HLS5, a Novel RBCC (Ring Finger, B Box, Coiled-coil) Family Member Isolated from a Hemopoietic Lineage Switch, Is a Candidate Tumor Suppressor*

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Hemopoietic cells, apparently committed to one lineage, can be reprogrammed to display the phenotype of another lineage. The J2E erythroleukemic cell line has on rare occasions developed the features of monocytic cells. Subtractive hybridization was used in an attempt to identify genes that were up-regulated during this erythroid to myeloid transition. We report here on the isolation of hemopoietic lineage switch 5 (Hls5), a gene expressed by the monocytoid variant cells, but not the parental J2E cells. Hls5 is a novel member of the RBCC (Ring finger, B box, coiled-coil) family of genes, which includes Pml, Herf1, Tif-1α, and Rfp. Hls5 was expressed in a wide range of adult tissues; however, at different stages during embryogenesis, Hls5 was detected in the branchial arches, spinal cord, dorsal root ganglia, limb buds, and brain. The protein was present in cytoplasmic granules and punctate nuclear bodies. Isolation of the human cDNA and genomic DNA revealed that the gene was located on chromosome 8p21, a region implicated in numerous leukemias and solid tumors. Enforced expression of Hls5 in HeLa cells inhibited cell growth, clonogenicity, and tumorigenicity. It is conceivable that Hls5 is one of the tumor suppressor genes thought to reside at the 8p21 locus.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF009514, AF009513, AAC17945, AF145374, AF944189, and AF942463.

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Differentiated cells for each of the hemopoietic lineages develop from pluripotent stem cells (1), a process orchestrated via specific combinations of transcription factors (2, 3). Lineage-restricted structural/functional genes are gradually expressed as cells display the phenotype of mature cells. Although the process of lineage commitment was once considered irrevocable, data over the past decade indicate that the hemopoietic system may be more plastic than previously thought (4). Leukemic cells apparently committed to one lineage can be “reprogrammed” or undergo “lineage switching” and develop the appearance of another cell type (5, 6).

Early descriptions of lineage switching involved the conversion of B lymphoma cells overexpressing the raf and myc oncogenes into macrophages (5, 7). Other B cell to macrophage transitions involved a number of different oncogenes (8–11), whereas mast cells transformed with Bcr-abl have produced erythroid and megakaryocytic cells (12). A B cell to granulocytic switch has also been observed previously (13). The recent data with Pax5+/− mice are particularly noteworthy as committed B cells lacking the Pax5 gene could develop into macrophages, osteoclasts, dendritic cells, granulocytes, natural killer cells, and T cells upon appropriate stimulation (14, 15).

The combination and concentration of transcription factors within hemopoietic cells play key roles in dictating lineage fate (3, 16). Indeed, by manipulating the levels of transcription factors, the plasticity of the hemopoietic system has been demonstrated, e.g. increasing the concentration of GATA-1 in myelomonocytic cells produced thrombocytes, eosinophils, and erythroblasts (6). Similarly, overexpression of GATA-1 or NF-E2 in M1 monoblastoid cells generated erythroid and megakaryocytic cells (17, 18). In addition, overexpression of PU.1 in murine erythroleukemia cells induced an erythroid to myeloid lineage switch (19).

One example of a spontaneous lineage switch was the conversion of J2E erythroleukemic cells into monocytoid cells (20). The parental J2E cells differentiated morphologically and synthesized hemoglobin in response to erythropoietin (21, 22), whereas the monocytic variants (J2E-m)1 lacked all of the

1 The abbreviations used are: J2E-m, monocytic variant of J2E cell; dpc, days post-coitum; HLS or Hls, hemopoietic lineage switch; MLF1, myeloid leukemia factor 1; RACE, rapid analysis of the cDNA ends;
erythroid characteristics and displayed myeloid surface markers (20). To search for genes involved in this breach of lineage commitment, we have utilized cDNA representational difference analysis (RDA) (23). This PCR-based form of subtractive hybridization identified hemopoietic lineage switch 7 (Hls7), the murine homologue of human myeloid leukemia factor 1 (MLF1) (24, 25), as one molecule expressed exclusively in the myeloid variant J2E (26). Ectopic expression of MLF1 in parental J2E cells recapitulated the phenotypic changes associated with the erythroid to myeloid switch. Moreover, exogenous MLF1 impeded the differentiation of normal and immortalized erythroid cells and promoted myeloid maturation (23). These observations implicated MLF1 as an important hemopoietic lineage-determining gene.

In this study, we describe the isolation of Hls5 from the RDA between J2E cells and a myeloid counterpart, J2E-m2. Hls5 is a novel member of the Ring finger, B box, coiled-coil (RBCC) family of genes, which includes the well characterized tumor suppressor gene Pml (27) as well as Tif1-α (28), Herf1 (29), and Rfp (30). The human HLS5 gene mapped to chromosome 8p21, a locus involved in numerous leukemias and cancers (31). Significantly, expression of exogenous HLS5 suppressed the tumor-igenic phenotype of HeLa cells. Therefore, it is plausible that HLS5 is one of the tumor suppressor genes postulated to reside at 8p21.

EXPERIMENTAL PROCEDURES

Cell Culture—Cells were maintained in Dulbecco’s modified Eagle’s medium with 5% fetal calf serum, and cell viability was determined by eosin exclusion (22). Total RNA and protein isolated from various tissues and cell lines were analyzed by Northern blotting and immunoblotting as described previously (23, 32).

cDNA RDA—The RDA used was based on the method of Hubank and Schatz (33) and modified as described previously (23). Poly(A+) RNA isolated from J2E driver and J2E-m2 tester cell lines was converted to double-stranded DNA, digested with Sau3AI, and subjected to three cycles of subtraction. Fragments enriched by this procedure were subcloned into pGEM-T (Promega, Madison, WI) for further analysis.

cDNA and Genomic Library Screening—A cDNA library from the murine lymphohematopoietic progenitor cell line EML C.115 (34) was screened for full-length HLS5 using the RDA fragment as a probe. To confirm that the 5′ end of the cDNA had been isolated, a rapid analysis of the cDNA ends (RACE) strategy was adopted using the Marathon cDNA amplification kit (Clontech, Palo Alto, CA). A 129 SvJ murine genomic library in Lambda DASHII (Stratagene, La Jolla, CA) was screened for clones containing the HLS5 gene. The complete genomic sequence was obtained from two independent clones. The human HLS5 cDNA was isolated from a human fetal liver cDNA library (Clontech BD Biosciences), and two independent clones were sequenced.

Hls5 Constructs and Infection of HeLa Cells—A Myc epitope tag was introduced at the C terminus of HLS5 and HLS5 and then subcloned into pcDNA3 (Invitrogen) and pBabe-puromycin (35) to generate the pcDNA3-Hls5-Myc, pcDNA3-HLS5-Myc, and pBabe-Hls5-Myc, respectively. The PA317-packaging cell line (36) was transfected with linearized pBabe-Hls5-Myc, and viral supernatants were used to infect HeLa cells. Transfected HeLa cells were selected by puromycin (35) or those treated with nocodazole (100 ng/ml) were incubated overnight before Cdk1 immunoprecipitation (anti-Cdc2 p34; sc-54, Santa Cruz Biotechnology) and then stained with propidium iodide before analysis on a FACScan II instrument (BD Biosciences) as described in detail elsewhere (22, 38). TUNEL (transferrase dUTP nick and label) assays were performed according to the manufacturer’s instructions (Roche Applied Science). Transfected HeLa cells or those treated with nocodazole (100 ng/ml) were incubated overnight before Cdk1 immunoprecipitation (anti-Cdc2 p34; sc-54, Santa Cruz Biotechnology, Santa Cruz, CA) and assayed for kinase activity as we have described previously (32) using [γ-32P]ATP and histone H1. Phosphorylated protein was separated by SDS-PAGE, transferred, and analyzed by phosphorimaging device.

**Fig. 1.** RDA conducted between the erythroid (J2E) and a myeloid (J2E-m2) cell types. A, the left panel shows the cDNA representations generated prior to the analysis. The right panel shows the eight individual clones obtained following three rounds of subtraction. A, homologies of Hls1–8 and expression patterns determined by Northern blotting; nd, not determined; MW, molecular weight.

**Table 1.** Homology and Expression of Hls1–8 in J2E and J2E-m2 Cells.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Homology</th>
<th>Expression (mRNA)</th>
</tr>
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<tbody>
<tr>
<td>Hls1</td>
<td>Cyclooxygenase 2</td>
<td>J2E</td>
</tr>
<tr>
<td>Hls2</td>
<td>Glutamate carboxypeptidase</td>
<td>J2E</td>
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<tr>
<td>Hls3</td>
<td>Mitochondrial DNA</td>
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<tr>
<td>Hls4</td>
<td>Reg. subunit PKC</td>
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<td>Hls5</td>
<td>Novel RBCC</td>
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<tr>
<td>Hls6</td>
<td>GDP dissociation inhibitor D4</td>
<td>J2E</td>
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<tr>
<td>Hls7</td>
<td>Myeloid Leukemia Factor-1</td>
<td>J2E</td>
</tr>
<tr>
<td>Hls8</td>
<td>Hls1,2 concatamer</td>
<td>nd</td>
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Clonogenicity and Tumorigenicity Assays—For clonogenicity assays, a layer of 0.8% agar was placed in 6-cm dishes and then 10⁶ HeLa cells were seeded in a 0.3% agar layer as described above (37). Colonies were counted 3 weeks after initial plating. Tumorigenicity was determined by injecting 10⁶ cells subcutaneously into 6–8-week-old female BALB/c nude mice. Tumor volume was determined twice a week using calipers.

**Cell Cycle and Apoptosis Analyses—**HeLa cells transfected with vector alone, pcDNA3-Hls5-Myc, or pcDNA3-HLS5-Myc using LipofectAmine 2000 were cultured for 24–72 h and then stained with propidium iodide before analysis on a FACScan II instrument (BD Biosciences) as described in detail elsewhere (22, 38). TUNEL (transferrase dUTP nick and label) assays were performed according to the manufacturer’s instructions (Roche Applied Science). Transfected HeLa cells or those treated with nocodazole (100 ng/ml) were incubated overnight before Cdk1 immunoprecipitation (anti-Cdc2 p34; sc-54, Santa Cruz Biotechnology, Santa Cruz, CA) and assayed for kinase activity as we have described previously (32) using [γ-32P]ATP and histone H1. Phosphorylated protein was separated by SDS-PAGE, transferred, and analyzed by phosphorimaging device.

**In Situ Hybridization and Fluorescent in Situ Hybridization—**Whole mount in situ hybridization on mouse embryos was performed as described previously (39). A digoxigenin-labeled riboprobe was prepared by transcription of a 903-bp fragment from the 3′ -untranslated region of HLS5. For fluorescent in situ hybridization, a 2.4-kb fragment of the human HLS5 gene was generated by PCR, labeled with biotin-14-dATP and hybridized to metaphase spreads from two normal males using a modification of the previously described technique (40). Images of met-
Hls5 is a novel member of the RBCC family. A, cDNA and amino acid sequence of murine Hls5. Sequence analysis of the cDNA (nucleotides numbered above) identified a 501 amino acid open reading frame (amino acids numbered below). The various domains are boxed and displayed at the right. B, structural organization of Hls5 and other members of the RBCC family of proteins. C, genomic structure of murine Hls5 with introns boxed. D, approximately 1 kb of Hls5 promoter region with potential transcription factor binding sites and predicted transcription initiation site (TIS) identified. UTR, untranslated region.
aprase preparations were captured by a cooled CCD camera using the ChromoScan image collection and enhancement system (Applied Imaging International Ltd.).

RESULTS

Isolation of Hls5, a Novel RBCC Family Member—In an attempt to identify genes that were activated when J2E cells became monocytic, an RDA was conducted between the erythroid and myeloid cell lines. Fig. 1A shows the representations from J2E and the myeloid variant J2E-m2. Following three rounds of PCR-based subtraction, eight clones were isolated, which were termed Hls1–8 (Fig. 1B). Hls1 and Hls6 represented previously characterized myeloid genes, whereas Hls7 is the murine homologue of MLF1 (23–25). Hls3, Hls4, and Hls8 were not studied further because they included a mitochondrial contaminant, a known signaling molecule and a concatamer.

When originally isolated, Hls2 was a novel molecule. Full-length Hls2 was represented by a 1,716-bp cDNA and contained an open reading frame encoding a protein of 433 amino acids (GenBank™ accession numbers AF009513 and AAC17945). However, overexpression and underexpression studies of Hls2 in a variety of hemopoietic cell lines failed to alter their phenotype. A yeast two-hybrid screen did not identify any binding partners for Hls2. This gene was subsequently shown to have 88% identity and 92% homology with the secreted protein, human plasma glutamate carboxypeptidase (41), and was not analyzed further.

The 361-bp Hls5 fragment (GenBank™ accession number AF009514) was used to screen a murine cDNA library, and then RACE was employed to delineate the 5′ end of the cDNA. These approaches generated a 3,687-bp cDNA (GenBank™ accession number AF145374) with a 501 amino acid open reading frame (Fig. 2A). The putative translation initiation site contained a consensus Kozak (42, 43) sequence.

Data base searches revealed that the predicted protein had significant homology with several members of the RBCC family of proteins (Fig. 2B). The highest similarity was found with the Rfp (30, 44), SS-a/Ro-52 (45), and Herf1 proteins (29). In addition, these molecules contained an SPRY/B30.2/NHL domain (46). Hls5 also displayed structural similarities to Pml (27) and Tfi1-α (28) with the exception that these molecules have two B-box domains and lack the SPRY region. KIAA1098 and TRIM55 represent Hls5-related expressed sequence tags (47, 48). While this paper was in preparation, Kimura et al. (49) reported the cloning of a cDNA induced by macrophage colony-stimulating factor they named MAIR, which is identical to Hls5.

Isolation of genomic clones revealed that the gene contained six exons and five introns ranging in size from 728 bp to 5.6 kb (Fig. 2C). Potential binding sites for numerous transcription factors were identified within the putative promoter region (Fig. 2D).

Expression of Hls5 and Intracellular Localization—Northern blotting was used to determine the organs in which Hls5 was expressed. Fig. 3A shows that Hls5 mRNA, ~4 kb in length, was detected in a wide variety of adult organs. Abundant mRNA was present in brain, heart, kidney, spleen, skeletal muscle, and thymus with lower levels found in stomach, large intestine, and bone marrow. Interestingly, Hls5 was not detectable in adult liver, whereas it was present in fetal liver.

As Hls5 was isolated from an erythroid to myeloid lineage switch, the expression pattern of Hls5 was examined in a panel of hemopoietic cell lines (Fig. 3B). Although the levels varied considerably, Hls5 mRNA was present in most erythroid, myeloid, and lymphoid lines tested; however, the transcript was not detected in J2E cells or the pre-B cell line 1-Bra. The difference in Hls5 expression between J2E cells and other erythroleukemic lines (F4N, 707) could be attributed to immortalization of these cells at different stages of red cell maturation in which J2E cells displayed a more mature phenotype than the other erythroid lines (23).

The predicted molecular mass of the Hls5 protein was 59 kDa; however, immunoblotting of transfected COS cells identified a 66-kDa protein (Fig. 3C). The endogenous protein could not be detected in a variety of cell lines, presumably because of low levels of expression.

Indirect immunofluorescence and confocal microscopy were then employed to determine the intracellular location of Hls5.
protein. As the antisera produced to Hls5 were unable to detect the subcellular localization of the endogenous protein, COS and HeLa cells were transiently transfected with a Myc-tagged Hls5 construct. Fig. 3D shows that Hls5 protein was detected in discrete cytoplasmic granules as well as punctate nuclear bodies. The cytoplasmic localization did not coincide with markers for Golgi, mitochondria, or lysosomes, and the nuclear spots were not PML bodies (data not shown). In cells expressing higher levels of Hls5, the protein also concentrated in the perinuclear region. Similar subcellular compartmentalization of Hls5 was observed in NIH3T3 and 293T cells (data not shown).

Expression of Hls5 during Embryogenesis—To pursue the expression profile of Hls5 during embryogenesis, whole mount in situ hybridization analysis was performed over a range of embryonic stages. At 10.5 days post-coitum (dpc), Hls5 expression was detected in branchial arches 1 and 2 and the frontonasal process, limb buds, spinal cord, and dorsal root ganglia (Fig. 4). There was also evidence of relatively weak somite-associated staining. Hemi-sectioning of the embryo confirmed staining in the dorsal root ganglia and revealed that neural expression was predominantly in the dorsal half of the spinal cord, although weaker expression was also detected in the ventral region (data not shown). At 12.0 dpc, Hls5 was expressed primarily in the limbs and transiently in the developing eye. By 13.5 dpc, expression in the limb was restricted to the interdigital regions and expression was evident in the telencephalic region of forebrain. This analysis suggests that expression of Hls5 in the developing embryo is far more restricted than that seen in adult organs.

Human HLS5 Localizes to Chromosome 8p21—A full-length human cDNA was isolated (GenBank™ accession number AF492463), and low levels of mRNA were detected in a number
of human organs and tissues (Fig. 5A). A 2.4-kb human genomic fragment (GenBank™ accession number AF494189) was used for fluorescent in situ hybridization, which showed that the gene was located on chromosome 8p21.1 (Fig. 5B). An analysis of the DNA databases and chromosome maps showed that expressed sequence tag KIAA1098 (47), which is identical to human HLS5, resided between markers D8S137GT and D8S131 (Fig. 5C). These data confirm that HLS5 is located at 8p21.1, a region that has been implicated in numerous leukemias and solid tumors, and is thought to contain at least one tumor suppressor gene (31).

**Hls5 Suppresses HeLa Cell Growth and Tumorigenicity**—As HLS5 resides within a tumor suppressor locus and is related to the known tumor suppressor gene Pml, which restricts cell growth (27, 37), the effects of ectopic Hls5 expression were investigated in HeLa cells. Using a retroviral vector, Hls5 protein expression was increased in several independent HeLa transfectants (Fig. 6A). Each clone expressing Hls5 grew slower (Fig. 6B) and generated fewer colonies in soft agar (Fig. 7, A and B). Significantly, the development of tumors in nude mice was reduced substantially in cells transfected with Hls5 (Fig. 7C). Together, these data indicate that Hls5 is capable of inhibiting the growth and tumorigenic phenotype of HeLa cells and is therefore a candidate tumor suppressor gene within the 8p21 locus.

It is noteworthy that within a few weeks of culture, Hls5 protein could no longer be detected in each of the Hls5 transfecants (data not shown). To circumvent the problem of reduced Hls5 expression following stable transfection, HeLa cells were also transiently transfected with Hls5. Fig. 8A shows that cells accumulated in G2/M phase of the cell cycle, indicating that one mechanism by which Hls5 may inhibit cell growth is by impeding passage through the cell cycle. Similar to nocodazole, a G2/M inhibitor, Hls5 increased the activity of cdk1 (Fig. 8B); however, no significant changes were observed in the levels of p53, cyclins A, B1, or E, Cdk1, Cdk2, p21 waf1, p27kip1, and p57kip2 or the tyrosine phosphorylation status of Cdk1 (data not shown).

Human HLS5 induced a similar build up of HeLa cells in G2/M 30 h post-transfection (Fig. 8C). Significantly, overexpression of both the murine and human genes ultimately led to increased cell death. Fig. 8C shows a marked accumulation of sub-G1 DNA 70 h after transfection, indicative of DNA breakdown and apoptosis. This effect was dependent on the level of HLS5, increasing the amount transfected correlated well with the degree of DNA degradation until saturation was reached (Fig. 8D). The increase in sub-G1 DNA coincided with reduced cell viability and increased TUNEL-positive cells (Fig. 8E). These data demonstrate that HLS5 overexpression induces cell death. Thus, the growth inhibitory effects of Hls5 may result from a combination of delayed transit through the cell cycle and elevated cell death.
In this paper, we report on the isolation of Hls5, a novel gene that was induced when the erythroleukemic J2E cell line spontaneously developed a myeloid phenotype. Hls5 is a new member of the growing family of RBCC proteins. Other members include Pml (27), Herf1 (29), TIF1-α (28, 50), SS-A/Ro (45), and Rfp (30, 44). The tripartite organization of the RBCC domains is evolutionarily conserved, suggesting that these proteins have functional similarities (48). It has been proposed that the coiled-coil domain of the RBCC family is required for homodimerization, whereas the other motifs are involved in recruitment of additional proteins, which specify intracellular compartmentalization (48). The subcellular localization of Hls5 in cytoplasmic granules and nuclear bodies is similar to that observed with a striking number of RBCC proteins (44, 48). Whereas these cytoplasmic vesicles or speckles remain undefined, some punctate nuclear structures have been well characterized, e.g. PML bodies (27, 51).

Pml, Rfp, and Tif-1α are RBCC family members involved in chromosomal translocations, which induce neoplasms (50–55), with PML being recognized as a bona fide tumor suppressor gene (27, 37, 38, 56). Efp, another RBCC family member, is associated with the development of breast cancer (57). The localization of human HLS5 to chromosome 8p21 is significant because this locus is implicated in several leukemias and solid tumors. It has been proposed that one or more tumor suppressor genes reside on the short arm of chromosome 8 (31, 58–65). This region has been associated with numerous cancers including prostate, breast, lung, bladder, ovary, and liver (58, 59, 61, 62, 64–68). Importantly, three studies on liver, breast, and colon cancer have mapped a deleted region, which overlaps with the location of HLS5 (59, 62, 68). Like Pml (69), Hls5 was able to reduce the growth of HeLa cells, inhibit colony formation, and suppress tumor development, indicating that HLS5 is a potential tumor suppressor gene at this locus.

The reduced growth and tumorigenicity of HeLa cells expressing exogenous Hls5 may be explained, at least in part, by the accumulation of cells in G2/M. Impaired transit through the cell cycle could result in Hls5-expressing cells displaying slower growth rates. Additionally, overexpression of Hls5 induced apoptosis in HeLa cells. This observation is compatible with the independent isolation of HLS5 as MAIR, a pro-apoptotic molecule in TF-1 and NIH3T3 cells. MAIR reduced mitochondrial membrane potential and activated caspases 7, 8, and 9 but not caspase 3; however, additional studies are required to define the precise biochemical mechanisms for Hls5/MAIR-inducing cell death (49). It is possible that the effects of Hls5 are concentration-dependent, i.e. at lower levels, Hls5 interferes with cell cycle passage, whereas at higher concentrations the protein promotes cell death. Alternatively, restricting movement through the cell cycle may be a prelude to apoptosis. Therefore, a loss of a gene at 8p21.1, which affects the G2/M transition and apoptosis, is potentially deleterious to cells.

**FIG. 8.** *Hls5 slows cell cycle and induces apoptosis.* A, cell cycle analysis of HeLa cells transfected with Hls5 (gray line) or vector alone (black line). B, HeLa cells were either transfected with vector alone or treated with nocodazole. Cells were lysed 24 h later, and then Cdk1 was immunoprecipitated and assayed for kinase activity using [γ-32P]ATP and histone H1. C, cell cycle analysis of HeLa cells transfected with murine Hls5 or human HLS5, 30 and 70 h post-transfection. D, increasing amounts (1–4 μg) of HLS5 DNA were transfected into HeLa cells, and the percentage of cells with sub-G1 DNA content was determined after 72 h. Immunoblotting (IB) of cell lysates revealed that the amount of HLS5 protein present in the cells compared with the loading control ERK2. Each value is the mean ± S.D. (n = 3), p < 0.0001 (ANOVA, analysis of variance). E, HeLa cell death after transfection with Hls5 or vector alone was determined by eosin exclusion, whereas DNA degradation was demonstrated by TUNEL staining. *, p < 0.05. Each value is the mean ± S.D. (n = 3).

Hls5, a Potential Tumor Suppressor Gene

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Hls5, a Potential Tumor Suppressor Gene

It is noteworthy that Hls5 was isolated from an erythroid to myeloid lineage switch and MAIR was cloned as a gene induced by macrophage colony-stimulating factor (49). The expression of Hls5 in bone marrow, spleen, and fetal liver is consistent with a role for this gene in hemopoiesis. Significantly, deletions, translocations, and inversions of 8p21 have resulted in acute myeloid leukemias (70) and a loss of this region has been implicated as a secondary event producing a worse prognosis in Mantle cell lymphoma (60). Intriguingly, the 8p21 locus is also involved in 8(15;17) (p22;q21), which produces rare acute promyelocytic leukemias (71, 72). Although the vast majority of acute promyelocytic leukemias are caused by the t(15;17) in acute promyelocytic leukemias (71, 72) and a loss of this region has been associated with a role for this gene in hemopoiesis. Significantly, deletions in these sites of the developing embryo suggest that Hls5 may be involved in remodeling of tissues and/or apoptosis. These observations support the notion that Hls5/MAIR may be pro-apoptotic (49) and that this effect may be cell type-specific. The role of Hls5 in development may become clearer with the completion of Hls5−/− mice.

It is interesting to note that two of the genes obtained from the erythroid to myeloid lineage switch are implicated in cancer development. Hls7 (23) was also identified as MLF1, a new oncogene involved in translocations between chromosomes 3 and 5, that causes acute myeloid leukemias (24). Furthermore, elevated levels of wild type MLF1 have been observed in non-t(3;5) leukemias and are associated with poor prognosis (75). Conversely, Hls5 bears features of a tumor suppressor gene. It would appear that the RDA performed on this hemopoietic lineage switch has isolated two important genes, which when dysregulated may induce neoplasia.

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