Expression of the Putative Proto-Oncogene *His*-1 in Normal and Neoplastic Tissues

Jie Li,* David P. Witte,† Terry Van Dyke,‡ and David S. Askew*

From the Department of Pathology and Laboratory Medicine,* University of Cincinnati, and the Department of Pathology,† Children's Hospital Medical Center, Cincinnati, Ohio, and the Department of Biochemistry and Biophysics,‡ University of North Carolina, Chapel Hill, North Carolina

*The **His-1** gene is expressed as a 3-kb spliced and polyadenylated RNA that is believed to function in the absence of an encoded protein. The precise function of the **His-1** gene is unknown, but its transcriptional activation in a series of mouse leukemias has implicated the **His-1** RNA in leukemogenesis when it is abnormally expressed. To study the oncogenic potential of this gene in more detail, we have examined the normal tissue distribution of **His-1** RNA during mouse embryogenesis and in various adult tissues. **His-1** expression was detected at low levels in the epithelia of the adult mouse stomach, prostate, seminal vesicle, and the developing choroid plexus by in situ hybridization. All other tissues examined lacked detectable levels of hybridizing RNA, suggesting that normal **His-1** gene expression is highly restricted to these epithelial sites. These transcripts were not detectable by Northern blot analysis of normal tissues but were readily identified in five mouse leukemias and in five carcinomas of the choroid plexus. These data indicate that the **His-1** gene expression is highly restricted and suggest that inappropriate activation of this gene may contribute to carcinogenesis. (Am J Pathol 1997, 150:1297–1305)*

The nonacute-transforming retroviruses induce neoplastic disease by integrating into genomic DNA and creating mutations at the site of insertion.[1] It is now well established that the oncogenic activity of these retroviruses is due to their ability to activate cellular proto-oncogenes that flank the insertion site, and this observation has been widely exploited to identify novel genes that function at multiple steps in the carcinogenic process.[2,3] We have previously identified a single-copy gene, *His*-1, that is a target for proviral insertion in a series of murine retrovirus-induced leukemias.[4,5] *His*-1 gene expression is transcriptionally activated in these leukemias, suggesting that *His*-1 is a proto-oncogene that is causally associated with leukemogenesis.[5]

The *His*-1 gene is unusual in that it is expressed as a 3-kb spliced and polyadenylated RNA that lacks an extensive open reading frame, a feature that is usually considered indicative of noncoding sequences.[5] The human homologue has been cloned from a conserved region of synteny on human chromosome 2, and the high degree of evolutionary conservation observed between these and other species homologues has suggested that the function of *His*-1 has been conserved throughout vertebrate evolution.[6] There is no evolutionary conservation of any of the multiple small open reading frames between mouse and human homologues, however, suggesting that the unidentified function of the *His*-1 RNA is unlikely to involve an encoded protein product. Based upon these observations, and the increasing precedence for functional RNA molecules in the literature,[7–15] we have hypothesized that RNA is the final and functional product from the *His*-1 gene.[6]

As the function of noncoding RNAs may have significance for the pathogenesis of human disease,[5,12,13,15] we have made a detailed examination of the expression pattern of the *His*-1 gene during mouse embryogenesis and in adult tissues. Our data indicate that most normal tissues lack detectable *His*-1 RNA, with the exception of the epithelial cells at

four tissue sites. We also provide evidence for the existence of alternatively spliced forms of the *His*-1 RNA in leukemic cells and in carcinomas of the choroid plexus epithelium. We hypothesize that the putative role of *His*-1 in oncogenesis may originate from the release of constraints that restrict transcription of this gene.

Materials and Methods

Cell Culture

The NFS and DA series of cell lines were derived from murine myeloid leukemias induced by the oncogenic retrovirus CasBrM-MuLV.[4] The C6, C10, 14-122, 14-166, 14-259, 7-M12, and 15-299 cell lines were derived from radiation-induced murine myeloid leukemias.[16,17] The Bac-1.2F5 cell line was derived from mouse macrophages transformed *in vitro*.[18] The RL-12 cell line was established from a radiation-induced T-cell leukemia.[19] All other mouse and human cell lines were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in either RPMI-1640 or Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 2 mmol/L L-glutamine. Bac-1.2F5 cell medium was supplemented with 20% L-cell-conditioned medium as a source of colony-stimulating factor-1.[18]

Northern Blot Analysis

Polyadenylated RNA was selected directly from cell or tissue lysates according to the procedure of Badley et al[20] or from total RNA preparations. Total RNA was isolated from cell lines and tissues by extraction in guanidinium isothiocyanate and pelleting through a cesium chloride gradient.[21] Either 20 μg of total RNA or 2 to 10 μg of poly(A)$^+$ RNA was analyzed by Northern blot hybridization after electrophoresis on 5.7% formaldehyde gels and transfer to either positively charged nylon membranes (Genescreen-Plus, Dupont/NEN, Boston, MA) or nitrocellulose. Hybridization membranes containing polyadenylated RNA from various human tissues (multiple tissue northern blot) were obtained from Clontech Laboratories (Palo Alto, CA). All filters were hybridized under stringent conditions to ^{32}P-labeled random-primed DNA probes in 50% formamide/5× standard saline citrate (SSC)/1× Denhardt's solution/10% dextran sulfate as previously described.[4] A 0.5-kb *Eco*R1-*Bam*H1 exon 3 fragment derived from the most evolutionarily conserved region of the mouse *His*-1 cDNA[5,6] was used to probe all mouse RNAs, and a 0.5-kb *Xba*I

fragment of the human gene (containing sequence homology to the same region used as the mouse probe) was used to probe all Northern blots containing human RNA.[6] Hybridization to a 1.2-kb *Eco*R1 fragment from the mouse *vav* cDNA was used as an RNA loading control for hematopoietic cells, as this gene is ubiquitously expressed throughout the hematopoietic lineage.[22] Hybridization to a 1.8-kb *Hin*dIII chicken β-actin probe was used to control for integrity and loading of RNA from nonhematopoietic cells.[21]

In Situ *Hybridization Analysis*

In situ hybridization of mouse tissues was performed as previously described.[23] Briefly, tissues were fixed in 4% paraformaldehyde/1× phosphate-buffered saline (PBS) and embedded in OCT or M1 embedding matrix. The 6- to 8-μm-thick frozen sections were cut and post-fixed again in 4% paraformaldehyde/1× PBS. Cultured cells were prepared by cytocentrifugation of cells onto silane-treated slides followed by fixation in 4% paraformaldehyde/1× PBS. Specimens were subsequently digested with 0.2 mg/ml proteinase K for 5 minutes. After acetylation and dehydration in graded ethanol, the slides were hybridized to a [^{35}S]UTP-labeled *His*-1 exon-3-specific riboprobe under high stringency conditions in a solution containing 50% formamide, 0.75 mg/ml denatured herring sperm DNA, 0.75 mg/ml yeast tRNA, 1× Denhardt's solution, 10% dextran sulfate, 0.1 mg/ml bovine serum albumin, and 5 mmol/L dithiothreitol (DTT). Slides were washed under conditions of high stringency as follows: three washes in 1× SSC/1 mmol/L DTT for 10 minutes at 50°C, one wash in ribonuclease A/T1 solution (50 mg/ml RNAse A, 100 U/ml RNAse T1) at 37°C for 30 minutes, two washes in 1× SSC/1 mmol/L DTT for 10 minutes at 50°C, two washes in 50% 2× SSC/1 mmol/L DTT and 50% formamide for 30 minutes at 50°C, one wash in 0.5× SSC/1 mmol/L DTT for 10 minutes at 50°C, one wash in 0.1× SSC/1 mmol/L DTT for 30 minutes at 55°C and then for an additional 10 minutes at room temperature, and finally sequential dehydration in 70, 90, and 100% ethanol. Exposure times were extended to maximize detection of weakly expressed transcripts (2 to 6 weeks at 4°C).

The antisense *His*-1 riboprobe was synthesized by *in vitro* transcription of the same *His*-1 DNA fragment cloned into the pBluescript vector that was used for Northern blot analysis (0.5-kb *Bam*H1-*Xba*I fragment from *His*-1-exon 3).[5] The 20-μl labeling reactions contained 200 μCi of [^{35}S]rUTP, 1 μl each of 10 mmol/L rATP, rCTP, and rGTP and 0.25 mmol/L

Table 1. *Summary of* His-1 *Expression by Northern Blot in Murine and Human Cell Lines and Tissues*

	His-1 expression	Cell lines or tissues
Mouse cell lines		
Leukemia/lymphoma*	Positive[†]	NFS-112, NFS-124, C10, Bac-1.2F5, RL-12.
	Negative[†]	NFS-22, NFS-36, NFS-56, NFS-58, NFS-60, NFS-61, NFS-70, NFS-78, NFS-107, DA-1, DA-2, DA-3, DA-8, DA-22, DA-29, DA-31, DA-34, EL-4, M1, C6, 14-122, 14-166, 14-259, 7-M12, 15-299, BXH2-4S
Fibroblast	Negative	NIH-3T3
Mouse tissues		
Choroid plexus carcinoma	Positive[†]	4/4
Normal adult tissues*	Negative	Oviduct, ovary, testis, intestine,[‡] skeletal muscle, heart, pancreas, liver,[‡] lung,[‡] kidney,[‡] brain,[‡] thymus,[‡] spleen,[‡] bone marrow, stomach, mammary gland, thyroid, esophagus
Human cell lines		
Leukemia/lymphoma	Negative	CEM, MOLT3, RAJI, Rwleu4, HL-60, U937
Prostate carcinoma	Negative	LNCaP-FGC, DU-145
Breast carcinoma	Negative	MCF-7
Lung carcinoma	Negative	A549, SCLC22, SCLC24
Colon carcinoma	Negative	Colo320
Human Tissues		
Normal adult tissues	Negative[†]	Foreskin fibroblasts, heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes

*Summarized in part from Ref. 5.
[†]Poly (A)[+] selected RNA.
[‡]RNA from these tissues was also isolated from embryonic (day 15) sources.

rUTP, and 10 U of T3 or T7 RNA polymerases (Stratagene, La Jolla, CA). The same riboprobe was also synthesized in the sense orientation and used as a control for background hybridization. In some instances, higher specific activity probes were generated by eliminating the nonradioactive rUTP from the labeling reaction.

Results

Northern Blot Analysis

We have previously reported that *His*-1 gene expression was detected by Northern blot analysis in a subset of mouse leukemic cell lines but not in a panel of normal mouse tissues.[5] We have subsequently expanded these studies to include RNA from multiple cell lines and tissues derived from both human and mouse sources, and a summary of these results is shown in Table 1. The focus of the study was on expression in mouse tissues as the *His*-1 cDNA was initially cloned from this species and the genomic organization of the mouse *His*-1 gene has been the most characterized.[5,6] To ensure the detection of *His*-1 RNA and not related sequences, all hybridizations in this study were performed at high stringency with a probe that is specific for a 400-bp region

comprising the most evolutionarily conserved segment of exon 3[6] (Figure 1, black box). This probe is highly specific for the *His*-1 gene as it does not detect any related sequences in the mouse genome by genomic Southern blot analysis, even under conditions of reduced stringency.[5] Hybridization of this probe to Northern blots of RNA from various murine tissue sources identified *His*-1 expression in five mouse leukemia cell lines and four choroid plexus carcinomas but not in any of the normal mouse tissues examined, including a select number of tissues from a day 15 mouse embryo (Table 1). Extended exposure times (1 week to 10 days) were performed for all Northern blots that contained tissues that were initially negative after overnight exposure. These data suggest that there are powerful constraints that limit the transcription of the gene in most murine tissues and that the ability to detect the RNA by Northern blot analysis is associated with the neoplastic phenotype.

As a human *His*-1 cDNA probe is not presently available, a direct comparison of the mouse expression data with that from human tissue cannot be made. However, as a first approach to examining expression in humans we performed a limited number of Northern blots on human RNA using either the mouse *His*-1 probe or a human genomic DNA frag-

A   **B**
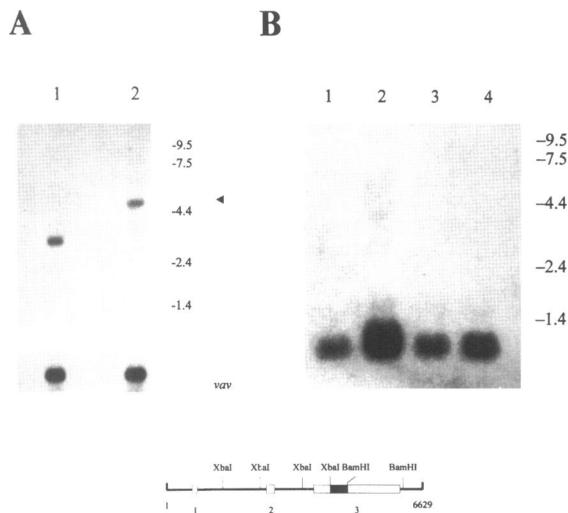
Figure 1. *Identification of alternative* His-1 *transcripts in murine tissues by Northern blot analysis.* **A:** *The previously identified 3-kb* His-1 *transcript[5] is shown in a myeloid leukemia* (**lane 1**) *as a comparison to the novel 5-kb* His-1-*related transcript that was identified in a T-cell leukemia* (**arrow, lane 2**). *Hybridization to the* vav *probe is shown as a loading control.* **B:** *A 1-kb* His-1-*related RNA was identified in four independently derived carcinomas of the choroid plexus* (**lanes 1** *to* **4** *contain 1, 10, 2, and 5 μg of poly* (A)⁺ *RNA from each individual carcinoma, respectively). Molecular weight markers (in kilobases) are indicated at the right of each panel. The black box on the schematic of the* His-1 *gene indicates the region of exon 3 used as a hybridization probe.*

ment that corresponded to the region contained by the mouse probe. No transcripts were detected in any of the human tissues examined using either of the two probes (Table 1). Although this may indeed indicate that the human RNA is absent from these tissues, these data must be interpreted with the caveat that the human gene may have significant differences in its genomic organization that would preclude detection by this probe.

The *His*-1 gene has been shown to express a single 3-kb RNA in mouse leukemias.[5] In this study we have identified a novel alternatively sized *His*-1-related transcript in a murine radiation-induced T-cell leukemia, RL-12.[18] A single 5-kb RNA was expressed in the RL-12 leukemia using the same mouse probe that was used to screen normal mouse tissues (Figure 1A, lane 2). This novel transcript was expressed at approximately the same level as the 3-kb *His*-1 RNA that has previously been identified in other murine leukemias[5] (Figure 1A, lane 1). We considered the possibility that the 5-kb transcript was derived from either the unspliced *His*-1 RNA, a chromosomal rearrangement, a related gene, or an alternatively spliced form of the *His*-1 RNA. As the 5-kb RNA was not detected with probes derived from either intron 1 or intron 2 (data not shown), it does not represent the full-length unspliced version of the published 3-kb transcript. Furthermore, no gross

structural rearrangements were detected by genomic Southern blot analysis in a 12-kb region that contains the entire *His*-1 gene, indicating that it is not due to a chromosomal rearrangement within the gene. Finally, it is unlikely that it represents a transcript from a related gene, as the experiments were performed at high stringency and this probe does not identify related sequences in the mammalian genome.[5,6] The data are therefore most consistent with an alternatively spliced form of the *His*-1 RNA that contains at least a portion of the *His*-1 sequences contained within the probe.[5,6]

In Situ *Hybridization Findings*

To determine whether the lack of expression by Northern blot analysis was a reflection of either low expression levels or a numerically infrequent cell population, we examined *His*-1 expression in a cell-type-specific manner by *in situ* hybridization. The *in situ* hybridization conditions were first optimized for the five leukemic cell lines that expressed *His*-1 transcripts at different levels by Northern blot analysis (Table 1). The NFS-124 cell line expressed the highest levels of *His*-1 RNA by Northern blot analysis,[5] and *in situ* analysis readily detected *His*-1 transcripts in these cells within 2 weeks of exposure (data not shown). The other four *His*-1-positive leukemic cell lines (Table 1) expressed lower levels by Northern blot analysis,[5] and *in situ* detection of *His*-1 RNA in these cells required extended exposure times (3 to 6 weeks). A summary of the exposure times required to identify the leukemia-associated *His*-1 transcripts by *in situ* hybridization is shown in Table 2. Based upon these observations, we chose to use the same conditions of extended exposure times to evaluate normal mouse tissue sections for expression of the *His*-1 RNA. A cytospin preparation of the NFS-124 leukemia was included in each experiment as a positive control.

As developmentally regulated genes can display oncogenic activity when inappropriately activated in adult tissues,[24] we evaluated both adult and fetal murine tissues for *His*-1 expression by *in situ* hybridization. Sections of the developing mouse embryo from days 10 through 19 of gestation were examined at 3, 4, 5, and 6 weeks of exposure. Tissues from whole embryo sections were evaluated up to day 15, but only abdominal sections of the embryo were examined beyond day 15. No hybridization was detected in any tissue after 3 weeks of exposure, indicating that high levels of *His*-1 gene expression were not present during these developmental stages. After 4 weeks of exposure, hybridization to the *His*-1

Table 2. *Summary of Exposure Times Required to Visualize* His-1 *Expression in Leukemic Cells by* in Situ *Hybridization*

	Antisense probe			Sense probe
Mouse leukemia	2W	4W	6W	6W
NFS-124	+	++	+++	−
NFS-112	−	+	++	−
RL-12	−	+	++	−
C10	−	+	++	−
Bac-1.2F5	−	+	++	−
C6*	−	−	−	−

W, weeks of exposure; +, weak positive; ++, positive; +++, strong positive.
*Negative control. No *His*-1 transcripts were detectable in these cells by Northern blot.

antisense probe was detected in the epithelial cells of the developing choroid plexus from a day 12 embryo (Figure 2A, panel 2) but was absent from other tissues at this developmental stage. An enlargement of the choroid plexus result is shown in panel 3 of Figure 2B to illustrate that the hybridization was specific for the epithelial cells lining the choroid plexus and was absent from both the inner choroid plexus stroma and the ependymal cells that are continuous with the choroid plexus epithelium. No hybridization was detected in the adjacent neuronal tissue. No *His*-1 transcripts were identified in any other embryonic tissue, although it is difficult to rule out the possibility that the gene is expressed during a brief stage of development that was not examined in this study.

None of the adult mouse tissues revealed high levels of *His*-1 transcripts (detectable within 2 weeks of exposure). However, after extended exposure times (4 to 6 weeks), low levels were identified in the epithelial cells of the prostate (Figure 3A, panel 2), seminal vesicle (Figure 3B, panel 2), and the nonglandular portion of the stomach (Figure 2B, panel 2). Hybridization in the stomach was restricted to the cells in the stratified squamous epithelium of the nonglandular forestomach and was notably absent from the outer keratinized layer (the keratinized layer is detached in the upper left-hand portion of the stomach section (Figure 2B, panel 1). The adjacent glandular epithelium of the mouse stomach lacked detectable *His*-1 transcripts (data not shown). A summary of the *in situ* hybridization findings on embryonic and adult mouse tissues is shown in Table 3. *In situ* hybridization was not performed on human tissues because a cDNA probe is not available for the human gene.

Northern Blot Analysis of Choroid Plexus Carcinomas

As the choroid plexus epithelium does not provide sufficient tissue for Northern blot analysis, we took advantage of a mouse model of choroid plexus epithelial carcinogenesis.[26] These transgenic mice develop carcinomas of the choroid plexus due to the targeting of the SV40 large T oncogene to the choroid plexus epithelium.[26] Northern analysis of RNA extracted from highly aggressive carcinomas that developed in four separate animals identified an alternatively sized *His*-1-related transcript in each of the four tumors (Figure 1B, lanes 1 to 4). This shorter *His*-1 transcript was not due to a structural rearrangement within the *His*-1 gene, as assessed by Southern blot analysis of genomic DNA digests that contained the entire *His*-1 gene in a 12-kb region (data not shown). The 1-kb choroid-plexus-specific transcript most likely represents alternative splicing into the region of the *His*-1 gene occupied by the probe, rather than a related gene, because the murine probe that detects it does not identify related genes in the vertebrate genome.[5,6]

Discussion

The *His*-1 gene has been previously shown to encode a 3-kb spliced and polyadenylated RNA that lacks protein coding potential.[5,6] This is an unusual feature that places *His*-1 in a category of RNAs that are attracting increasing interest because they are believed to function in the absence of a protein product.[7-15] The *His*-1 gene was first identified as a target for retroviral activation in mouse retrovirus-induced leukemias.[4,5] This observation, combined with the apparent noncoding nature of the *His*-1 RNA, suggests that further analysis of this gene may shed insight into novel pathways of gene function and oncogenesis. In this report, we have shown that the *His*-1 RNA has a highly restricted pattern of gene expression and that its expression level is generally low. The ability to detect *His*-1 transcripts by Northern blot analysis was clearly associated with the transformed phenotype in mouse tissues. *His*-1 expression was identified in five leukemic cell lines and
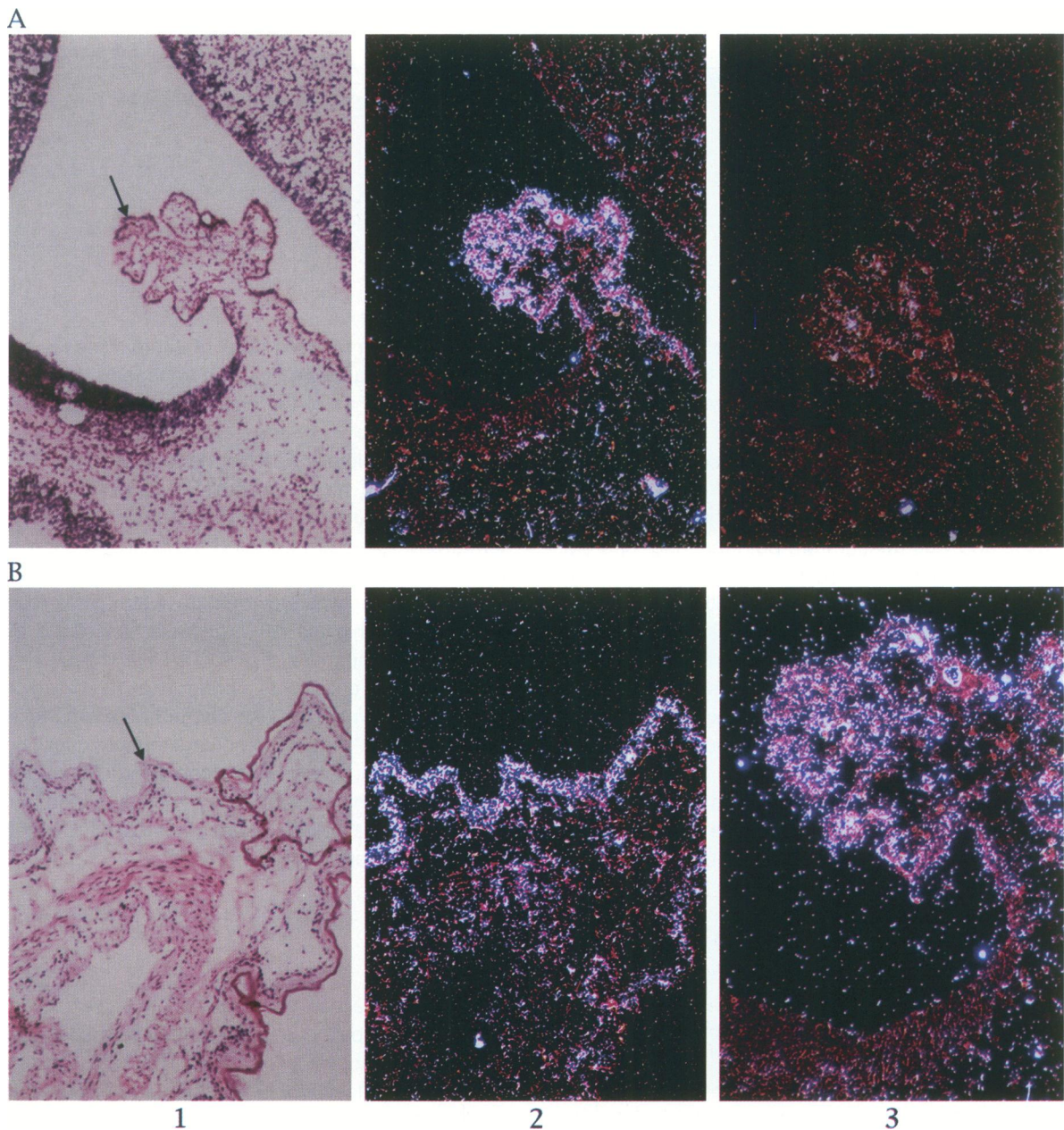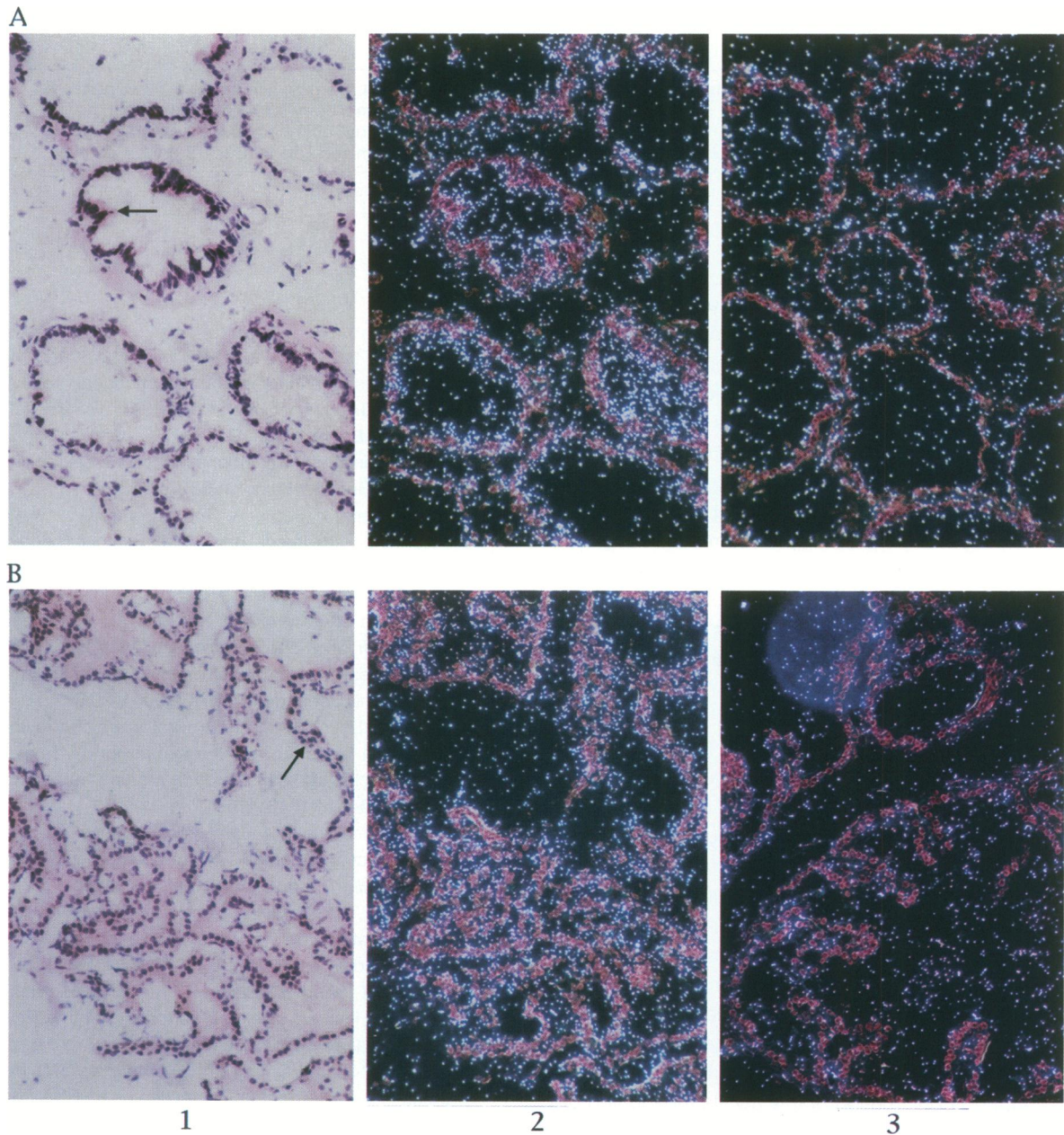
Figure 2. *Identification of* His-1 *transcripts by* in situ *hybridization of the developing choroid plexus* (**A1**) *and adult mouse stomach* (**B1**). *Hybridization to the antisense* His-1 *riboprobe was identified by dark-field microscopy in the epithelial cells of the choroid plexus from a day 12 embryo* (**A2** *and* **B3**). *Background hybridization to the control riboprobe in the sense orientation is shown for the choroid plexus section in* **A3**. *Hybridization to the antisense probe was also identified in the stratified epithelium of the nonglandular stomach* (**B2**). *The* **arrows** *indicate the location of the epithelial cells* (**A1** *and* **B1**).

four choroid plexus carcinomas but was not detected in all other normal tissues examined by Northern analysis. This expression was composed of the 3-kb *His*-1 transcript that has been previously published,[5] in addition to a novel 5-kb *His*-1-related RNA in a radiation-induced mouse T-cell leukemia and a novel 1-kb *His*-1-related RNA in four murine choroid plexus carcinomas (Figure 1). As the *His*-1 probe did not detect any rearrangements of the gene and is

known to exclusively detect the *His*-1 gene,[5] we conclude that these different sized RNAs are most likely derived from alternative splicing events within the *His*-1 gene.

We considered the possibility that the *His*-1 gene might be developmentally regulated, as two other noncoding RNA molecules have been reported to play a role in mammalian embryogenesis.[14,26] *His*-1 RNA was not detected in the majority of murine em-

Figure 3. *Identification of* His-*1 transcripts by* in situ *hybridization of the adult mouse prostate* (row **A**) *and seminal vesicle* (row **B**). *Hybridization to the antisense* His-*1 riboprobe was identified by dark-field microscopy in the epithelial cells of the prostate* (**A2**) *and the seminal vesicle* (**B2**). *The* arrows *on the bright-field images indicate the location of the hybridizing epithelial cells in prostate and seminal vesicle* (**A1** *and* **B1***, respectively*). *Background hybridization to the control riboprobe in the sense orientation is shown in* panel 3 *for each tissue.*

bryonic tissues by *in situ* hybridization, with the striking exception of the developing choroid plexus. Hybridization was specific to the choroid plexus epithelium and was absent from the adjacent neuronal tissue. Although the choroid plexus epithelium is contiguous with the ependymal lining of the ventricle, it is interesting that hybridization was restricted to the specialized ependymal cells that line the choroid plexus, suggesting that its function is specific to this

epithelium. These *in situ* hybridization results were supported by Northern blot analysis, which identified a *His*-1-related RNA in four independently arising choroid plexus carcinomas. We utilized a transgenic mouse model of choroid plexus carcinoma,[26] as human malignancies of the choroid plexus are rare, accounting for less than 1% of all human brain tumors.[27] The identification of this choroid-plexus-specific RNA is a striking result as the ability to detect

Table 3. *Summary of* His-1 *Gene Expression by in Situ Hybridization in Normal Mouse Tissues*

	Positive	Negative
Adult tissues	Stomach, seminal vesicle, prostate	Bone marrow, spleen, thymus, lymph node, liver, pancreas, duodenum, jejunum, ileum, large intestine, brain, lung, heart, kidney, tongue, ovary, uterus, testis, skin
Embryonic tissues	Day 12 choroid plexus	All other tissues examined (days 10 to 12 and 14 to 19)

*Only segments of abdominal tissues were represented in embryonic specimens beyond day 15.

His-1 transcripts by Northern blot analysis has to date been limited to mouse leukemias (Table 1).

The epithelium of the normal choroid plexus undergoes active proliferation and differentiation during embryonic development, and this activity ceases by 2 weeks after birth.[28] As the *His*-1 gene has already been implicated in carcinogenesis,[4,5] it is interesting to speculate that its expression in the developing choroid plexus epithelium and in choroid plexus carcinomas may have relevance to its putative role as an oncogene. Our studies also identified *His*-1 expression in the epithelial cells of the prostate, seminal vesicle, and nonglandular stomach, although the expression levels in the male reproductive epithelia were less pronounced.

Sequence comparisons between the human and mouse homologues of the *His*-1 gene have suggested that the gene possesses an important function that has limited its divergence during vertebrate evolution.[6] Although this function is presently unknown, the data presented in this study suggest that its function is restricted to epithelial cells and is present in only a limited number of tissues. Although the data showed no expression in any of the human tissues examined, it is difficult to rule out the possibility that differential splicing of the human gene would have prevented detection by the mouse probe, and confirmation of this awaits the cloning of a human *His*-1 cDNA. We are currently testing the hypothesis that the *His*-1 gene is under powerful constraints that limit transcription of its promoter and that release of these constraints may contribute to carcinogenesis. Further analysis of this unusual RNA may generate important information about carcinogenic pathways that are shared between epithelial and hematopoietic cells.

Acknowledgments

References

1. Tsichlis PN, Lazo PA: Virus-host interactions and the pathogenesis of murine and human oncogenic retroviruses. Curr Top Microbiol Immunol 1991, 171:95–171
2. Askew DS, Bartholomew C, Ihle JN. Insertional mutagenesis and the transformation of hematopoietic stem cells. Hematol Pathol 1993, 7:1–22
3. van Lohuizen M, Berns A. Tumorigenesis by slow-transforming retroviruses: an update. Biochim Biophys Acta 1990, 1032:213–235
4. Askew DS, Bartholomew C, Buchberg AM, Valentine MB, Jenkins NA, Copeland NG, Ihle JN. *His*-1 and *His*-2: identification and chromosomal mapping of two commonly rearranged sites of viral integration in a myeloid leukemia. Oncogene 1991, 6:2041–2047
5. Askew DS, Li J, Ihle JN. Retroviral insertions in the murine *His*-1 locus activate the expression of a novel RNA that lacks an extensive open reading frame. Mol Cell Biol 1994, 14:1743–1751
6. Li J, Rhodes JC, Askew: Evolutionary conservation of putative functional domains in the human homolog of the murine *His*-1 gene. Gene 1997 (in press)
7. Rastinejad F, Blau H. Genetic complementation reveals novel regulatory role for 3′ untranslated regions in growth and differentiation. Cell 1993, 72:1–20
8. Tycowski KT, Shu M-D, Steitz JA. A mammalian gene with introns instead of exons generating stable RNA products. Nature 1996, 379:464–466
9. Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the *H19* gene may function as an RNA. Mol Cell Biol 1990, 10:28–36
10. Brockdorff N, Ashworth A, Kay GF, McCabe VM, Norris DP, Cooper PJ, Swift S, Kastan S: The product of the mouse *Xist* gene is a 15kb inactive X-specific transcript containing no conserved orf and located in the nucleus. Cell 1992, 71:515–526
11. Brown CJ, Hendrich BD, Rupert JL, Lafreniere RG, Xing Y, Lawrence J, Willard HF. The human *XIST* gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 1992, 71:527–542
12. Hao Y, Crenshaw T, Moulton T, Newcomb E, Tycko B. Tumour-suppressor activity of *H19* RNA. Nature 1993, 365:764–767
13. Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A, Tilghman SM. Disruption of imprinting caused by

deletion of the *H19* gene region in mice. Nature 1995, 375:34–39

14. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. Requirement for *Xist* in X chromosome inactivation. Nature 1996, 379:131–137

15. Wevrick R, Kerns JA, Francke U. Identification of a novel paternally expressed gene in the Prader-Willi syndrome region. Hum Mol Genet 1994, 3:1877–1882

16. Trakhtenbrot L, Krauthgamer R, Resnitzky P, Haran-Ghera N. Deletion of chromosome 2 is an early event in the development of radiation-induced myeloid leukemia in SJL/J mice. Leukemia 1988, 2:545–550

17. Azumi JI, Sachs L. Chromosome mapping of the genes that control differentiation and malignancy in myeloid leukemic cells. Proc Natl Acad Sci USA 1977, 74:253–257

18. Schwarzbaum S, Halpern R, Diamond B. The generation of macrophage-like cell lines by transfection with SV40 origin defective DNA. J Immunol 1984, 132:1158–1162

19. Lieberman M, Declève A, Ricciardi-Castagnoli P, Boniver J, Finn OJ, Kaplan HS. Establishment, characterization and virus expression of cell lines derived from radiation- and virus-induced lymphomas of C57BL/Ka mice. Int J Cancer 1979, 24:168–177

20. Badley JE, Bishop GA, John T, Frelinger JA: A simple rapid method for the purification of polyA⁺ RNA. Biotechniques 1988, 6:114–116

21. Askew DS, Ashmun RA, Simmons BC, Cleveland JL. Constitutive *c-myc* expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. Oncogene 1991, 6:1915–1922

22. Katzav S, Martin-Zanca D, Barbacid M. *Vav*, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. EMBO J 1989, 8:2283–2290

23. Witte DP, Aronow BJ, Stauderman ML, Stuart WD, Clay MA, Gruppo RA, Jenkins SH, Harmony JA. Platelet activation releases megakaryocyte-synthesized apolipoprotein J, a highly abundant protein in atheromatous lesions. Am J Pathol 1993, 143:763–773

24. Wilkinson DG, Bhatt S, McMahon AP. Expression pattern of the FGF-related protooncogene *int*-2 suggests multiple roles in fetal development. Development 1989, 105:131–136

25. Brunkow ME, Tilghman SM. Ectopic expression of the *H19* gene in mice causes prenatal lethality. Genes Dev 1991, 5:1093–1101

26. Van Dyke TA, Finlay C, Miller D, Marks J, Lozano G, Levin AJ. Relationship between simian virus 40 large tumor antigen expression and tumor formation in transgenic mice. J Virol 1987, 61:2029–2032

27. Pierga J-Y, Kalifa C, Terrier-Lacombe M-J, Habrand J-L, Lemerle J. Carcinoma of the choroid plexus: a pediatric experience. Med Pediatr Oncol 1993, 21:480–487

28. Symonds H, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T, Van Dyke T. p53-dependent apoptosis suppresses tumor growth and progression *in vivo*. Cell 1994, 78:703–711