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Original Research Article

Role of mRNA Binding Protein (HuR) Expression Level in Cancer Cells

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Abstract

HuR is a mRNA-binding protein. Intracellular localization of HuR is mainly found within nucleus, but it could be translocate between the nucleus and cytoplasm. In the cytoplasm HuR canincrease half-life of certain mRNA target. Since cytoplasmic localization of HuR is essential for its activity, thus, HuR translocation in malignant cells could have prognostic indication. In the present study we aimed to evaluate the significance importance of HuR in the aggressiveness ofcolorectal adenocarcinoma. To achieve this goal, we have investigated itsexpression level in adenocarcinoma sample from Iraqi patients, 7through linking its expression with tumor histopathological variables (stage, grade, grade, and lymph node involvement), by using Immunohistochemical staining method. Study done on 40 colorectal cancer samples and their respective resection margins. Present study demonstrated that, the positive expression rate of integrin HuR in non-tumor colorectal mucosa was significantly lower than that of the colorectal cancer (CRC) tissue (P<0.005). Moreover, when CRC samples breakdown according to histopathological variables, significant differences in expression level of HuR protein when compared with different tumor stage, grade, and LN involvement depending on mean expression ±SE value (P< 0.05, P< 0.05, and p<0.05 respectively). Our results show that high cytoplasmic HuR expression is associated with a poor histologic differentiation, large tumor size, and poor prognosis in colorectal adenocarcinoma.

Key words : Colon cancer, HuR, prognosis, tumor progression, HuR translocation, IHC, histopathology.

الخلاصة

HuR هو بروتين ملازم للحامض النووي الرايبوزي الناقل. الموضع الخلوي لهذا البروتين يكون داخل النواة بصوره اساسيه، إلا أنه يمكن ينتقل بين النواة والسيتوبلازم .في السيتوبلازم HuRله القدرة على زيادة نصف عمر الحامض النووي الناقل الذي يرتبط به . لكون التموضع السايتوبلازميلHuR ضروري لفعاليته الخلوية، وبالتالي، فأن الانتقال الداخل خلوي من النواه للسايتوبلازملHuR في الخلايا السرطانيه قد يكون له اهميه معنويه في تحديد مأل المرض. نهدف من خلال الدراسةالحالية لتحديد ف الأهميةالمعنويةلـ HuR في ضراوة الورم القولونيالمستقيمي. ولتقيق هذا الهدف فقد قمنا بقياس التعبير الموضعي لهذا البروتين في الخلايا السرطانيه لـ 40 عينه من للمرضى العراقيين باستخدام تقنيه التصبيغ المناعي النسيجي من خلال مقارنه قيمه التعبير الموضعي لهذا البروتين مع التغيرات النسيجيةالمرضية للورم. علما انه تم استخدام حافه النسيج المرضي الخاليه من الورم كمجموعه سيطرة.اظهرت الدراسةالحالية ان معدل التعبير الايجابي لـ HuR في مجموعه السيطرة هوه اقل وبصوره معنويه بالمقارنة مع النسيج المرضي (P<0.005). بالإضافة الى ذالك عندما قورن التعبير الخلوي لـ HuR مع التغييرات المرضية للورم القولونيالمستقيمي وجدت هنالك فروقات ذات دلاله إحصائية معنويه في مستوى تعبير هذا البروتين بالمقارنة مع مراحل تطور الورم ، مستوى ظراوه الورم ، و انتشار الورم للعقد اللمفاوية (P<0.005، P<0.005، و P<0.005 على التوالي). كانت الاستنتاجات من هذه الدراسة ان التعبير الخلوي المرتفع لـ HuR له علاقه بالتميز النسيجي الردئ ، حجم الورم، والتوقع السيئ لمأل المرض.

الكلمات المفتاحية:سرطان القولون ، مأل الورم، تطور الورم ، التصبيغ المناعي النسيجي.

Introduction

Colorectal cancer is one of common malignancies in the world. The incidence of colorectal cancer ranks 3rd among malignancies, and the mortality is only inferior to that of lung cancer, gastric cancer and liver cancer [1]. Distant metastasis is the main cause of death in patients with colorectal cancer, in which liver metastasis is the most common. The liver metastasis rate of colorectal cancer is up to 20-70%, in which 1/3 is with simple liver metastasis [2]. Therefore, to further study the mechanism of liver metastasis of colorectal cancer, and seek more accurate predictors of liver metastasis are particularly important for establishment of more targeted and individualized prevention, treatment and follow-up measures. Traditional clinical and pathological indicators for prognosis of colorectal cancer include tumor invasion [3], Aberrant expression of gene products is a general feature of tumorigenesis. Gene expression can be regulated at the level of DNA, RNA or the protein itself. RNA regulation occurs through alterations in translational efficiency and in mRNA stability. mRNA stability depends on cis-elements in the RNA and trans-acting factors. A well-studied mRNA instability element is the AU-richelement (ARE) in the 3' untranslated region (3'UTR). HuR, amember of the Hu/ELAV (embryonic lethal abnormal vision family of RNA-binding proteins, can bind ARE-containingmRNAs through its RNA recognition motif. It is postulated thatHuR binds these mRNAs in the nucleus and accompanies theminto the cytoplasm (i.e. nucleocytoplasmic translocation), therebyprotecting the mRNA from degradation, and affecting the proteinexpression levels of target genes [4]. It has been hypothesized that HuRcontributes to neoplastic growth by regulating expression of genesinvolved in carcinogenesis that harbor an ARE in the 3'UTR,such as COX-2, β-catenin and vascular endothelial growth factor (VEGF) [5,6,7]. HuR is known to be an important post-transcriptionalregulator of VEGF. Although the native half-life of VEGFmessenger RNA (mRNA) is under 1 h, the half-life mayincrease by 2.5–8-fold when VEGF mRNA is bound byHuR. [8,9]. This finding, combined with the observation thatHuR expression is augmented in times of hypoxic stress,supports the hypothesis that HuR may be an upstream mediator of tumor angiogenesis [10,11].Although no HuR mutations have been found to be associated with cancer, links between HuR and malignancies of breast, colon, lung, and ovary have beensuggested [12]. Importantly, it has been shown in small series that HuR expression tends to correlate with degree of transformation, that is, levels of HuR are lower in normal mucosa than in adenomas, which in turn have lower levels ofHuR expression than carcinomas [12].We were, therefore, interested in determining whether the expression pattern of HuR in colorectal tumors correlated with histological type or grade.In this set of experiments we attempted to investigate whether high cytoplasmic HuR signal was predictive of poor prognosis, as it has been shown in other cancer models.

Patients And Methods

Patients and Sampling

Forty patients with colorectal adenocarcinoma, who were confirmed histopathologicaly, were included in this study. Their age were ranged from 20- 80 years. Paraffin embedded blocks of tumor and resection margins were retrieved along with the histopathological report of each patient from histopathological laboratory. For staging of the cancer, astler-coller staging system was adopted in this study [13]. In addition, resection margins were confirmed again to be free of malignancy. Adequate thin paraffin embedded sections (5μm thick) of tumor and resection margins were prepared on positively charged slides

for the immunohistochimistrey Technique (IHC).

Immunohistochemical Detection of HuR

A primary monoclonal antibody against HuR(US biological, USA) reacts with it's antigen. biotinylated secondary antibody then reacts with the primary antibody. This is followed by the attachment of an enzyme-conjugated streptavidin to the biotins on the secondary antibody. The enzyme converts a substrate to a colored reaction product. And the procedude has been done according to manufactural instructions (US biological, USA).There after slides were examined by histopathologist under light microscope (40x).

Results

HistopathologicalData

Forty patients with colorectal adenocarcinoma were investigated. According to the histological differentiation, tumors were broken down in to three groups, well differentiated (WD, n=10), moderately differentiated (MD, n=20), and poorly differentiated (PD, n=10), moreover, patients were further grouped according to their histopathological criteria, as follow: tumor stage (A, n=9, B, n=10, C, n=11, and D, n=10), lymph node involvement (free, n=19, and involved, n=21).

Tumor sites versus resection margins

Based on immunohistochemical staining method, the present study found that, there were significant difference in the mean expression level of HuR protein between tumor sites and their resection margins (79.45714± 2.29, 32.4± 1.39), respectively (P<0.005),(table 1). A noteworthy here is that high intensity of staining is found in the periphery of necrotic foci of the colorectal cancer, labeling with the HuR antibody distinctly intensified in tumor epithelium adjacent to the necrotic foci. HuR, IHC staining in tumor sample and their resection margin are shown in figure 1.

Correlation among protein expression of HuR with different histopathological variables

HuR proteinexpression in colorectal adenocarcinoma was analyzed against the different histopathological features of the tumors based on Spearman's statistical correlation. As shown in Table 2, 3, and 4, current study demonstrated that there were significant differences in expression level of HuR protein when compared with different tumor stage, grade, and LN involvement depending on mean expression ±SE value (P< 0.05, P< 0.05, and p<0.05 respectively).

Table 1: HuR, protein expression in tumor sites and their resection margins, based on *t.test*

Sample	No.	HuR Mean ± SE
Normal	40	**32.4± 1.392040868**
Tumor	40	**79.45714± 2.29370**
*** (P<0.005).**		

Table 2: Expression level of HuR protein along different stage group

Stage	No.	Mean ± SE
		HuR
A	9	**55.16 ± 1.99**
B	10	**74.40 ± 1.62**
C	11	**86.82 ± 1.49**
D	10	**94.87 ± 0.74**
*** (P<0.05).**		

Table 3: Expression level of HuR protein along with different tumor grade

Grade	No.	Mean ± SE
		HuR
WD	10	**62.00 ± 4.47**
MD	20	**78.79 ± 2.72**
PD	10	**92.00 ± 2.06**
*** (P<0.05).**		

Table 4: Effect of LN involvement on HuR expression level

LN	No.	Mean ± SE
		HuR
Free	19	**67.18 ± 2.69**
Involved	21	**90.21 ± 1.29**
*** (P<0.05).**		

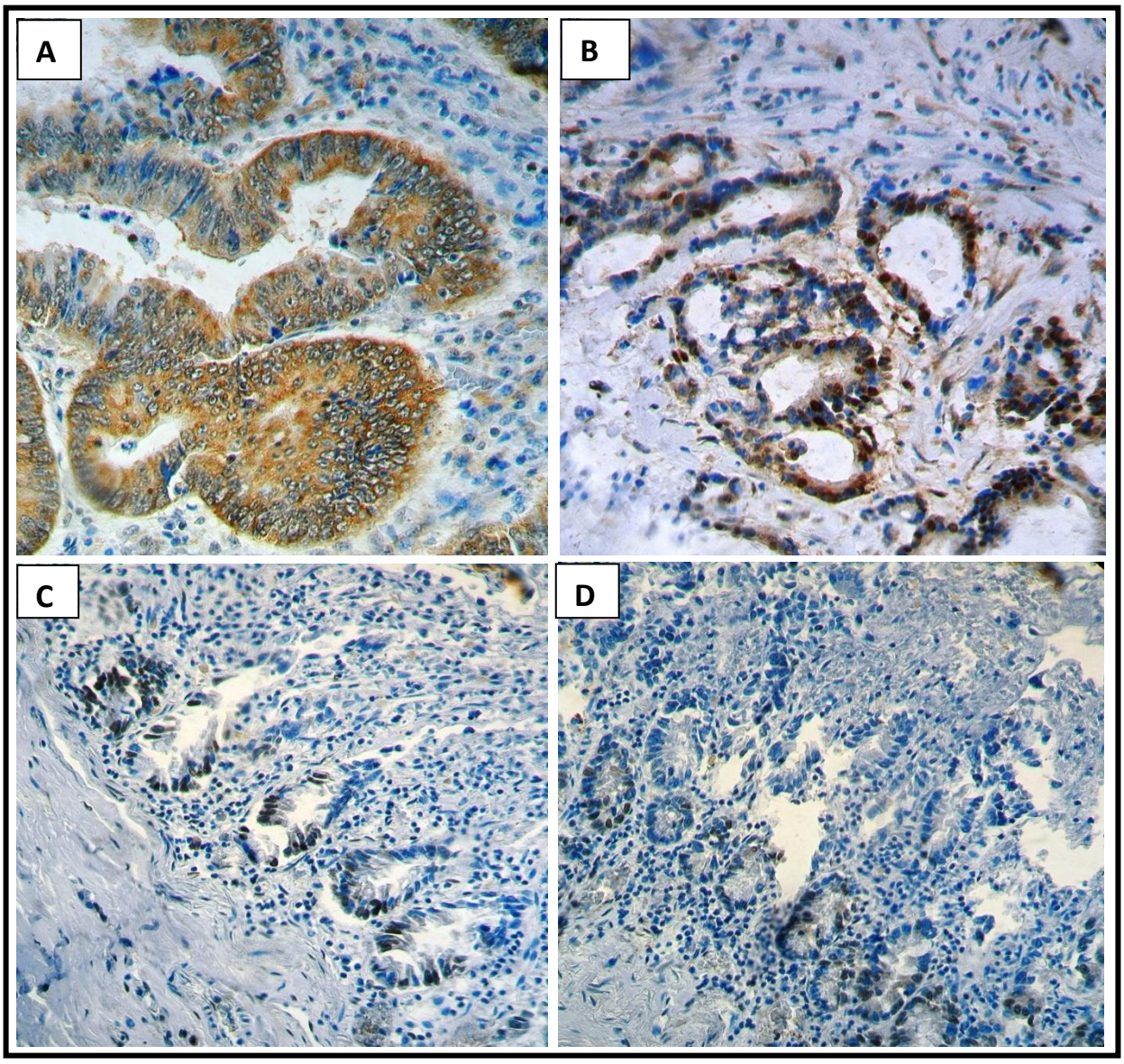

Figure1: Immunohistochemichal staining of HuR in colorectal adenocarcinoma section and their resection margins by DAP (brown color) counterstained with hematoxylin. (A) HuR protein expression in high grade tumor cells which show predominant cytoplasmic staining. (B) HuR protein expression in low grade tumor cells which show nuclear stain. (C and D) Resection margin stained with HuRaintegrin. Magnification power (40X).

Discussion

HuR expression was investigated in normal colorectal mucosa, and patients with C colorectal adenocarcinomas. We found increasing cytoplasmic HuRimmunoreactivity and nucleocytoplasmic translocation of HuR in tumor tissue compared with their respective resection margins (79.45714± 2.29, 32.4± 1.39; P<0.005) However, when HuR expression was analyzed according different tumor histopathological variables, current study showed that HuRprotein expression rate was significantly associated with tumor stage, poor differentiation, and lymphoid node invasion, ($P< 0.05$, $P< 0.05$& $P< 0.05$respectively), in addition to that we demonstrated a cytoplasmic immuno-reactivity or translocation gradually increased subsequently along with tumor grade from well differentiated in to poorly differentiated (figure 1), also we noticed same translocation associated with tumor stage progression,and lymph node metastasis.Whereas, nuclear expression of

HuR showed to be limited to tumor free resection margin, and tumor with low grade.Our results are in accord with the previous studies that reported that the HuR protein translocates from the nucleus to the cytoplasm during tumorigenesis [14-16].Denkert et al. found a gradual increase of cytoplasmic HuR expression in subsequent Dukes stages: 38% of Dukes A carcinomas and 50% of Dukes B carcinomas, 58% of Dukes C carcinomas and 85% of Dukes D carcinomas exhibited cytoplasmic HuR expression.8 Taken together, cytoplasmic HuR expression appears associated with progression to metastasis and poor prognosis [17].

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. Some studies have shown that mitogenic-activated protein kinase-2 increased cytoplasmic translocation of HuR and the stability of ARE-containing mRNAs [18]. 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 [19-21]. There are some other mRNAs, such as the mRNAs for tumor necrosis factor α, cyclin A, cyclin B1, VEGF, and uPAreceptor that are stabilized by HuR [22-24]. 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 some mRNA target in colorectalcancer. There are several hundred putative targets for HuR, relatively few of these interactions are well characterized.We elected to investigate the interaction between HuR and VEGF. Additional studies with a larger number of specimens arerequired to determine if HuR might be a potential target for tumorcontrol.

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