. Cyclooxygenase-2 expression correlates with angiogenesis and apoptosis in gastric cancer tissue. *Hum Pathol.* 2004;35:488-95.
- Tsuji M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA.* 1997;94:3336-40.
- Sutton KM, Wright M, Fondren G, Towle CA, Mankin HJ. Cyclooxygenase-2 expression in chondrosarcoma. *Oncology.* 2004;66:275-80.
- Do SI, Do IG, Kim GY, Lee S, Kim YW, Park YK, et al. Correlation between cyclooxygenase-2 expression and HuR cytoplasmic translocation of breast cancer. *Korean J Pathol.* 2008;42:75-80.
- Mrena J, Wiksten JP, Thiel A, Kokkola A, Pohjola L, Lundin J, et al. Cyclooxygenase-2 is an independent prognostic factor in gastric cancer and its expression is regulated by the messenger RNA stability factor HuR. *Clin Cancer Res.* 2005;11:7362-8.
- Denkert C, Weichert W, Pest S, Koch I, Licht D, Köbel M, et al. Overexpression of the embryonic-lethal abnormal vision-like protein HuR in ovarian carcinoma is a prognostic factor and is associated with increased cyclooxygenase 2 expression. *Cancer Res.* 2004;64:189-95.
- Lim SJ, Kim HJ, Kim JY, Park K, Lee CM. Expression of HuR is associated with increased cyclooxygenase-2 expression in uterine cervical carcinoma. *Int J Gynecol Pathol.* 2007;26:229-34.
- Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. *CA Cancer J Clin.* 2005;55:10-30.
- Korea National Statistical Office. Death by cause. www.nso.go.kr: Korean Statistical Information Service (KOSIS).
- Subbaramaiah K, Marmo TP, Dixon DA, Dannenberg AJ. Regulation of cyclooxygenase-2 mRNA stability by taxanes: evidence for involvement of p38, MAPKAPK-2, and HuR. *J Biol Chem.* 2003;278:37637-47.
- Wang W, Furneaux H, Cheng H, Caldwell MC, Hutter D, Liu Y, et al. HuR regulates p21 mRNA stabilization by UV light. *Mol Cell Biol.* 2000;20:760-9.
- Atasoy U, Watson J, Patel D, Keene JD. ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is upregulated during serum stimulation and T cell activation. *J Cell Sci.* 1998;111:3145-56.
- Wang W, Fan J, Yang X, Fürer-Galban S, Lopez de Silanes I, von Kobbe C, et al. AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol.* 2002;22:3425-36.
- Wang W, Caldwell MC, Lin S, Furneaux H, Gorospe M. HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. *EMBO J.* 2000;19:2340-50.
- Wein G, Rössler M, Klug R, Herget T. The 3'-UTR of the mRNA coding for the major protein kinase C substrate MARCKS contains a novel CU-rich element interacting with the mRNA stabilizing factors HuD and HuR. *Eur J Biochem.* 2003;270:350-65.
- Tran H, Maurer F, Nagamine Y. Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen-activated protein kinase-activated protein kinase 2. *Mol Cell Biol.* 2003;23:7177-88.