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HuR and TTP:

Two RNA Binding Proteins That Deliver Message From the 3' End

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Colorectal carcinoma (CRC) is a leading cause of cancer deaths worldwide, and identification of new therapeutic targets remains an important endeavor.¹ In this regard, molecular analyses regulating tumorigenesis have identified three classes of molecules broadly classified as receptors and signal transduction molecules (eg, epidermal growth factor receptor, vascular endothelial growth factor receptor, Ras),^{2,3} transcription factors (peroxisome proliferator activated receptor- γ , nuclear factor [NF]- κ B),^{4,5} and inflammatory mediators (interleukin [IL]-6, IL-8). A central player in all these activities is cyclooxygenase-2 (COX-2), which catalyzes the key step in the conversion of free arachidonic acid to prostaglandins. Many studies have demonstrated that unregulated COX-2 expression is a rate-limiting step that occurs early in carcinogenesis. However, many side effects (eg, gastrointestinal ulceration and bleeding, platelet dysfunctions) have been ascribed to the COX-2-specific inhibitors. Consequently, the notion that down-regulating COX-2 gene regulation will provide therapeutic effects similar to the inhibitors without causing the unwanted side effects is appealing and is likely to have important therapeutic implications.

Most of the studies on COX-2 expression have dealt with transcriptional regulation through elements in the COX-2 promoter (Figure). For example, the β -catenin/TCF complex in the presence of Wnt signaling induces COX-2 transcription.^{6,7} Similarly, hypoxia induces COX-2 through the activation of NF- κ B.^{8,9} Other factors such as C-EBP have also been shown to regulate COX-2 gene transcription.^{10,11} However, the level of steady-state mRNA is governed by a balance between gene transcription and mRNA degradation. Posttranscriptional regulation at the level of mRNA decay and translation is often aberrant in cancer cells, and is a hallmark of COX-2 expression.¹²

Many oncogenes, growth factor, cytokine, and cell-cycle genes are regulated at the posttranscriptional level. Immediately after transcription, the native mRNAs undergo splicing. In addition, they are modified to include a 7-methylguanosine cap structure at the 5' end and a poly(A) tail at the 3' end, both of which increase stability of the transcript.¹³ In addition, continuing translation enhances mRNA stability, whereas premature and aberrant translation results in termination or impaired translation initiation, which promotes mRNA degradation. The major mechanism is by controlling the rate of mRNA turnover for transcripts bearing

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destabilizing *cis*-elements. One such element, present in COX-2 3' untranslated region (UTR) is called AU-rich element ARE, which consists of AUUUA repeats and is broadly conserved in the human and mouse COX-2 3'UTRs (76% identity over 116-nt).¹⁴

To date, only a handful of regulatory factors have been identified that seem to control a large pool of target mRNAs, suggesting that a slight perturbation in the control mechanism may generate wide-ranging effects that could contribute to the development of a complex disorder such as cancer. ARE binding proteins (ARE-BPs) bind the AU-rich or U-rich sequences in the 3'UTR to regulate localization, translation and degradation of mRNAs encoding critical growth-response genes, cytokines, and cell-cycle regulatory proteins.¹⁵ At least 5 ARE-BPs have been identified that bind to the COX-2 ARE and affect stability and translation of COX-2 mRNA, including HuR, TIA-1, CUGBP2, RBM3, and Tristetraprolin (TTP; Table).¹⁶⁻²⁴ HuR, which was first identified as tumor antigens in lung carcinomas of individuals with paraneoplastic neurologic disorder, is ubiquitously expressed.²⁵ Dixon et al²⁶ have previously demonstrated that HuR overexpression increases the stability of COX-2, VEGF, and IL-8 mRNAs.²⁶ Moreover, HuR overexpression in LoVo cells increased COX-2 protein and enhanced the expression of a reporter gene containing the COX-2 3'UTR.²⁶ Young et al²⁷ have now cast light on the expression of HuR and TTP. The TTP protein has been shown to reduce granulocyte-macrophage colony-stimulating factor mRNA stability via deadenylation, and also binds the COX-2 3'UTR.^{28,29} Sawaoka et al²⁹ identified 2 COX-2 mRNAs in HCA-7 human CRC cell line differing in the length of their 3'UTRs. The longer one binds TTP and is repressed more rapidly than the shorter one that does not bind TTP. Thus, the shorter COX2 mRNA sustains a stronger COX2 expression.²⁹ Other RNA binding proteins have also been demonstrated to bind to COX-2 3'UTR, but the functional significance of the interactions is currently not known (Table 1).

In this issue of *GASTROENTEROLOGY*, Young et al²⁷ have determined the expression of HuR and TTP during colon carcinogenesis.²⁷ They demonstrate that HuR levels were increased, whereas TTP levels were reduced as early as in adenomas, which correlated well with expression of COX-2. Furthermore, there was an increase in the cytoplasmic localization of HuR. These confirm previous studies demonstrating that cytoplasmic localization rather than a significant overall increase in HuR expression is important in increasing the translation of ARE-containing mRNAs. This is also reminiscent of our work demonstrating that demonstrated increased CUGBP2 expression and subsequent cytoplasm localization of the protein.³⁰ However, although HuR increased COX-2 mRNA translation, CUGBP2 was demonstrated to reduce the translation of COX-2 mRNA.

Thus, direct evidence is provided for HuR- and TTP-mediated regulation of COX-2 in this manuscript. When HuR levels go up, TTP levels are reduced. TTP is the only protein currently known to reduce COX-2 mRNA stability, which could be the reason for the reduced mRNA translation. Katsanou et al³¹ have demonstrated that HuR does not alter levels of target cytokine mRNAs in the absence of the destabilizing functions of TTP but that synergizes with the translational silencer TIA-1 to reduce the translation of these mRNAs.³¹ Similarly, Sureban et al³² showed that HuR and CUGBP2 interact and bind to COX-2 mRNA with a 1:1 stoichiometry, and this leads to translation inhibition. Furthermore, HuR interactions with another RNA-binding protein RBM3 significantly enhanced the translation of COX-2 mRNA. Hence, it should be noted that the context of HuR binding to the ARE sequences is essential for its translation function.

Demonstration of a reduction in TTP levels also emphasizes the need for more investigation of the role of the HuR, TTP and other RNA binding proteins in the field of gastrointestinal cancers. Clearly, COX-2 is a major factor in CRC. However, there are multiple transcripts that encode AREs in their 3'UTR. A question that remains is whether these proteins function in a

similar manner with other such transcripts. Although some evidence suggests that these proteins also regulate inflammatory factors, it is sparse and further studies are warranted, especially in inflammation-mediated tumorigenesis. Also, the current study did not provide any evidence of mechanisms by which expression of HuR and TTP are regulated. The authors have alluded to promoter regions in the genes that encode these 2 proteins. An interesting observation is that the promoter for TTP consists of CpG islands, something we have observed in one of the promoters that encode CUGBP2 (Ramalingam S, Anant S, unpublished observations). Although the significance of this is currently unknown, it begs the question of whether factors that negatively affect COX-2 expression have similar regulatory mechanisms to silence their expression. Addressing this issue requires additional experiments and have potentially far-reaching implications at the basic science level, in addition to COX-2 in colon cancer.

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Figure. Differential modulation of COX-2 mRNA translation by HuR and TTP. After transcription of the COX-2 gene, which can be induced by the actions of transcription factors NF- κ B, C/EBP and β -catenin/TCF-4, the transcript undergoes capping, splicing and polyadenylation, after which it is transported to the cytoplasm presumably by proteins involved in trafficking. Once in the cytoplasm, the transcript undergoes normal translation and subsequent degradation. ARE sequences in the 3' UTR assume a stem loop structure and interact with ARE-BPs to modulate the stability and translation. In this issue, Young et al demonstrate that in colon cancers HuR levels is increased resulting in increased HuR binding to COX-2 mRNA and consequent increase in stability and translation of COX-2 mRNA. In contrast, expression of TTP reduces in colon cancers, TTP binding would increase mRNA decay, which has been previously shown to occur through exonuclease-mediated deadenylation.

Table 1

RNA Binding Proteins That Interact With COX-2 3' UTR

RNA Binding Protein	Known mRNA function	Regulate COX-2 mRNA stability	Regulate COX-2 mRNA translation	Ref
HuR	Increase Stability and Translation	?	?	26
CUGBP2	Increase Stability and inhibits Translation	?	?	30
RBM3	Increase Stability and increases Translation	?	?	24
TTP	mRNA destabilizing	?	?	21
CPF-A	Increases Stability	?	ND	17
TIA-1	Inhibit translation	ND	ND	22
TIAR	Inhibit translation and alternative RNA processing	ND	ND	17
AUF1	Reduce Stability	ND	ND	17
hnRNP A0	Increase Stability	ND	ND	17
hnRNP A2/B1	Cytoplasmic trafficking	ND	ND	17
hnRNP A3	Cytoplasmic trafficking	ND	ND	17

ND, Not determined