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Characterization of the promoter of *1A6/DRIM*, a novel cancer-related gene and identification of its transcriptional activator

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Abstract

1A6/DRIM (Down-regulated in Metastasis) has been reported to express at a high level in the gastric cancer tissues and the premalignant lesions implicating the involvement of *1A6/DRIM* in cell transformation. Although the information regarding the putative functions and distribution of the *1A6/DRIM* in different tissues and cell lines has been increasing recently, its promoter and promoter-regulating factors remain unknown. In this study, the transcription initiation site of *1A6/DRIM* was confirmed to be located at 147 bp upstream of the ATG codon using the primer extension analysis. The minimal promoter region of the *1A6/DRIM* is located between −47 and +42 of the transcription initiation site measured by luciferase reporter assays using a set of deletion constructs. In addition, an E-box is shown to be an essential element for transcriptional regulation of *1A6/DRIM* demonstrated by luciferase assay with different deletion and mutation constructs. Finally, a transcription factor, upstream stimulatory factor 2 (USF2) was found to be an activator of the *1A6/DRIM* through binding to the E-box demonstrated by luciferase reporter assay, electrophoretic mobility shift assay, and chromatin immunoprecipitation (ChIP) assay. The structural analysis of the *1A6/DRIM* promoter and the identification of its potential regulatory effecter may help us to understand its biological functions in regulating cancer development.

Keywords: Transcriptional regulation; E-box; Upstream stimulatory factor; Tumorigenesis

1. Introduction

DRIM (Down-regulated in Metastasis) (NC000012) gene spans approximately 106 kb in length and consists of 62 exons and 61 introns. The *1A6* gene was originally identified from a fetal gastric epithelial cell line GES-1, named *Key-1A6* (AF072718), using differential display technology (Ke et al., 1996). The GES-1 was established by infection of the primary fetal epithelial cells with SV40 virus (Ke et al., 1994). The sequence of the *Key-1A6* is 100% identical to the 3′ terminal sequence of *DRIM* (Schwirzke et al., 1998). Therefore, the gene is designated as *1A6/DRIM* in this study. Fluorescent in situ hybridization (FISH) mapped a single *1A6/DRIM* gene locus to human chromosome 12 q23.2–23.3 (Zheng and Ke, 2001). The *1A6/DRIM* gene encodes a protein comprising of 2785 amino acids with significant homology to the protein in *S. cerevisiae* and *C. elegans* (Schwirzke et al., 1998). The immunohistochemical study

Abbreviations: DRIM, down-regulated in metastasis; USF, upstream stimulatory factor; FISH, fluorescent in situ hybridization; EST, expressed sequence tag; SV40, simian virus 40; PCR, polymerase chain reaction; FBS, fetal bovine serum; EMSA, electrophoretic mobility shift assay; DTT, dithiothreitol; ChIP, chromatin immunoprecipitation; PMSF, phenylmethylsulfonyl fluoride; HLH, helix loop helix.

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has shown that 60.8% of gastric cancer tissues expressed *1A6/DRIM* whereas only 12.2% out of adjacent normal gastric epithelium and superficial gastritis were *1A6/DRIM* positive (Liu et al., 2003). The same study also demonstrated that the positive rate of *1A6/DRIM* increased along with the gastric epithelial pathological progression.

It was reported that the expression of *1A6/DRIM* is different among the tissues and cell lines by Northern blot analysis, and it may be associated with metastasis (Schwirzke et al., 1998). Our previous work (Zheng and Ke, 2001) and Goodison's recent report (Goodison et al., 2003) revealed that the *1A6/DRIM* expression and its mRNA level did not correlate with the gene copy numbers. Furthermore, no *1A6/DRIM* amplification was found in gastric cancer tissues by FISH (Zheng and Ke, 2001). These results imply that the activation of *1A6/DRIM* might be regulated at the transcription level. Therefore, understanding how the *1A6/DRIM* is expressed and which factor(s) influences its transcription expression will help us to understand its biological function and its roles in cancer development.

In the present study, we identified the transcription initiation site and the promoter region that is responsible for the basal transcription of *1A6/DRIM*. The transcription factor binding site and the transcriptional activator for *1A6/DRIM* were further determined.

2. Material and methods

2.1. Primer extension analysis

Total RNA was isolated from HeLa and BGC-823 (Deng et al., 1989) cells using Trizol Reagent (Invitrogen) based on the manufacturer's instruction. An antisense oligodeoxynucleotide, 5′ GAA GGG ATG CAA CCG ACA GT 3′ corresponding to the sequence from 43 to 62 bp upstream of the translation start codon (Fig. 2) was labeled with FAM (Beijing AuGCT Biotechnology). Primer extension assay was performed using the superscript III (Invitrogen) as previously described (Dhandayuthapani et al., 1998) with modifications.

2.2. Cloning of the 1A6/DRIM promoter region and construction of a series of luciferase reporter vectors

A 1079-bp putative *1A6/DRIM* promoter region was amplified by PCR using primers based on the genomic DNA sequence of the human *1A6/DRIM* gene. The forward primer (primer 2 listed in Table 1) contained the *Kpn*I restriction enzyme site while the reverse primer (primer 1 in Table 1) carried a *Bgl*II site. The PCR reaction was carried out at 94.5 °C, for 1 min; 57 °C, for 1 min; and 72 °C, for 1 min for total of 35 cycles. The PCR product was purified with glass milk and subcloned into the *Kpn*I/*Bgl*II site in pGL3-Basic reporter vector (Promega, Madison, WI) to obtain pLUC-1037.

The *1A6/DRIM* promoter deletion luciferase reporters were also generated by PCR. The primers were listed in Table 1. The primers 1 and 3 were used for pLUC-847; the primers 1 and 4 for pLUC-642; the primers 1 and 5 for pLUC-439; the primers 1 and 6 for pLUC-247; the primers 1 and 7 for pLUC-90. The primers 14 and 10 were used for pLUC-64; the primers 14 and 11 for pLUC-47; the primers 14 and 12 for pLUC-34. To obtain two luciferase reporter plasmids, which contain the downstream region of transcription start site, a 102-bp (pLUC-10) and a 138-bp (pLUC-147) fragments were amplified, respectively. The primers 14 and 13 were used for pLUC-10 and the primers 8 and 9 for pLUC-147. Two of the pLUC-64 mutants, named pLUC-64-m1 and

Table 1
The primers for series of luciferase reporter constructs

Name	Position	Sequence
Primer1	+23 to +42	5′-gatc aga tct GAT GCC CAG ACT TTC TCA CG-3′
Primer2	−1037 to −1019	5′-gatc ggt acc CGT TTG GCT CCC AGA ACA G-3′
Primer3	−847 to −829	5′-gatc ggt acc ACC AAA CAT TAA CAG CGG T-3′
Primer4	−642 to −624	5′-gatc ggt acc TGG CTC ACA CCT GTA ATC C-3′
Primer5	−439 to −421	5′-gatc ggt acc GGA GAT TGT AGT GAG CCG A-3′
Primer6	−247 to −229	5′-gatc ggt acc AGG TAC GTA GTG AGG GAC A-3′
Primer7	−90 to −72	5′-gatc ggt acc TAG GCC CCA CCC CAC CAA A-3′
Primer8	+147 to +128	5′-gatc aga tct GGC TGC AGA GGG CCA GTG GC-3′
Primer9	+10 to +28	5′-gatc ggt acc GGC TCA AGC CGC ACG TGA G-3′
Primer10	−64 to −45	5′-gatc ggt acc CCT GCG CCG TTC TTT TTT CC-3′
Primer11	−47 to −29	5′-gatc ggt acc TCC GTC **CAC GTG** ACC CAC T-3′
Primer12	−34 to −15	5′-gatc ggt acc CCC ATC CAG GCT CCT CCT TG-3′
Primer13	+10 to +29	5′-gatc ggt acc GGC TCA AGC CGC ACG TGA GA-3′
Primer14	*	5′-gatc cca tgg TGG CTT TAC CAA CAG TAC CG-3′
Primer15	−64 to −35	5′-gatc ggt acc CCT GCG CCG TTC TTT TTT CCG TCC **ACG GTA** A-3′
Primer16	−64 to −35	5′-gatc ggt acc CCT GCG CCG TTC TTT TTT CCG TCC **ACG TA**A-3′
Primer17	−64 to −34	5′-gatc ggt acc CCT GCG CCG TTC TTT TTT CCG TC_____A CCC AC-3′

The restriction enzyme site was underlined. The boldface oligonucleotides correspond to the normal or mutated E-box. The nucleotide substitutions and deletions in the E-box were underlined. *Corresponding to the sequence 91 to 66 in pGL3-Basic reporter vector.