American Journal of Pathology, Vol. 152, No. 1, January 1998

Developmentally Regulated Expression of the Novel Cancer Anti-Apoptosis Gene *Survivin* in Human and Mouse Differentiation

Colette Adida,*† Paul L. Crotty,* James McGrath,‡ Dominique Berrebi,§ Jacques Diebold,¶ and Dario C. Altieri*†

From the Departments of Pathology and Genetics,‡ Boyer Center for Molecular Medicine,† Yale University School of Medicine, New Haven, Connecticut; and the Departments of Pathology, Hopital Robert Debré§ and Hospital Hotel Dieu,¶ Paris, France*

Inhibitors of programmed cell death (apoptosis) may regulate tissue differentiation and aberrantly promote cell survival in neoplasia. A novel apoptosis inhibitor of the IAP gene family, designated survivin, was recently found in all of the most common human cancers but not in normal, terminally differentiated adult tissues. The expression of survivin in embryonic and fetal development was investigated. Immunohistochemistry and *in situ* hybridization studies demonstrated strong expression of survivin in several apoptosis-regulated fetal tissues, including the stem cell layer of stratified epithelia, endocrine pancreas, and thymic medulla, with a pattern that did not overlap with that of another apoptosis inhibitor, *bcl*-2. A sequence-specific antibody to survivin immunoblotted a single ~16.5-kd survivin band in human fetal lung, liver, heart, kidney, and gastrointestinal tract. In mouse embryo, prominent and nearly ubiquitous distribution of survivin was found at embryonic day (E)11.5, whereas at E15 to -21, survivin expression was restricted to the distal bronchiolar epithelium of the lung and neural-crest-derived cells, including dorsal root ganglion neurons, hypophysis, and the choroid plexus. These data suggest that expression of survivin in embryonic and fetal development may contribute to tissue homeostasis and differentiation independently of *bcl*-2. Aberrations of this developmental pathway may result in prominent re-expression of survivin in neoplasia and abnormally prolonged cell viability. *(Am J Pathol 1998, 152:43–49)*

Regulation of cell proliferation by programmed cell death (apoptosis) contributes to tissue and organ homeostasis during development and differentiation.[1] This process involves an evolutionarily conserved multistep cascade[2] and is controlled by proteins that promote or counteract apoptotic cell death. Specifically, inhibitors of apoptosis, most notably of the *bcl*-2 family,[3,4] maintain lymphoid homeostasis and morphogenesis in adult[5] and fetal[6] tissues. Deregulated expression of *bcl*-2 has also been implicated in cancer, by aberrantly prolonging cell survival and facilitating the insurgence of transforming mutations.[3,4]

In addition to *bcl*-2, a novel class of apoptosis inhibitors related to the Baculovirus *iap* gene[7] has been recently identified in human, mouse, and *Drosophila*. Highly evolutionarily conserved, these molecules are structurally organized in two to three Cys/His baculovirus IAP repeats (BIR) and a carboxyl-terminal RING finger.[8–13] Recombinant expression of IAP proteins counteracted various forms of apoptosis *in vitro*[9,11,13] and *in vivo*.[12] Recently, a novel gene encoding a structurally unique IAP apoptosis inhibitor, designated *survivin*, has been identified. Survivin is a ~16.5-kd cytoplasmic protein containing a single BIR and no RING finger, which inhibits apoptosis induced by growth factor (interleukin (IL)-3) withdrawal when transfected in B cell precursors.[14] At variance with *bcl*-2[3,4] or other IAP[9,11–13] proteins, survivin is undetectable in adult tissues but becomes prominently expressed in all of the most common human cancers of lung, colon, breast, pancreas, and prostate and in ~50% of high-grade non-Hodgkin's lymphomas *in vivo*.[14] Intriguingly, the coding strand of the survivin gene was highly homologous to the sequence of effector cell protease receptor-1 (EPR-1),[15] but oriented in the opposite direction,[14] thus suggesting the existence of two separate genes duplicated in a head-to-head configuration. To elucidate the potential role of survivin in cancer, we sought to investigate the expression of this novel apoptosis inhibitor in human and mouse development and the relationship with its complementary transcript EPR-1.[15]

Supported by NIH grants HL43773 and HL54131 and was done during the tenure of an American Heart Association Established Investigatorship Award to Dr. Altieri.

Accepted for publication October 17, 1997.

Address reprint requests to Dr. Dario C. Altieri, Yale University School of Medicine, BCMM 436B, 295 Congress Avenue, New Haven, CT 06536. E-mail: dario.altieri@yale.edu.

Materials and Methods

Tissue Procurements

Tissue samples from 10- and 21-week-old human fetuses were collected after therapeutic abortion. Samples were fixed in formalin and embedded in paraffin blocks, and 5-μm tissue sections were cut and put on high adhesive slides. Mouse embryos of embryonic day (E) 11.5, 15, or 21 were collected, fixed, and paraffin embedded before preparation of tissue slides, as described above.

Synthetic Peptides and Antibodies

A peptide duplicating the EPR-1 predicted cytoplasmic sequence[15] A^{306}KSGSFSVGQWMKPASAIRS^{325} was synthesized, confirmed for amino acid composition and mass spectrometry, coupled to keyhole limpet hemocyanin in a 1:1 ratio (100 μg), and injected subcutaneously into a rabbit in complete Freund's adjuvant. After a 4-week interval, animals were boosted with subcutaneous injection of 100 μg of peptide in incomplete Freund's adjuvant and sequentially boosted and bled at alternate weeks. Purification of the anti-EPR-1 antibody, designated TAIL, was carried out by affinity chromatography on a peptide-Sepharose matrix (5 mg/ml peptide) with elution of specific IgG in 1 mmol/L glycine, pH 2.5. An affinity-purified antibody raised against the survivin sequence A^{3}PTLPPAWQPFLKDHRI^{19} was used and characterized previously.[14]

Immunoblotting

Aliquots of detergent-solubilized extracts of human T leukemia Jurkat, monocytic THP-1, or 293 human embryonic kidney cells (American Type Culture Collection, Rockville, MD) were separated by electrophoresis on a 5 to 20% SDS-polyacrylamide gradient gel under nonreducing conditions. Samples were electroblotted to Immobilon membranes (Millipore Corp., Bedford, MA) at 450 mAmps for 2 hours at 4°C, blocked in TBS plus 5% dried milk, and incubated with 10 μg/ml nonimmune rabbit IgG (Tago, Burlingame, CA) or anti-EPR-1 TAIL antibody for 2 hours at 22°C. After washes, the membrane was incubated with a 1:5000 dilution of peroxidase-conjugated goat anti-rabbit IgG, and binding of the primary antibody was visualized by enhanced chemiluminescence (ECL, Amersham International, Little Chalfont, UK), according to the manufacturer's specifications. Homogenized tissue samples of human fetal gastrointestinal tract, liver, heart, lung, and kidney were processed for immunoblotting as described above, except that binding of the anti-survivin antibody (10 μg/ml) was revealed by addition of alkaline-phosphatase-conjugated goat anti-rabbit IgG and tetrazolium salts (Promega Corp., Madison, WI), as described.[14]

Immunohistochemistry and in Situ Hybridization

Five-micron sections from formalin-fixed, paraffin-embedded human fetus or mouse embryo tissues were deparaffinized in xylene and rehydrated in graded alcohol with quenching of endogenous peroxidase activity by treatment with 2% H_2O_2 in methanol. For immunostaining, the slides were boiled for 5 minutes in a standard pressure cooker, blocked in 10% normal goat serum, and incubated with affinity-purified anti-survivin or anti-EPR-1 TAIL antibody (20 μg/ml) for 14 hours at 4°C. After washes, the slides were incubated with biotin-conjugated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 30 minutes at 22°C, followed by streptavidin-conjugated peroxidase (Boehringer Mannheim, Indianapolis, IN) and 3′,3′-diaminobenzidine, and counterstained with hematoxylin. In control experiments, the primary antibody was substituted with normal goat serum. For *in situ* hybridization, 1 μg of the survivin cDNA in pcDNA3 (Invitrogen Corp., San Diego, CA) was completely digested with *Eco*RI and transcribed in the antisense orientation using T7 RNA polymerase in the presence of digoxigenin 11-uridine-5′ triphosphate (Boehringer Mannheim). Tissue slides were coated with 1% gelatin, 0.1% chrome-alum, baked at 120°C for 2 hours, and stored dust-free at 22°C. Sections were deparaffinized and rehydrated through graded alcohol, digested with proteinase K (1 μg/ml in 100 mmol/L Tris/HCl, pH 8.7, 50 mmol/L EDTA) for 30 minutes at 37°C, and acetylated in 0.25% acetic anhydride acid and 100 mmol/L triethanolamine, pH 8.0, for 10 minutes at 22°C. Detection of survivin mRNA in human or mouse tissues was carried out by *in situ* hybridization of the survivin riboprobe in 4X SSC, 1X Denhardt's solution, 50% deionized formamide, 250 μg/ml yeast tRNA, 500 μg/ml salmon sperm DNA, and 5% dextran for 16 hours at 50°C. After washes in 2X SSC for 90 minutes at 48°C, immobilized digoxigenin was detected using an anti-digoxigenin monoclonal antibody (Boehringer Mannheim) at a 1:3000 dilution and revealed by alkaline phosphatase staining with nitroblue tetrazolium/5′-bromo-4′-chloro-3′-indolyl phosphate cytochemical stain.

Results

Immunochemical Characterization of Anti-EPR-1 TAIL Antibody

In immunoblotting, the affinity-purified anti-EPR-1 sequence-specific TAIL antibody recognized a single band of ~62 kd from detergent-solubilized extracts of T-lymphoblastoid Jurkat and monocytic THP-1 cells (Figure 1), in agreement with previous observations.[15] In contrast, the TAIL antibody did not recognize specific bands in human embryonic 293 cells, and no bands were immunoblotted by control rabbit IgG in any cell type tested under the same experimental conditions (Figure 1).

Differential Expression of Survivin and EPR-1 in Human Fetal Tissues

Consistent with the presence of survivin transcripts in human fetal mRNA by Northern hybridization,[14] a se-

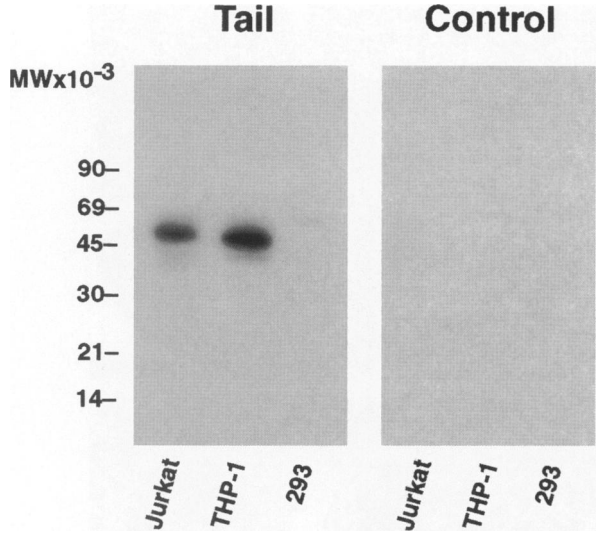

Figure 1. Immunochemical characterization of anti-EPR-1 TAIL antibody. Detergent-solubilized extracts of T-lymphoblastoid Jurkat, monocytic THP-1, or embryonic 293 cells were separated on a 5 to 20% SDS-polyacrylamide gradient gel, electroblotted to nylon membranes, and incubated with 10 μg/ml nonimmune rabbit IgG (control) or affinity-purified sequence-specific anti-EPR-1 TAIL antibody (TAIL) for 2 hours at 22°C. After washes, the membrane was incubated with goat anti-rabbit IgG, and binding of the primary antibody was visualized by enhanced chemiluminescence. Molecular weight markers in kilodaltons are on the left.

quence-specific antibody to survivin reacted with many fetal tissues by immunohistochemistry (Table 1). The expression of the survivin-related complementary gene product EPR-1[14] in fetal tissues was also investigated with the TAIL antibody by immunohistochemistry (Table 1). Prominent staining for survivin was observed in proximal tubules of the kidney, lung alveolar epithelium, endometrial glands, epidermis, thymic medulla, endocrine pancreatic islets, the neurons of the spinal cord, and the axial mesenchymal space surrounding the somites (Table 1 and see below). In the skin, survivin staining was particularly accentuated in the basal layer of the interdigital space. A potential reciprocal relationship between EPR-1 and survivin in 21-week fetal tissues was investigated. In the canalicular stage of fetal lung, survivin was prominently expressed in the acinar tubules lined by alveolar cells but not in the epithelium of large bronchioles or the lung mesenchyme (Figure 2a, inset). In pancreas, survivin was present in endocrine islets, and absent in the exocrine epithelium (Figure 2c, inset). In contrast, no expression of EPR-1 was detected in fetal lung epithelium or mesenchyme (Figure 2b) or exocrine or endocrine pancreas (Figure 2d) under the same experimental conditions. In 21-week fetal kidney, expression of survivin was limited to the epithelial cells of proximal tubules, with no staining of glomeruli or Bowman's capsule (Figure 2e). In control experiments, immunoreactivity of survivin in fetal kidney was abolished by antibody preadsorption with the cognate immunizing peptide (Figure 2g). As a mirror image of this pattern, EPR-1 expression was observed in distal collecting tubules (Figure 2f) and in scattered mesangial cells in the glomeruli (Figure 2f and data not shown). In 21-week fetal liver, EPR-1 was strongly expressed in 50 to 80% of cells,

Table 1. Expression of Survivin/EPR-1 in Human Fetal Tissues

Tissue distribution	Survivin	EPR-1
Liver		
Hepatocytes	+(20%)	++(50–80%)
Bile duct	–	+
Kidney		
Proximal tubules	+++	+/–
Glomeruli	–	+/–(mesangial cells)
Collecting tubules	–	+++
Urothelium	–	+/–
Adrenal cortex	–	–
Lung		
Alveolar duct	++	–
Bronchial epithelium	–	–
Mesenchyme	–	–
Skin		
Epidermis	++	+
Interdigital space	++	ND
Dermis	–	–
Spinal cord		
Vertebral chondroblasts	++	+/–
Axial mesenchyme	++	–
Neurons	–	–
Placenta		
Endometrial glands	++	+
Placental villosity	–	–
Trophoblast	–	–
Eye		
Retina	–	–
Corneal epithelium	++	ND
Stroma	–	ND
Gastrointestinal tract		
Early crypts	++	ND
Villi	–	ND
Spleen	–	–
Thymus		
Medulla	++	–
Cortex	–	–
Pancreas		
Epithelial cells	–	–
Islets	+++	–

Immunohistochemical staining was carried out with affinity-purified anti-survivin or anti-EPR-1 TAIL antibody on paraffin-embedded sections of 21-week-old fetal tissues followed by 3′-3′-diaminobenzidine and hematoxylin counterstaining. ND, not determined.

whereas staining for survivin was observed in only 10 to 20% of liver cells, with a characteristic periportal and perivenular distribution (not shown). Finally, and consistent with its distribution in stratified epithelia (Table 1), strong expression of survivin mRNA was detected in epithelial cells at the bottom of early crypts in the fetal gastrointestinal tract by *in situ* hybridization (Figure 2h). In Western blots, the anti-survivin antibody recognized a single band of ~16.5 kd in tissue extracts of fetal gastrointestinal tract, liver, heart, lung, and kidney (Figure 3), in agreement with the predicted size and molecular organization of survivin.[14] In contrast, no specific bands were immunoblotted by nonimmune rabbit IgG under the same experimental conditions (Figure 3).

Expression of Survivin in Mouse Tissues

Similar to human fetal tissues, survivin was prominently expressed in the epithelium of the primitive gastrointestinal tract at E11.5 (Figure 4a, inset). At E15 to -21,

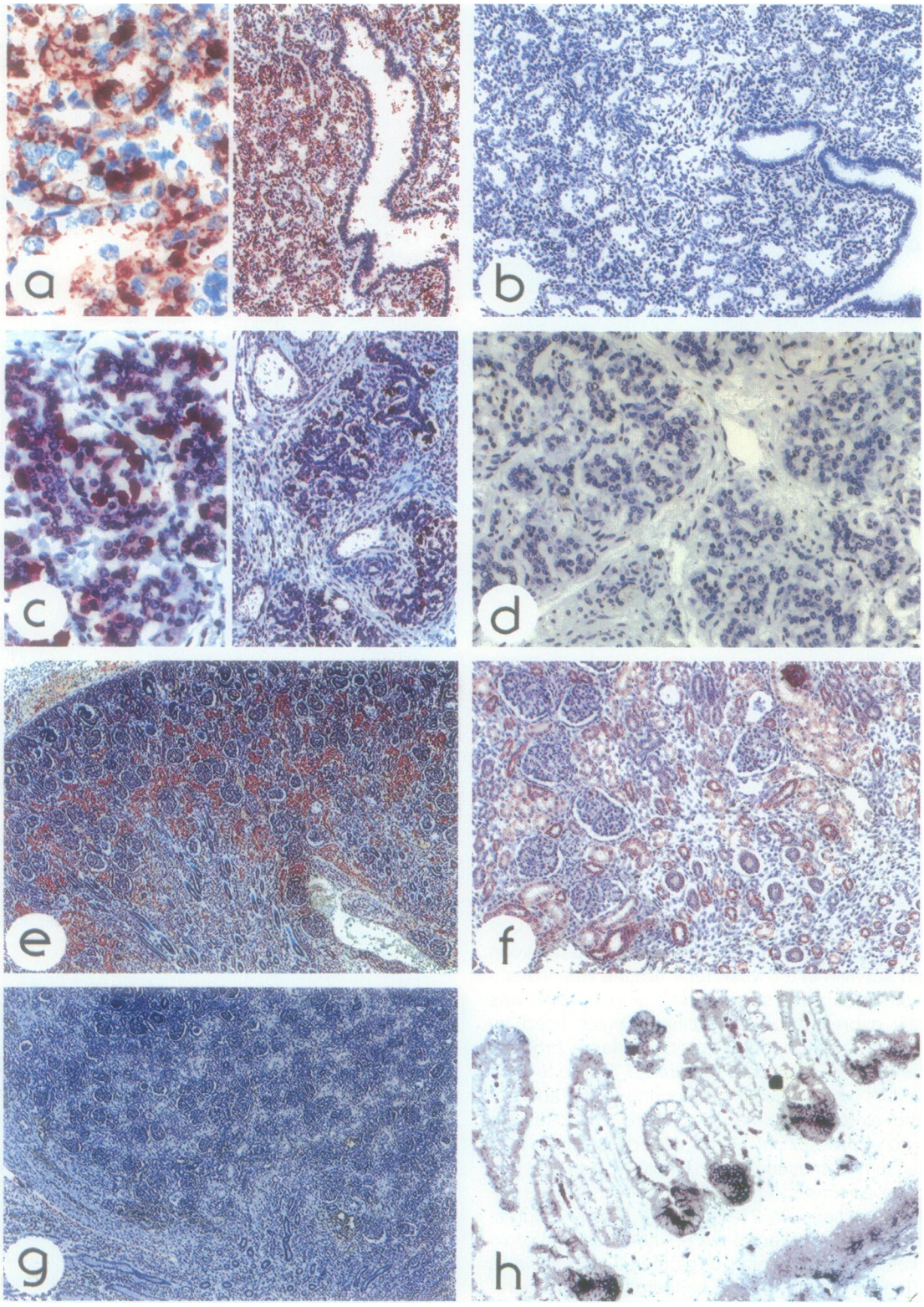

Figure 2. Differential developmental expression of EPR-1 and survivin in human fetal tissues. Tissue samples were fixed, paraffin embedded, and analyzed for EPR-1 or survivin expression by immunohistochemistry. Expression of survivin (**a**, **c**, and **e**) or EPR-1 (**b**, **d**, and **f**) in human fetal lung (**a** and **b**), pancreas (**c** and **d**), and kidney (**e** and **f**) by immunohistochemistry. **g:** Inhibition of survivin immunoreactivity with fetal kidney by antibody preadsorption with the cognate immunizing peptide. **h:** *In situ* hybridization of survivin mRNA in the epithelium of primitive crypts of fetal gastrointestinal tract. Original magnification, ×100 (**a–e** and **g**), ×200 (**f** and **h**), and ×400 (**insets**).

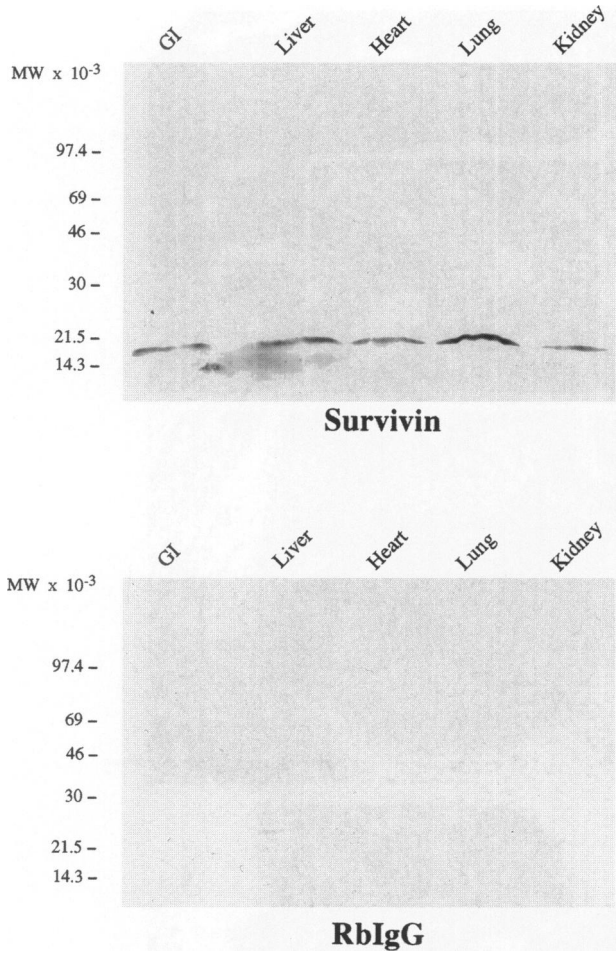

Figure 3. Immunoblotting of survivin expression in fetal tissues. The indicated homogenized tissue samples were separated on a 5 to 20% polyacrylamide gradient gel, transferred to nylon membranes, and immunoblotted with nonimmune rabbit IgG (RbIgG) or the anti-survivin antibody followed by alkaline-phosphatase-conjugated goat anti-rabbit IgG and tetrazolium salts. GI, gastrointestinal tract. Molecular weight markers in kilodaltons are on the left.

expression of survivin was observed in the distal bronchiolar epithelium of the lung and weakly in the lung mesenchyme (Figure 4b, inset). In neural-crest-derived cells, survivin was also prominently expressed in dorsal root ganglion neurons by *in situ* hybridization (Figure 4c) and immunohistochemistry (Figure 4d) in 60 to 70% of cells in the hypophysis (Figure 4e, inset) and in the choroid plexus (Figure 4f) by immunohistochemistry. At variance with this selected distribution at late embryonic stages, survivin was ubiquitously found in mouse tissues at the earlier stage of E11.5 by immunohistochemistry. Particularly, immunoreactivity for survivin was diffusely positive in primitive mouse brain at E11.5 (Figure 4g, inset) and decreased to undetectable levels at E15 to -21 (Figure 4h).

Discussion

In this study, we have shown that a novel apoptosis inhibitor, survivin,[14] was expressed in human fetus and mouse embryo in a developmentally regulated fashion. Its distribution in apoptosis-regulated tissues was distinct from that of *bcl-2*,[6] and its expression in fetal kidney, liver, pancreas, and lung was discordant and nonoverlapping with that of its related complementary transcript EPR-1.[15]

Survivin was recently described as a structurally unique IAP apoptosis inhibitor,[9,11–13] undetectable in normal adult tissues but prominently expressed in virtually all of the most common human cancers *in vivo*.[14] Consistent with the role of *bcl-2* in follicular lymphoma,[3,4] these findings suggested that apoptosis inhibition might be a more general feature of neoplasia, potentially contributing to disease progression and resistance to therapy.[14] Here, analysis of human fetal tissues and mouse embryo revealed that the selective distribution of survivin in cancer might reflect aberrations of a developmentally controlled pathway of gene expression. In this context, survivin was abundantly found in apoptosis-regulated fetal tissues, such as thymic medulla, and in the basal layer of stratified epithelia of skin and gastrointestinal tract, thus potentially preserving the viability of regenerating stem cells. Consistent with a key role of apoptosis in neuroectodermal development,[16] survivin was also expressed in fetal endocrine pancreas and mouse neural-crest-derived cells, including the dorsal root ganglion neurons, hypophysis, spinal cord, and the choroid plexus. As judged from sequential stage analysis of mouse embryo, survivin expression was strictly developmentally controlled, with a nearly ubiquitous presence of the molecule at early gestational stages of E11.5 and a more selective neuroendocrine distribution at E15 to -21. In this context, regulation of apoptosis by survivin may cooperate with growth factors,[17] neurotrophins,[18] and extracellular matrix signals[19] to control growth and differentiation of specific nuclei of the hypothalamus[20] and dorsal root ganglion cells.[21] However, the distribution of survivin in fetal and embryonic tissues was distinct from that of *bcl-2*,[6] with undetectable levels in adrenal glands, retina, and trophoblast and expression in *bcl-2*-negative epidermis and distal bronchiolar epithelium of the lung and in ~20% of periportal and perivenular fetal liver cells. This suggests that IAP and *bcl-2* proteins may mediate nonoverlapping anti-apoptosis mechanisms, a prediction recently corroborated by the ability of XIAP to function as a direct caspase inhibitor in a pathway unaffected by *bcl-2*.[22]

Second, the pattern of survivin expression in fetal kidney, lung, liver, and pancreas was discordant and mutually exclusive with that of EPR-1. As one of the most intriguing features of the survivin gene,[14] its coding sequence was highly homologous and complementary to that of the factor Xa receptor EPR-1.[15] Northern hybridization studies with single-strand-specific probes demonstrated the existence of separate transcripts for EPR-1 (1.3 kb) and survivin (1.9 kb), and Southern blots of human genomic DNA were consistent with the presence of multiple, evolutionarily conserved, EPR-1/survivin-related genes (unpublished observations). Although the mutually exclusive distribution of EPR-1 and survivin in fetal tissues suggested a coordinated mechanism of gene regulation, it is not known whether an EPR-1 mRNA

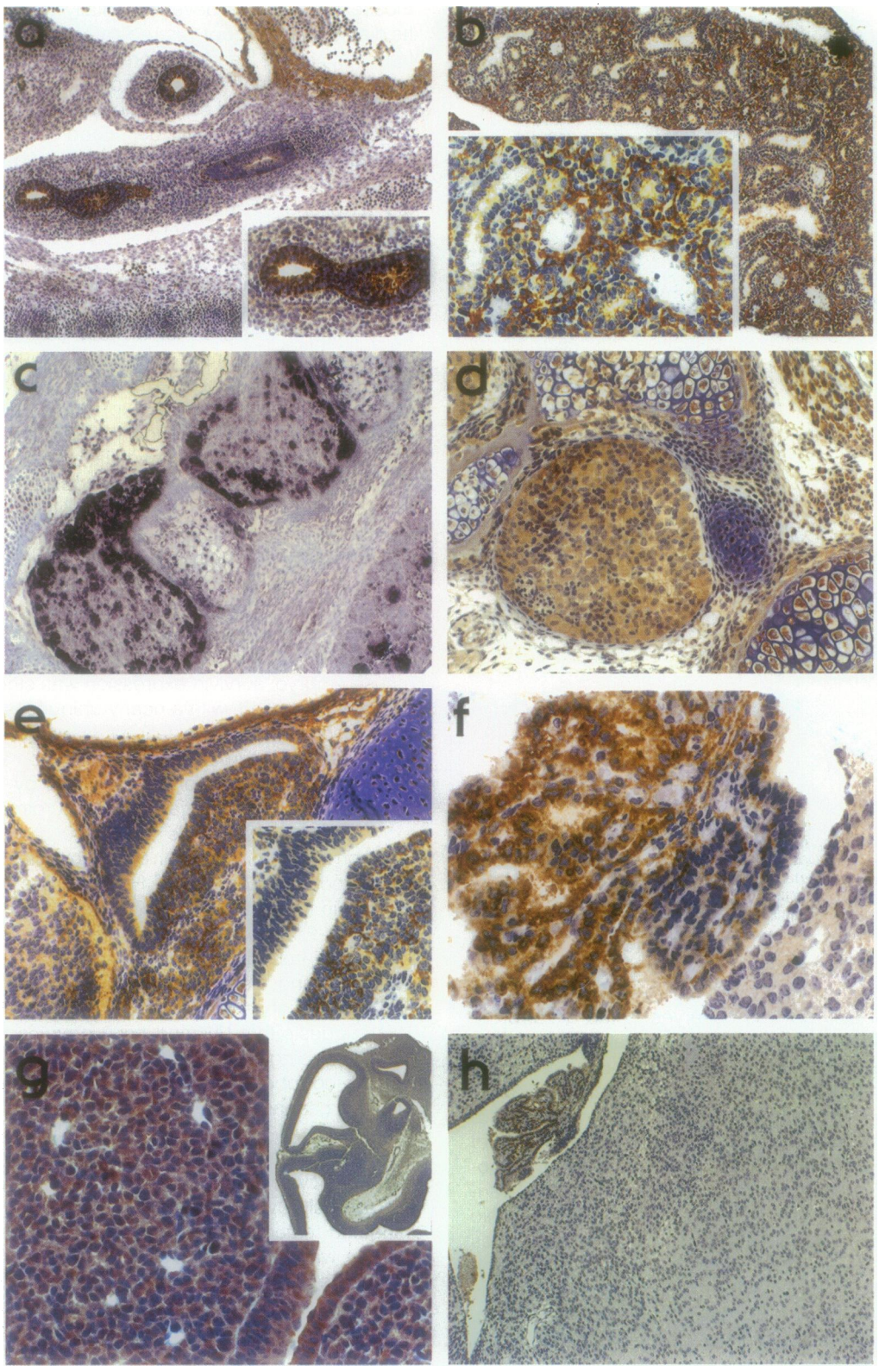

Figure 4. Developmentally regulated expression of survivin in mouse embryo. **a** and **b:** Immunostaining of survivin in epithelial cells of gastrointestinal tract (**a**) and distal bronchiolar lung (**b**) at E11.5 (**a**) and E15 to -21 (**b**). **c** and **d:** *In situ* hybridization (**c**) and immunohistochemistry (**d**) of survivin expression in dorsal root ganglion neurons at E15 to -21. **e** and **f:** Immunostaining of survivin hypophysis (**e**) and the choroid plexus (**f**) by immunohistochemistry at E15 to -21. **g** and **h:** Survivin expression in mouse brain at E11.5 (**g, inset**) and E15 to -21 (**h**). Original magnification, ×100 (**a**, **b**, **h**, and **g inset**), ×200 (**c–f** and **a inset**), and ×400 (**g** and **b and e insets**).

can down-regulate survivin expression by acting as a natural antisense transcript.[23–26] Furthermore, the embryologically distinct distribution of survivin in proximal tubules (metanephric mesoderm) and of EPR-1 in collecting ducts (metanephric diverticulum) of fetal kidney suggests a potential involvement of additional, cell-type-specific mechanism(s) of gene regulation.

In summary, these studies reiterate the role of apoptosis inhibitors in morphogenesis[6] and propose a new role for the IAP protein survivin[14] in developmentally regulated mechanisms of tissue and organ differentiation. Dissection of this pathway of gene expression should help define the potential contribution of survivin to apoptosis inhibition in cancer.[14]

References

1. Vaux DL, Haecker G, Strasser A: An evolutionary perspective on apoptosis. Cell 1994, 76:777–779
2. Oltvai ZN, Korsmeyer SJ: Checkpoints of dueling dimers foil death wishes. Cell 1994, 79:189–192
3. Reed JC: Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994, 124:1–6
4. Yang E, Korsmeyer SJ: Molecular thanatopsis: a discourse on the BCL2 family and cell death. Blood 1996, 88:386–401
5. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ: BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc Natl Acad Sci USA 1991, 88:6961–6965
6. LeBrun DP, Warnke RA, Cleary ML: Expression of *bcl-2* in fetal tissues suggests a role in morphogenesis. Am J Pathol 1993, 142:743–753
7. Clem RJ, Miller LK: Control of programmed cell death by the baculovirus genes *p35* and *iap*. Mol Cell Biol 1994, 14:5212–5222
8. Rothe M, Pan M-G, Henzel WJ, Ayres TM, Goeddel DV: The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 1995, 83:1243–1252
9. Duckett CS, Nava VE, Gedrich RW, Clem RJ, Van Dongen JL, Gilfillan MC, Shiels H, Hardwick JM, Thompson CB: A conserved family of cellular genes related to the baculovirus *iap* gene and encoding apoptosis inhibitors. EMBO J 1996, 15:2685–2694
10. Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Besner-Johnston A, Lefebvre C, Kang X, Salih M, Aubry H, Tamai K, Guan S, Ioannou P, Crawford TO, de Jong PJ, Surh L, Ikeda J-E, Korneluk RG, MacKenzie A: The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 1995, 80:167–178
11. Liston P, Roy N, Tamai K, Lefebvre C, Baird S, Cherton-Horvat G, Farahani R, McLean M, Ikeda J-E, MacKenzie A, Korneluk RG: Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 1996, 379:349–353
12. Hay BA, Wassarman DA, Rubin GM: Drosophila homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. Cell 1995, 83:1253–1262
13. Uren AG, Pakusch M, Hawkins CJ, Puls KL, Vaux DL: Cloning and expression of apoptosis inhibitory proteins homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. Proc Natl Acad Sci USA 1996, 93:4974–4978
14. Ambrosini G, Adida C, Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nature Med 1997, 3:917–921
15. Altieri DC: Xa receptor EPR-1. FASEB J 1995, 9:860–865
16. Oppenheim RW: Cell death during development of the nervous system. Annu Rev Neurosci 1991, 14:453–501
17. Jung W, Castren E, Odenthal M, Vande Woude GF, Ishii T, Dienes H-P, Lindholm D, Shirmacher P: Expression and functional interaction of hepatocyte growth factor-scatter factor and its receptor c-*met* in mammalian brain. J Cell Biol 1994, 126:485–494
18. Davies AM: Switching neurotrophin dependence. Curr Biol 1994, 4:273–276
19. Zhang Z, Vuori K, Reed JC, Ruoslahti E: The $\alpha 5\beta 1$ integrin supports survival of cells on fibronectin and up-regulates *bcl-2* expression. Proc Natl Acad Sci USA 1995, 92:6161–6165
20. Davis EC, Popper P, Gorski RA: The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 1996, 734:10–18
21. Coggeshall RE, Pover CM, Fitzgerald M: Dorsal root ganglion cell death and survivin cell numbers in relation to the development of sensory innervation in the rat hindlimb. Dev Brain Res 1994, 82:193–212
22. Deveraux QL, Takahashi R, Salvesen GS, Reed J: X-linked IAP is a direct inhibitor of cell-death proteases. Nature 1997, 388:300–304
23. Celano P, Berchtold CM, Kizer DL, Weeraratna A, Nelkin BD, Baylin SB, Casero RA Jr: Characterization of an endogenous RNA transcript with homology to the antisense strand of the human c-*myc* gene. J Biol Chem 1992, 267:15092–15096
24. Khochbin S, Lawrence J: An antisense RNA involved in p53 mRNA maturation in murine erythroleukemia cells induced to differentiate. EMBO J 1989, 8:4107–4114
25. Kimmelman D, Kischner MW: An antisense mRNA directs the covalent modification of the transcript encoding fibroblast growth factor in *Xenopus* oocytes. Cell 1989, 59:687–696
26. Krystal GW, Armstrong B, Battey JF: N-myc mRNA forms an RNA-RNA duplex with endogenous antisense transcripts. Mol Cell Biol 1990, 10:4180–4191