Cloning and Characterization of Two Promoters for the Human HSAL2 Gene and Their Transcriptional Repression by the Wilms Tumor Suppressor Gene Product*

HSAL2 is a member of a gene family that encodes a group of putative developmental transcription factors. The HSAL gene complex was originally identified on the basis of DNA sequence homology to a region-specific homeotic gene (SAL) in Drosophila. This study reveals a novel, functional 5’ exon for HSAL2 and demonstrates that two distinct HSAL2 gene transcripts arise from two overlapping transcription units, resulting in proteins that differ by 25 amino acids. By utilizing functional luciferase reporter assays, two distinct promoters for HSAL2, P1 for the proximal promoter (upstream of exon 1) and P2 for the distal promoter (upstream of exon 1A), were identified. Evaluation of mRNA prevalence and tissue specificity, with particular focus on adult tissues, revealed that production of mRNA from P1 was selective and relatively rare. Production of mRNA from P2 was demonstrably higher and was expressed by a greater number of tissues. In contradistinction, HSAL2 expression directed by P2 was undetectable in some malignant populations as opposed to their normal human counterparts, suggesting a potential role as a tumor suppressor gene. Consensus-binding sites were identified for several transcriptional factors, with multiple sites for WT-1, and Hox-1.3 present within both the P1 and P2 regions. In transient transfection assays, transcription from both HSAL2 P1 and P2 was strikingly repressed by the WT-1 tumor suppressor protein. These findings suggest that an intracellular WT-1/HSAL2 pathway may play a role in development and hematopoiesis.

SAL-related genes have been isolated from Caenorhabditis elegans (3), fish (4), Xenopus (5), mouse (6), and human (1, 7). Each of these homologues is expressed during embryonic development as well as in certain adult tissues. In humans, HSAL1 is mutated in patients with Townes-Brocks syndrome with features including urogenital, limb, anal, and cardiac malformation (8, 9). HSAL2 is expressed in a number of mice and human tissues and is considered a potential tumor suppressor gene for ovarian cancer. The gene has been mapped to 14q11.1–13, which is a region associated with LOH in 49% of human ovarian cancers (10). Furthermore, alterations in the expression of HSAL2 have been observed in human ovarian carcinomas. In mouse, both in vitro and in vivo assays demonstrate a specific interaction between HSAL2 and the large T antigen of polyoma virus (38). This interaction is critical for susceptibility to polyoma-induced neoplasia, including mammary tumors. When HSAL2 is transfected into the ovarian cancer cells, the tumor population exhibits a significant reduction in growth rate and colony formation in soft agar. Furthermore, mutations in the HSAL2 gene have been associated with ovarian cancer. HSAL3 has been mapped to chromosome 18q23, and it has been suggested that this isoform is involved in the phenotype of patients with 18q deletion syndrome characterized by developmental delay, hypotonia, growth retardation, midface hypoplasia, hearing loss, and tapered fingers (7).

SAL proteins belong to a group of C2H2 zinc finger transcription factors and are characterized by multiple domains distributed over the entire protein (11). The structural characteristics of SAL2 seen in vertebrates involve a C2H2 finger near the N terminus and a cluster of C2H2 motifs distributed over the remaining portion of the protein, considered essential for DNA binding. HSAL2 proteins have glutamate-, proline-, and alanine rich sequences, suggestive of transcriptional activator and repressor functions (1).

The downstream target genes of the HSAL family remain to be identified. However, two important signaling pathways upstream of SAL have been identified. In fish, SAL expression is regulated by hedgehog (Hh) activity (4). In addition, Decapentaplegic (Dpp), a homologue of transforming growth factor-β (TGF-β), also acts as an upstream regulator of the Drosophila sal gene (12–15). The Dpp and Hh signaling pathways are highly conserved throughout the animal kingdom and are critical for embryonic pattern formation. Most of the genes in the

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Dpp and Hh pathways have been shown to play similarly essential roles in development (15, 16). Importantly, a remarkable number of these genes in humans are either tumor suppressor genes or oncogenes. For instance, homologues of the components of the Wingless and Dpp signaling pathways (PTC, APC, and MAD) are tumor suppressors, whereas homologues of CI and Wingless (GLI1 and WNT) are oncogenes in the human (17–20).

Taken together, the HSAL gene family appears to be essential for normal embryonic development, and genetic alterations of the family members have been linked to human congenital defects and cancer. However, there is little known about the mechanism of HSAL gene regulation. Prior experimentation has suggested that the HSAL2 gene putatively possesses two alternative 5′ exons (exons 1 and 1A) (21). To elucidate some of the mechanisms of HSAL gene control, and to identify cis- and trans-acting regulatory elements/factors that direct the complex pattern of HSAL gene expression in normal human tissues as well as in human cancer, we have cloned and characterized the 5′-flanking region(s) of the HSAL2 gene. The results suggest that there are two promoters, P1 and P2, responsible for controlling the alternative usage of 5′ exons, leading to the production of two distinct mRNAs. P1 and P2 exhibited different tissue specificity and resulted in generation of HSAL2 isoforms differing at the N terminus. In addition, HSAL2 transcripts were undetectable or silent in some of eight well-characterized human tumors. Our findings should facilitate the understanding of the transcriptional regulation of HSAL2 in embryonic development and human cancer.

MATERIALS AND METHODS

Cell Culture—Cell lines 293 (derived from human embryonic kidney cells) and U2OS (derived from osteosarcoma cells) were purchased from the American Type Culture Collection (Manassas, VA). Cells were maintained in media supplemented with 10% fetal calf serum.

WT-1 Constructs—WT-1 expression constructs were kindly provided by Dr. Jonathan D. Licht and contained two isoforms of murine WT-1 cDNA constructs. The expression of WT-1 constructs was directed by the RSV promoter (22, 23). The B isoform includes an N-terminal splice that codes for 17 additional amino acids. The D isoform is the same as the B isoform except for an additional 3 amino acids inserted between zinc fingers 3 and 4. WT-1 expression proteins were detectable from each construct by immunohistochemistry (22).

5′-Extension—These procedures were performed using the avian myeloblastosis virus-reverse transcriptase primer extension system (Promega) following the manufacturer’s instructions. 1 μg of poly(A)+ RNA from human brain and kidney was transcribed to cDNA with a 32P-end-labeled exon 1-specific primer (5′-GGATATGGGATTGAGGAGGCCAAGGATTGGAGGAGGCCGATG-3′). The products were analyzed on an 8% denaturing polyacrylamide gel.

Identification of Exon 1-Exon 2 and Exon 1A-Exon 2 Transcripts—Total RNA of specific human tissues (see under "Results") was obtained from Stratagene (La Jolla, CA), Ambion (Austin, TX), or the Department of Pathology, Roger Williams Medical Center (Providence, RI) subsequent to pathologic diagnosis. RNA (5 μg) was reverse-transcribed after annealing with 0.1 μg oligo(dT) for priming of cDNA synthesis in a 20-μl reaction using the SuperScript Preamplification System (Life Technologies, Inc.). cDNAs for specific human tissues and selected tissues were obtained from CLONTECH (Palo Alto, CA). One common primer located in exon 2 was used as the 5′ downstream primer in combination with one or the other of two 5′ upstream primers specific for exon 1 and exon 1A, respectively. PCR amplification was constructed to ensure the product crossed over an intron. The primer sets were as follows: exon 2 common primer, N1 (5′-GGATATGGGATTGAGGAGGCCAAGGATTGGAGGAGGCCGATG-3′); exon 1-specific primer, N2 (5′-CCACAAGGTAATCTCGCGACTCC-3′); and exon 1A-specific primer, N3 (5′-CCACAAGGTAATCTCGCGACTCC-3′). PCR amplification was performed in 50-μl reaction volumes containing 5 μl of cDNA, 10 μl Tris-HCl (pH 8.3), 50 μl KCl, 2 μl MgCl2, 0.2 μl T3 primers, and 1.25 units of Taq DNA polymerase (PerkinElmer Life Sciences). In some experiments, 3 μCi of [32P]dCTP were added to each PCR sample. After initial denaturation at 94 °C for 10 min, amplification was performed under the following conditions: 1 min at 94 °C, 1 min at 57 °C, and 1 min at 72 °C (exon 1-exon 2 primer pair); 1 min at 94 °C, 1 min at 64 °C, and 1 min at 72 °C (exon 1A-exon 2 primer pair); 1 min at 94 °C and 1.5 min at 72 °C (GAPDH primer pair). Amplification was performed within the linear range for the exon 1- exon 2 transcript and for the exon 1A-exon 2 transcript, as indicated in the figure legends. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used to control for template loading. PCR products were electrophoretically separated on 2% agarose gels. Radioactive PCR products were electrophoretically separated on 5% polyacrylamide gels. The gels were dried and exposed to Biomax MR-2 (Kodak) autoradiography film. DNA sequencing was used to confirm amplification products.

Isolation of BAC Clones for HSAL2—A collection of human BAC library was performed in collaboration with the Research Genetics Institute (Huntsville, AL). Three positive clones were identified. The isolated clones were verified by direct nucleotide sequencing of the BAC DNA.

Chromosomal Localization—Chromosomal localization was determined by fluorescence in situ hybridization. DNA isolated from an HSAL2 BAC clone was labeled with digoxigenin and hybridized to human peripheral blood lymphocytes in metaphase. Signals were detected via rhodamine-conjugated avidin. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole dihydrochloride.

HSAL2 Promoter Constructs—The 5′-flanking region of exon 1A was amplified with primers 5′-GGATATGGGATTGAGGAGGCCAAGGATTGGAGGAGGCCGATG-3′ and 3′-GGATATGGGATTGAGGAGGCCAAGGATTGGAGGAGGCCGATG-3′ to generate a fragment from −3 to −2278 bp upstream of the first ATG, with HindIII and Xhol sites at each end, respectively. After HindIII and Xhol digestion, this fragment was cloned into the promoter-less pG3-Luc basic luciferase reporter plasmid (Promega) at the HindIII and Xhol site to generate −2278-LUC. −429-LUC was generated by digesting −2278-LUC with PstI and Xhol followed by blunt end with T4 DNA polymerase and ligating overnight. −331-LUC and −214LUC were created in the same manner, except the parental −2278-LUC was digested with Xhol/SacI and Xhol/BglII, respectively.

Transfection—Transfection was performed using LipofectAMINE 2000 (Life Technologies, Inc.) following the manufacturer’s instructions. Cells were plated in 12-well plates at a density of ∼1 × 104 cells/well. Cells were harvested 48 h after transfection. Plasmid DNA for transient transfection was prepared using the Qiagen Plasmid Midi Kit (Qiagen). To analyze the HSAL2 promoter constructs, 1 μg of HSAL2 reporter plasmid plus 0.2 μg of internal control β-galactosidase plasmid, CMV-β-gal (Life Technologies, Inc.), was used. For co-transfection experiments with WT-1, a total of 1.3 μg of plasmid DNA was used. The reaction contained 0.3 μg of HSAL2 promoter-reporter plasmid and either 0.9 μg of PcDNA 3 vector DNA or 0.9 μg of WT-1 expression plasmid plus 0.2 of internal control of CMV-β-gal plasmid.

β-Galactosidase Assay and Luciferase Assay—The cells were extracted using 100 μl of luciferase cell culture lysis reagent (Promega) 48 h post-transfection. The β-galactosidase assay was performed with 10 μl of cell extract using the β-Galactosidase Enzyme Assay System (Promega) and the standard assay protocol provided by the manufacturer (except that 1 μl Tris base was used as stopping buffer instead of sodium carbonate). Five microfilters of extract were used for the luciferase assay using a Luciferase Assay kit (Promega), following the manufacturer’s instructions. After subtracting background, the luciferase activity (arbitrary units) was normalized to β-galactosidase activity (arbitrary units) for each sample.

RESULTS

Detection of Two mRNA Transcripts from the HSAL2 Gene—Given a high degree of homology between mouse and human cDNA for SAL2, primers were constructed to evaluate message production from the human HSAL2 gene. One common primer, located within exon 2, was used as the 3′ downstream primer (reverse) in combination with exon 1-1 specific primers, as demonstrated in Fig. 1A. RT-PCR was performed using cDNAs transcribed from fetal kidney and intestinal mRNAs. As shown in Fig. 1B, a product encompassing sequences derived from exon 1A and exon 2 was amplified from the kidney and intestine when using the exon 1A primer in conjunction with the exon 2 primer. A product containing exon 1 and exon 2 sequences was seen when specific primers for exon 1 and exon 2 were applied. Sequencing of the amplified PCR products revealed that when primers for exon 1 and exon 2 were utilized,
a product resulted that bypassed exon 1A. The PCR product using primers for exon 1A and exon 2 were devoid of exon 1 sequences. Sequence analysis of the amplified PCR products showed that consensus splice sites were used in the processing of exon 1-exon 2 and exon 1A-exon 2 mRNA transcripts (data not shown). Determination of deduced amino acid sequence reveals that the protein derived from exon 1-exon 2, as opposed to exon 1A-exon 2, differed by up to 25 amino acids at the N terminus (Fig. 1).

Identification of HSAL2 Transcription Start Sites for Exon 1A-Exon 2 mRNA

The position of one of the HSAL2 transcription start site(s) was next determined using 5' extension. Human fetal kidney and brain were chosen to be suitable tissues to provide mRNA because both of these tissues have been shown to strongly express HSAL2 (1). By using a primer specific for exon 1A, one major transcriptional start site, 120 nucleotides upstream of the ATG codon (~120) was identified, as seen in Fig. 1D. Three minor transcriptional start sites were also located at positions ~125, ~170, and ~195 nucleotides (Fig. 1D). The numerical identification of the transcriptional start sites was based upon the A of the ATG translational start site being given the number +1.

Cloning and Characterization of the HSAL2 Gene Promoters

Cloning and Sequencing of the Human HSAL2-Flanking Region—To identify the promoter region(s) upstream of HSAL2, three BAC clones were isolated and found to contain the required full-length HSAL2 gene, as determined by direct nucleotide sequencing. To confirm further the specificity of the HSAL2 gene, DNA from the HSAL2 BAC clone was labeled with digoxigenin-11-dUTP by nick translation and hybridized in situ to normal human chromosomes as substrate. Fluorescence in situ hybridization revealed that the BAC clone 2 containing HSAL2 localized to the proximal long arm of chromosome 14 (q11.1–q13) (Fig. 2).

The Human HSAL2 P2 Promoter—Evaluation of the P2 promoter was first to be undertaken, given the relative abundance
of the mRNA product controlled therefrom. Sequencing of the 5'-flanking region of exon 1A did not reveal classical TATA or CAAT boxes. However, this region was GC-rich and contained several GC boxes and potential binding sites for multiple general transcription factors including AP1, AP4, and Sp1 within 429 bp upstream of the major translation start site (Fig. 3A). A data bank search using the Transfac transcription factor database revealed several putative binding sites for regulatory transcription factors scattered within 2.3 kb upstream of the first ATG of exon 1A, including two sites for TGF-β/Smad, one site for Hox-1.3, and seven sites for WT-1 (Fig. 3B). To determine whether this region has significant promoter activity, the 2.3-kb upstream region was cloned in front of a promoterless luciferase reporter (pGL3-Basic; Promega). Luciferase expression in transient transfection assays was next evaluated in the presence of a constant amount of the internal plasmid expressing β-galactosidase. In NIH-3T3 cells, P19 embryonic carcinoma cells, and 293 human kidney cells, luciferase activities were 6-, 23-, and 10-fold greater than the promoterless vector, respectively (Fig. 4A).

To identify the minimal DNA sequences required for HSAL2 promoter activity and to analyze DNA sequences important for the high level of HSAL2 transcription, transient transfection assays were further carried out on the 293 kidney cell line. This was performed using a series of promoterless luciferase reporter constructs containing up to 2.3 kb of 5'-flanking sequence surrounding the transcription start sites. The series of deleted promoter reporter constructs used in the transfections are shown in Fig. 4B. The constructs were co-transfected with a β-galactosidase expression vector (pCMV gal), and luciferase activities were normalized in reference to the respective β-galactosidase activity. All five promoter constructs demonstrated functional activity in 293 cells, in contrast to the promoterless construct pGL3-basic that served as a control. The most significant luciferase activity observed was 17-fold greater than that found in pGL3-basic. The shortest promoter construct contained 219 bp upstream of the first ATG and demonstrated approximately an 8-fold increase in luciferase activity compared with that of the promoterless vector.

The Human HSAL2 P1 Promoter—As a result of the reporter

**Fig. 3.** Sequence analysis of the HSAL2 P2 promoter region. A, common general transcription factor binding sites are marked. Stimulating protein 1 (Sp1) sites, circled; activator protein 4 (AP4) sites, underlined; activator protein 1 (AP1) sites, asterisks. Arrows and numbers refer to the 5' end of the promoters used in luciferase constructs. B, putative regulatory transcription factor binding sites in the HSAL2 P2 promoter region. Arrow indicates the major transcription start site.

**Fig. 4.** Characterization of transcriptional activity of the HSAL2 P2 promoter. A, HSAL2 P2 promoter activities in 3T3, P19, and 293 cells. The 5'-flanking region of HSAL2 exon 1A, from -3 to -2281 upstream from the first ATG codon, was used to drive the expression of a luciferase reporter in the HSAL-luc construct. Fold differences, i.e. relative luciferase activity, were calculated as arbitrary luciferase activity of HSAL-luc over that of the vector, pGL3-Basic (Promega). Luciferase expression in transient transfection assays was next evaluated in the presence of a constant amount of the internal plasmid expressing β-galactosidase. In NIH-3T3 cells, P19 embryonic carcinoma cells, and 293 human kidney cells, luciferase activities were 6-, 23-, and 10-fold greater than the promoterless vector, respectively (Fig. 4A).

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The Human HSAL2 P1 Promoter—As a result of the reporter
gene and 5’ extension assays detailed above, a functional promoter located in the 5’-flanking region of exon 1A, 2.1 kb away from a start site for exon 1A-exon 2 mRNA, was demonstrated. In addition, our studies demonstrated the presence of an additional exon, exon 1, ∼71 kb upstream of exon 1A. Exon 1 contains a potential translation start site that, when spliced to exon 2, forms an open reading frame. As a result, exon 1A was absent from the exon 1-exon 2 transcript. These findings prompted an investigation into an alternative promoter, P1, for the exon 1-exon 2 mRNA. Evaluation of the 5’ region flanking exon 1 revealed known consensus sites for transcriptional regulatory elements including three sites for vitamin D receptor/retinoid X receptor, one site for WT-1, one site for PAX-3, and three sites for Hox-1.3 (Fig. 5A). To determine whether this region bears significant promoter activity, ∼2 kb of genomic DNA starting upstream of the translation start site for exon 1 was subcloned into a promoterless pGL3-Basic vector. Transient transfection was performed using the 293 renal cell line. P1 demonstrated a 4-fold increase in luciferase activity (Fig. 5B) compared with the control vector, pGL3-Basic. The full-length P2 construct containing ∼2 kb upstream of the translation start site showed an 8-fold increase in luciferase activity under the same conditions.

**P1 and P2 Promoters Are Repressed by WT-1**—Sequence analysis of the two identified HSAL2 promoters revealed multiple potential binding sites for WT-1. Given the known repressive actions of WT-1, it was next evaluated whether these DNA-binding proteins altered transcription from the human HSAL2 P1 and P2 promoters. For these studies, an osteosarcoma cell line, U2OS, was co-transfected with two distinct WT-1 constructs. The B isoform of WT-1 includes an N-terminal splice that codes for 17 additional amino acids. The D isoform of WT-1 is identical to the B isoform except for an additional 3-amino acid insertion between zinc fingers 3 and 4. The HSAL2 P1 promoter was repressed 7–9-fold by co-transfection with a 3-fold molar excess of the WT-1 expression plasmids (Fig. 6A). When a similar experiment was conducted utilizing the P2 promoter construct, the activity of the P2 promoter was repressed 4–10-fold (Fig. 6B). Removal of four of the five WT-1-binding sites from the P2 promoter resulted in decreased suppression in transfectedants, as expected (Fig. 6B).

**Utilization of the P1 and P2 Promoters as a Function of Cell Type and Tissue Origin**—Transcriptional activities of the P1 and P2 promoters were then tested by RT-PCR using cDNAs prepared from various human cells and tissues. A fragment of the ubiquitous GAPDH gene cDNA was amplified as a control. As seen in Fig. 7, the activity of the P2 promoter was apparently stronger than that of P1, because the P1 transcript required significant amplification and film exposure to be detected. The PCR product derived from the P2 promoter was found to be present in multiple tissues, although with varying levels of expression. The PCR product of the P1 promoter was substantially more restricted in its distribution, and its scarcity was confirmed by the degree of amplification needed for its detection. It should be noted that in addition to the tissues showing expression of the P1 transcript, as seen in Fig. 7, data shown in Fig. 1 reveal a P1-directed PCR product from both the kidney and small intestine. Also, prior experimentation has revealed significant expression of P1 from mouse embryonic tissue (21).

**HSAL2 mRNA Transcripts Were Undetectable from Some Human Tumors**—Expression of HSAL2 transcripts was then tested in eight characterized human tumors (Table I) to determine whether a disturbance of HSAL2 regulation might be present in the neoplasms. The cDNAs were obtained from human tumors propagated in athymic nude mice as xenografts (CLONTECH). Unlike cell lines propagated in vitro, xenografts are regulated by interactions with the host organism in a similar environment to that where the tumor originated (24). Each cDNA sample was carefully normalized to allow accurate assessments of relative abundance of target mRNAs (CLONTECH). Interestingly, the HSAL2 exon 1A-exon 2 transcript was not detectable in some lung carcinomas (two of two tested) and adenocarcinoma of the colon (one of two tested) and prostate (one of one tested) (Fig. 8). The failure of expression in some of the tumors analyzed is in contradistinction to their normal human counterparts, where these tissues expressed HSAL2 P2 transcripts (data not shown). The expression of a tumor suppressor gene can be silenced by deletion or DNA methylation. Many tumors show a wide range of genomic disturbances of DNA methylation, especially in CpG islands within the promoter region (25–28). It remains to be determined what is the cause(s) of the silent expression of HSAL2 in the neoplasms examined.
including several Sp1 and Ap4 sites. The feature of TATA-less factors that are essential components of eukaryotic promoters, sites but contained multiple binding sites for transcription TATA or CCAAT boxes in the vicinity of the transcription start.

WT-1 protein as described under "Materials and Methods." A, transcriptional repression effect of WT-1 (B) and (D) on HSAL2 P1 promoter. The construct was used as described in Fig. 5. B, transcriptional repression effects of WT-1 (B isosform) and (D isosform) on HSAL2 P2 deletion constructs. The HSAL2 P2 intact promoter construct contained a 2281-bp sequence upstream of the HSAL2 P2 translation start site. This construct included five potential WT-1-binding sites. The HSAL2 P2 (−219) promoter construct contained a 219-bp sequence upstream of the translation start site and bore only one potential WT-1-binding site.

**DISCUSSION**

Our results have revealed an interesting aspect of gene expression in eukaryotic cells. For the production of two distinct mRNAs by alternative promoters, one transcript was found in adult tissue. Analysis of human mRNAs by RT-PCR confirmed that the three exons of HSAL2 are utilized in the production of two distinct, mature transcripts. By using reporter assays, an additional alternative promoter P1, 11 kb away from P2, was identified and found to be involved in the regulation of exon 1-exon 2 mRNA expression. Compared with P2, P1 exhibited a weaker, yet significant, promoter activity in 293 renal cell and osteosarcoma cell lines. Despite the activity of both promoters, as evaluated utilizing in vitro cell lines, the results of our RT-PCR analysis of mostly adult tissue-derived mRNA showed that P1 is restricted in its tissue distribution and is scarce in amount. The tissues chosen for evaluation were relatively broad in scope and may be divided into hematopoietic/immunologic and general organ systems. Of interest is the apparent differential utilization of the P1 and P2 promoters as a function of tissue origin. The difference in activity of the P1 and P2 promoters in vivo could be a result of altered DNA methylation (32), yet this remains to be determined and may have important implications for gene regulation with particular emphasis on the hematopoietic tissues.
Potential binding sites for transcription factors in the sequenced promoter regions are of substantial interest. The presence of consensus sites for TGF-β/Smad, Hox-1.3, and WT-1 in the HSAL2 promoters are noteworthy. In Drosophila, SAL is activated in response to hedgehog signaling mediated by the TGF-β-like protein, DPP. These control pathways have not yet been demonstrated for mammalian SAL homologues, such as found in mouse and human. Given the activation of the Drosophila SAL gene in response to TGF-β-like signals, and the fact that it is highly conserved through the process of evolution, and the presence of putative Smads/TGF-β response elements in the HSAL2 promoter region, it is conceivable that HSAL2 is positioned within the TGF-β signal cascade. A consensus sequence for Hox-1.3 was identified at position −1736. The Hox family of transcription factor genes is a key element in the establishment of mammalian body patterns. The coordinated regulation of homeobox genes plays an important role in human hematopoietic and embryonic development. It will be important to determine whether Hox genes control the expression of HSAL2 as morphogenesis proceeds.

The WT-1 gene is essential for kidney development, and it is mutated in some Wilms tumors and specific types of acute leukemia (33–35). By utilizing a reporter gene assay, WT-1 can markedly repress HSAL2 promoter activity in several cell lines, suggesting that HSAL2 is negatively regulated by WT-1. We hypothesize that an intracellular WT-1/HSAL2 circuit is important in kidney development and hematopoiesis. This may be similar to the well established WT-1/PAX-2 system, in which these factors exhibit complementary expression patterns and functionally interact during renal development (34). Evidence for a WT-1/HSAL2 pathway is presently suggested by the following. 1) There is an inverse correlation between HSAL2 levels in CD34+ primitive hematopoietic cells and increasing levels of WT-1. 2) HSAL1 was found to be expressed, together with WT-1, during kidney development, and HSAL1 was specifically overexpressed in Wilms tumors (37). 3) The HSAL2 promoter(s) contained multiple potential WT-1-binding sites. 4) WT-1 was able to repress transcription in transfected cells that express a reporter gene under control of HSAL2 promoter sequences.

Recently, the usage of multiple promoters has been recognized as an important transcriptional mechanism creating diversity and flexibility of gene expression (36). Alternative promoters could exhibit tissue specificity, generate protein isoforms, and react differently to signals (36). We have demonstrated for the first time in the literature that a homeotic gene, HSAL2, belongs to that group of genes bearing features of tissue-dependent promoter usage as a means to generate different protein isoforms. Further studies are needed to determine whether this important transcription regulatory mechanism of alternative promoter usage could play a biological role in controlling HSAL2 expression in human development.

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