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Differential expression of *hDAB2IPA* and *hDAB2IPB* in normal tissues and promoter methylation of *hDAB2IPA* in hepatocellular carcinoma

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***Background/Aims*: *hDAB2IP* is a candidate tumor suppressor gene. We studied the expression of its two variants, *hDAB2IPA* and *hDAB2IPB*, in normal tissues, and the expression and methylation status of *hDAB2IPA* in hepatocellular carcinomas (HCC) and cell lines.**

***Methods*: Conventional or real-time RT-PCR was performed in normal tissue samples, cell lines and HCC samples, and sequencing analysis and methylation-specific PCR in cell lines and HCC samples.**

***Results*: *hDAB2IPA* was the predominant isoform, being expressed in the majority of tissues examined. The expression of *hDAB2IPA* was silenced or down-regulated but could be restored by 5-aza-2′-deoxycytidine treatment in liver cancer cell lines. The reactivation of *hDAB2IPA* was associated with promoter demethylation. The correlation between promoter methylation and *hDAB2IPA* expression was confirmed in eight pairs of matched HCC samples. Further, the methylation of the *hDAB2IPA* promoter in HCC was confirmed in an additional 53 pairs of patient samples. More than 80% of HCC samples showed *hDAB2IPA* promoter methylation, compared to 11.5% in the corresponding adjacent normal tissue ($p < 0.0001$, χ^2).**

***Conclusions*: Our data suggest that *hDAB2IPA* is the dominant isoform expressed in normal tissues. Its expression is suppressed in HCC, consistent with its role as a tumor suppressor gene, mainly by promoter methylation.**

***Keywords*: Differential expression; Hypermethylation; *hDAB2IPA*; Hepatocellular carcinoma**

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third most common cause of cancer-related deaths in the world. It is a common cancer in Asia and Africa [1,2] often associated with poor prognosis due to late diagnosis and limited therapeutic options [3]. The molecular mechanisms underlying hepatocarcinogenesis are, for the most part, unknown. Tumor suppressor genes such as *RASSF1A* [4,5] and *NORE1B* [3] have been implicated in hepatocarcinogenesis. NORE1B is one of the isoforms of the Ras effector NORE1, which shares almost 60% homology with RASSF3, a member of RASSF family [6]. NORE1 binds directly to Ras and several other Ras GTPases and antagonizes the growth-enhancing effect of Ras [7].

The *hDAB2IP* gene is a candidate tumor suppressor gene. *hDAB2IP* [human DOC-2/DAB2 (differentially expressed in ovarian carcinoma-2) interacting protein]

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is located on chromosome 9q33.1–q33.3 and spans approximately 86 kb according to the human DNA sequence obtained from clone RP11-298A17 (Accession No.: AL365274). At least 2 transcripts *hDAB2IPA* (NM_032552.1, AF367051.1) [8] and *hDAB2IPB* (NM_138709.1, AY032952.1) [9] have been identified at this locus, comprising 5.2 kb with 15 exons and 3.5 kb with 14 exons, respectively. They are generated by splicing from different first exons to a common second exon and hence, are driven from different promoters (Fig. 1A). Both transcripts share exons 2–13 (Fig. 1B). There is a CpG island in the 5′ region of *hDAB2IPA* but not in that of *hDAB2IPB*. The translation of *hDAB2IPA* starts at exon 3, while that of *hDAB2IPB* starts at exon 2. The open reading frame in *hDAB2IPB* encodes a longer peptide (1065aa), which is almost identical to the product of *hDAB2IPA* (967aa) except for additional amino acids at N- and C-termini (Fig. 1C). The transcription of *hDAB2IPA* may start further at upstream, since the transcript of its mouse counterpart is 45 bp longer than *hDAB2IPA* at 5′ end [10]. However, there is no direct evidence currently from the cDNA database to support the existence of this transcript in human. Although the 2 isoforms of this gene were identified several years ago, their expression and regulation have not been studied separately. Most published studies have not differentiated them and it has been difficult to determine which of the two isoforms was studied. In this study, we examined the expression of both isoforms in normal adult and fetal tissues to determine whether the isoforms were regulated distinctly. In addition, we explored the regulation of *hDAB2IPA* in HCC samples and cell lines to determine whether its regulation is consistent with a tumor suppressor role. hDAB2IPA functions in a similar manner to NORE1B, a protein that activates ras-GTPase activity. The hDAB2IPA protein interacts directly with DOC-2 to inhibit Ras signaling. Its rat homolog, Dip1/2, has been shown to inhibit the growth rate and colony formation of the human prostate cancer cell line C4-2 [11]. As has been shown for other tumor suppressor genes [3–5,12], aberrant methylation of the *hDAB2IPA* promoter has been detected in prostate, breast, gastrointestinal and lung cancers by methylation-specific PCR (MSP) analysis [13–16]. The hypermethylation occurs in the P2 region (−598 to +44) of the promoter, which contains a CpG island [8]. Transcriptional silencing by hypermethylation of this promoter region is a critical event in prostate carcinogenesis [16]. Other studies have also demonstrated the relationship between *hDAB2IPA* promoter hypermethylation and cancer stage and lymph node metastasis [14,15]. However, the methylation status of *hDAB2IPA* in HCC has not been investigated. Here we show that *hDAB2IPA* is down-regulated in liver cancer cell lines and HCC samples by promoter hypermethylation, supporting its function as a tumor suppressor gene.

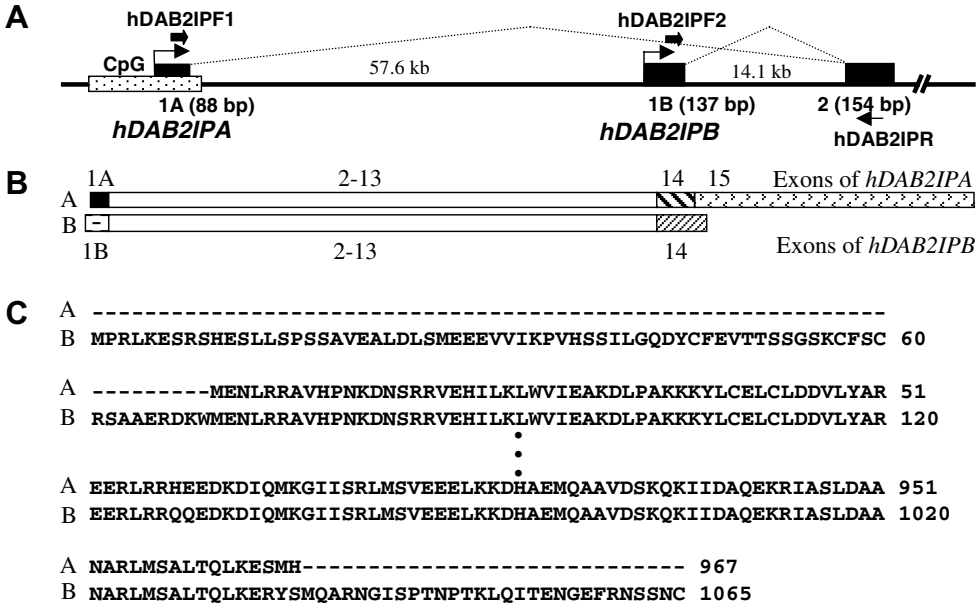

Fig. 1. Schematic of the genomic organization of the two *hDAB2IP* gene transcripts. (A) Transcription map showing the 5′ region of *hDAB2IP* gene locus on chromosome 9q33. *hDAB2IPA* and *hDAB2IPB* are generated by respective splicing from exons 1a and 1b to a common exon 2. Hence, they are driven by different promoters. Black boxes indicate exons, whereas the dotted box indicates the CpG island at the promoter region of *hDAB2IPA*. The transcription start sites are shown using bent arrows. The relative positions of the hDAB2IPF1, hDAB2IPF2 and hDAB2IPR primers are shown by straight arrows. Relative positions and sizes of the introns and exons are also indicated. (B) mRNA comparison of *hDAB2IPA* and *hDAB2IPB*. The two transcripts share exons 2–13 but are different at both the 5′- and 3′-ends. (C) Amino acid sequence of the N- and C-termini of the two isoforms. 960 out of 964 amino acids of hDAB2IPA are identical to that of hDAB2IPB. Figure shown is not to scale.